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The Radical Scavenging Effects of Stilbene Glucosides from *Polygonum multiflorum*

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The extract of the root of *Polygonum multiflorum* exhibited a significant antioxidant activity assessed by the DPPH radical scavenging activity *in vitro*. The bioassay-guided fractionation of the extract yielded a stilbene glucoside, (*E*)-2,3,5,4'-tetrahydroxystilbene-2-*O*-β-d-glucopyranoside (**1**) as an active constituent responsible for the antioxidant property. Compound **1** demonstrated a moderate DPPH radical scavenging activity (IC_{50}, 40 μM), while the corresponding deglucosylated stilbene **2** exhibited a much higher activity (IC_{50}, 0.38 μM).

Key words: Stilbene glucoside, (*E*)-2,3,5,4'-Tetrahydroxystilbene-2-*O*-β-D-glucopyranoside, *Polygonum multiflorum*, DPPH Radical scavenging activity.

INTRODUCTION

Stilbene glucosides have attracted a great deal of interest because relatively high quantities are found in grapes, wine, and some medicinal plants (Goldberg, 1995; Mattivi *et al.*, 1995; Frankel *et al.*, 1993; Jang *et al.*, 1997; Teguo *et al.*, 1996). They seem to have a variety of biological activities such as antioxidant activity (Teguo *et al.*, 1996; Teguo *et al.*, 1996; Teguo *et al.*, 1998), antiplatelet aggregation activity (Verotta *et al.*, 1994; Orsini *et al.*, 1997; Chen *et al.*, 1994), coronary vasodilator activity (Inamori *et al.*, 1989), antileukemic (Mannila *et al.*, 1993), antifungal activity (Langcake, *et al.*, 1979), and protein-tyrosine kinase (Jayatilake *et al.*, 1993), monoamine oxidase A (Ryu *et al.*, 1988) and gastric H^+, K^+-ATPase inhibitory activity (Murakami *et al.*, 1992). Interestingly, many medicinal plants contain large amount of antioxidants other than vitamin C, vitamin E and carotenoids. The antioxidant effect is mainly due to the phenolic components such as flavonoids, phenolic acid, and phenolic diterpenes (Pietta *et al.*, 1998). As part of our continuous search for bioactive components from Korean medicinal plants, we have focused on the antioxidant effect of the methanol extract of *Polygonum multiflorum* (Polygonaceae). Bioassay-guided isolation of the extract afforded a stilbene glucoside **1** as an active compound of the antioxidant property.

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MATERIALS AND METHODS

General experimental procedures

UV spectrum was recorded with a HP8453 UV/VIS spectrophotometer. IR spectrum was performed on a Perkin-Elmer model 1750 FT-IR spectrophotometer. MS spectra were measured on a JEOL JMX-SX 102 mass spectrometer. High resolution mass measurement was done with a JEOL AX-505H mass spectrometer at high resolution using NBA or glycerol as a matrix. ^1H NMR and ^{13}C NMR spectra were recorded in CD_3OD or $CDCl_3$ at 25°C on a Brucker ARX-400 NMR spectrometer. Chemical shifts (δ) are given relative to TMS, using the solvent peaks [CD_3OD (δ_H 3.30, δ_C 49.0) and $CDCl_3$ (δ_H 7.26, δ_C 77.1)] as the internal standards.

Plant materials

The root bark of *Polygonum multiflorum* was supplied from Hankook Sinyak Pharmaceutical Co., LTD, Taejon, Korea. A voucher specimen (KM-98203) was deposited at

the Laboratory of Natural Products Chemistry, Hanbat National University.

DPPH radical scavenging assay

Radical scavenging effects on DPPH (α,α′-diphenylpicrylhydrazyl) radical were determined by the method previously described (Ryu *et al.*, 2001).

Extraction and isolation

The dry root bark of *Polygonum multiflorum* (200 g) was extracted with MeOH at room temperature for a day. The MeOH extract (36 g) with the radical scavenging effect of IC_{50}, 26 mg/mL was partitioned between 30% MeOH and $CHCl_3$ (4.8 g, 13.3% yield); the former layer was further partitioned between *n*-BuOH (8.3 g, 23%) and H_2O (23.4 g, 65%). The bioactive *n*-BuOH fraction was subjected to an ODS flash chromatography with aqueous MeOH [0, 20, 40, 60, 100%] to give five fractions. The most active 40% MeOH fraction was gel-filtered with MeOH to afford a crude phenolic glycoside fraction (IC_{50}, 4.6 μg/mL). This fraction was finally purified by reversed-phase HPLC with 25% MeCN to yield a glycoside **1** (74 mg, 0.89%).

(*E*)-2,3,5,4'-Tetrahydroxystilbene-2-*O*-β-D-glucopyranoside (1)

Brown powder; $[\alpha]_D^{23}$+28.9° (*c* 1.2, MeOH); UV λ_{max} (log ε) 215 nm (4.16), 306 (4.35) and 318 (4.36); IR (film) ν_{max} cm^{-1} 3650 (OH), 1630 and 1540 (aromatic); FABMS (pos) *m/z* 407 $[M+H]^+$, 429 $[M+Na]^+$; HRFABMS (pos) *m/z* $[M+H]^+$ 407.1353 (calcd 407.1345 for $C_{20}H_{23}O_9$); ^1H NMR (CD_3OD, 400 MHz) δ 7.70 (2H, d, 8.2 Hz, H-2' and H-6'), 7.45 (1H, d, 16.4 Hz, H-8), 6.91 (2H, d, 8.2 Hz, H-3' and H-5'), 6.76 (1H, d, 16.4 Hz, H-7), 6.59 (1H, d, 1.7 Hz, H-6), 6.24 (1H, d, 1.7 Hz, H-4), 4.50 (1H, d, 7.8 Hz, H-1"), 3.77 (2H, m, H-6"), 3.57-3.54 (2H, overlapped, H-2" and H-4" , 3.44 (1H, m, H-3"), 3.27 (1H, m, H-5"); ^{13}C NMR (CD_3OD, 100 MHz) δ 157.8 (C4'), 155.3 (C5), 152.2 (C3), 137.5 (C2), 132.9 (C1'), 130.0 (C8), 129.2 (C1), 128.5 × 2 (C2' and C6'), 121.0 (C7), 115.8 x 2 (C3' and C5'), 107.6 (C1" , 103.1 (C4), 101.6 (C6), 77.5 (C5"), 77.3 (C3"), 74.8 (C2" , 70.1 (C4"), 61.4 (C6").

Enzymatic hydrolysis of 1

Stilbene glucoside **1** (20 mg) was dissolved in 3mL of AcOH-AcONa buffer solution (pH 5), to which β-glucosidase (10 mg) was added to lay aside at 37°C for 3 days. The reaction mixture was adjusted to neutral with $AgNO_3$ and then filtered. The filtrate was partitioned between AcOEt (10 mL × 3) and H_2O (10 mL). The organic layer was concentrated *in vacuo* to give (*E*)-2,3,5,4'-tetrahydroxystilbene (**2**, 8.2 mg), while the aqueous layer was further neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, concentrated (dried overnight), then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 hours. After the excess reagent was decomposed with H_2O, the reaction product was extracted with *n*-hexane (2 mL × 2). The TMSi derivative of the monosaccharide was identified to be d-glucose by co-GLC analysis with the TMSi derivative of a standard monosacchride.

2: colorless solid; ^1H NMR (CD_3OD, 400 MHz) δ 6.94 and 6.98 (2H, d, 16.4 Hz, H-7 and H-8), 7.51 (2H, d, 8.3 Hz, H-2' and H-6'), 6.82 (2H, d, 8.3 Hz, H-3' and H-5'), 6.45 (1H, d, 1.7 Hz, H-6), and 6.18 (1H, d, 1.7 Hz, H-4); FABMS (pos) *m/z* 245 $(M+H)^+$ and 267 $(M+Na)^+$.

Acetylation of 1

A mixture of stilbene glucoside **1** (18 mg), Ac_2O (1.0 mL), and pyridine (1.0 mL) was stirred at room temperature overnight. The reagents were evaporated *in vacuo* and the residue was subjected to SiO_2 column chromatography [CH_2Cl_2/MeOH (95:5)] to yield a heptaacetyl stilbene glucoside **3** (28 mg).

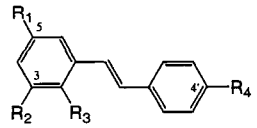
3: white solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.44 (2H, d, 8.2 Hz, H-2' and H-6'), 7.27 (1H, d, 16.3 Hz, H-8), 7.00 (2H, d, 8.2 Hz, H-3' and 5'), 6.93 (1H, d, 16.3 Hz, H-7), 6.77 (1H, br s, H-6), 6.49 (1H, br s, H-4), 5.22 (1H, t, 7.8 Hz, H-3"), 5.15 (2H, overlapped, H-2" and H-4"), 4.86 (1H, d, 7.9 Hz, H-1"), 4.22 and 3.85 (2H, br d, 12.6 Hz, H-6"); 3.54 (1H, m, H-5"), and 2.25, 2.22 × 2, 2.00, 1.95, 1.92, and 1.86 (7 singlet acyl methyls); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7-168.6 (7 carbonyls), 150.8, 147.6, 144.2, 142.6, 134.9, 133.8, 130.8, 130.3, 128.1 × 2, 122.4 × 2, 116.6 116.4, 101.6, 73.2, 72.4, 71.9, 68.1, 61.6, and 21.3-20.7 (7 acyl methyls); FABMS (pos) *m/z* 701 $(M+H)^+$.

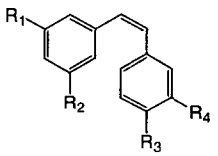
RESULTS AND DISCUSSION

Compound **1** was obtained as a brown powder: $[\alpha]_D^{23}$ +28.9 (*c* 1.2, MeOH). A colorizing reaction of **1** with $FeCl_3$ showed dark blue and the UV spectrum of **1** exhibited bands at 214 nm (log ε 4.16), 306 (4.35) and 318 (4.36) to suggest the presence of a stilbene skeleton (Hata *et al.*, 1975). The ^1H NMR spectrum of **1** was similar to that of resveratrol glucoside **5** isolated from *Erythrophleum lasianthum* (Langcake *et al.*, 1979). Enzymatic hydrolysis of **1** yielded a glucose and compound **2**, which was identified as 2,3,5,4'-tetrahydroxystilbene by spectral analysis and chemical modification (compound **3**). Thus, **1** was identified as *trans*-2,3,5,4'-tetrahydroxystilbene-2-*O*-β-d-glucopyranoside, which was previously reported by Hata *et al.*

Stilbene glucose **1** is structurally similar to resveratrol glucoside **4** (Langcake *et al.*, 1979; Nyemba *et al.*, 1995) and piceatannol glucoside **5** (Geahlen *et al.*, 1989; Thakkar *et al.*, 1993; Oliver *et al.*, 1994). Compounds **1** and **5** exhibited moderate radical scavenging effect on

Table 1. Antioxidant activity of phenolic compounds





1 R_1=R_3=R_4=OH, R_2=*O*-β-D-Glu
2 R_1=R_2=R_3=R_4=OH
3 R_1=R_3=R_4=OAc, R_2=tetraacetyl-β-D-Glu
4 R_1=R_2= OH, R_3=H, R_4=*O*-β-D-Glu
5 R_1=*O*-β-D-Glu, R_2=R_3= R_4=OH

Compound	IC_{50} (μM)
(*E*)-2,3,5,4'-tetrahydroxystilbene-2-*O*-β-D-glucopyranoside (**1**)	40
(*E*)-2,3,5,4'-tetrahydroxystilbene (**2**)	0.38
heptaacetyl stilbene glucoside (**3**)	> 100
resveratrol-4'-*O*-β-D-glucopyranoside (**4**)	1000 ± 95[a]
piceatannol-3-*O*-β-D-glucopyranoside (**5**)	29 ± 1.9[a]
BHT	1.44
ascorbic acid	0.04
catechin	0.3

[a]These values were cited from a reference (Teguo *et al.*, 1998).

DPPH radical with IC_{50} values of 40 μM and 29 μM, respectively, while compound **4** exhibited a poor activity (IC_{50}, 1000 μM) (Table 1). Rice-Evans *et al.* asserted that the catechol moiety attached on stilbene skeleton is essential for antioxidant activity of stilbenes (Rice-Evans *et al.*, 1995), and Merillon *et al.* reported that the glycosylation of stilbene reduces their activity compared to the corresponding aglycone, but stilbenes including the catechol structure dramatically increase antioxidant activity (Teguo *et al.*, 1998). It has also been reported that the antioxidant activity of polyphenols is related to the position of hydroxyl groups. The presence of a second hydroxyl group in the *ortho* or *para* position is known to increase the antioxidant activity due to additional resonance stability and *o*-quinone or *p*-quinone formation (Chen and Ho, 1997; Bouchet *et al.*, 1998). Their assertions were supported by the fact that deglucosylated stilbene **2** (IC_{50}, 0.38 μM), having a catechol moiety, was nearly a hundred times as potent as stilbene glucoside **1**. As expected, acetylated stilbene **3** showed a poor antioxidant activity.

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