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A New Stilbene Glucoside Gallate from the Roots of *Polygonum multiflorum*

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A new stilbenoid (**1**) was isolated from the root extract of *Polygonum multiflorum* together with eight known constituents (**2**~**9**). The chemical structure of **1** was established as the 6''-O-monogalloyl ester of (*E*)-2,3,4',5-β-tetrahydroxystilbene-2-β-D-glucopyranoside based on physicochemical and spectroscopic analyses, particularly by NMR spectroscopic data, *i.e.*, COSY, HMQC and HMBC. Compound **1** weakly inhibited acetylcholinesterase *in vitro*.

Key words: *Polygonum multiflorum,* Polygonaceae, (*E*)-2,3,4',5-tetrahydroxystilbene-2-β-D-glucoside, Stilbene glucoside gallate

INTRODUCTION

Polygonum multiflorum (Polygonaceae) is a traditional Chinese herbal medicine common in northeast Asia. The roots have been used as anti-allergy, anti-tumor, antibacterial, hemostatic, spasmolytic, and analgesics in Korean traditional medicine. The genus *Polygonum* is the source of a wide range of phenolic compounds, flavonoids, anthraquinones, stilbenes, and tannins (Lin et al., 2003), including a number of anthraquinones in the stilbene class such as (*E*)-2,3,4',5-tetrahydroxystilbene-2-β-D-glucoside, rhein, emodin, aloe-emodin, chrysophanol, physcion, and their derivatives (Yi et al., 2005). During the phytochemical survey of the extract of the species, a new stilbene glucoside gallate (**1**) was isolated together with eight known constituents (**2**~**9**).

MATERIALS AND METHODS

General experimental procedures

Optical rotations were determined using a Rudolph Autopol IV polarimeter. MS spectra were measured on Varian CP3800-1200L (EI-MS), Voyager PE Biosystems USA (MALDI-TOF/MS) and Jeol JMS-DX303 (FAB-MS) Spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brucker AM-300 and DMX-600 NMR spectrometer using TMS as an internal standard. Column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh, Merck), Sephadex LH-20 (25-100 mm, Pharmacia), and Lichroprep RP-18 (40-63 mm, Merck). TLC analysis was performed on Kieselgel 60 F_{254} plates (silica gel, 0.25 mm, Merck).

Plant material

The roots of *Polygonum multiflorum* (Polygonaceae) were collected in Chungnam Province on September, 2005, and were authenticated by Prof. Kang Ro Lee, College of Pharmacy, Sungkyunkwan University, Korea. A voucher specimen (KR0353) has been deposited in the herbarium of the Korea Research Institute of Chemical Technology, Daejeon 305-606, Korea.

Extraction and isolation

The dried roots of *P. multiflorum* (12 kg) were extracted twice with MeOH by maceration at room temperature for 7 days. The MeOH solution was combined and evaporated to dryness to give 1.8 kg of dark syrupy extract. The MeOH extract was suspended in H_2O (30 L) and partitioned with an equal volume of methylene chloride (MC, 3 × 30 L), EtOAc (3 × 30 L), and *n*-BuOH (3 × 30 L) successively, which afforded a methylene chloride

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soluble fraction (130 g), an EtOAc soluble fraction (359 g), an *n*-BuOH soluble fraction (477 g) and a aqueous fraction (810 g), respectively.

The EtOAc soluble fraction (120 g) was separated on a silica gel (70-230 mesh, 2.5 kg), eluted with a gradient solvent system (MeOH in MC 1% to 50%) to afford 6 fractions (Fr. A - Fr. F). Fr. F (21 g) was further purified by repeated column chromatography with Lichroprep RP-18 (MeOH in H_2O 0% to 40%), Sephadex LH-20 (100% MeOH) and silica gel (MeOH in MC 10% to 50%) which afforded compounds **1** (25 mg), **2** (120 mg), **3** (5.3 g), **4** (8 mg), **7** (50 mg), and **8** (49 mg).

The methylene chloride soluble fraction was also subjected to column chromatography on a silica gel (70-230 mesh) and eluted with a gradient solvent system (MeOH in MC 1% to 50%) to afford 10 fractions (Fr. a - Fr. j), from which compounds **5** (490 mg) and **6** (6.6 g) were isolated. The *n*-BuOH soluble fraction was also separated on a silica gel (70-230 mesh) in a similar manner to afford compound **9** (20 mg).

(*E*)-2,3,5,4'-tetrahydroxystilbene-2-β-D-(6''-galloyl)-glucoside (1)

Brown amorphous powder, $[\alpha]_D^{20}$ + 30.18 (*c* 0.1, MeOH), MALDI-TOF/MS *m/z*: 581 ($[M+Na]^+$; $C_{27}H_{26}O_{13}Na$), FAB-MS m/z: 558 [M^+], ^{1}H-NMR (Acetone-d_6, 600 MHz), ^{13}C-NMR (Acetone-d_6, 150 MHz); see Table I.

(*E*)-2,3,5,4'-tetrahydroxystilbene-2-β-D-(2''-galloyl)-glucoside (2)

Brown amorphous powder, $[\alpha]_D^{20}$ - 2.09 (*c* 0.5, MeOH), FAB-MS m/z: 558 [M^+], ^{1}H-NMR (Acetone-d_6, 600 MHz) δ: 3.51-3.94 (5H, m, sugar H), 5.03 (1H, d, *J* = 8.0 Hz, H-1''), 5.34 (1H, t, *J* = 9 Hz, H-2''), 6.32 (1H, d, *J* = 2.8 Hz, H-4), 6.65 (1H, d, *J* = 2.8 Hz, H-6), 6.75 (2H, d, *J* = 8.5 Hz, H-2',6'), 6.93 (1H, d, *J* = 16.4 Hz, H-β), 7.17 (1H, d, *J* = 16.4 Hz, H-α), 7.20 (2H, d, *J* = 8.5 Hz, H-3',5'), 7.27 (2H, s, H-2''',6'''), ^{13}C-NMR (Acetone-d_6, 150 MHz); see Table II.

(*E*)-2,3,5,4'-tetrahydroxystilbene-2-β-D-glucoside (3)

Brown amorphous powder, $[\alpha]_D^{20}$ + 20.91 (*c* 0.5, MeOH), EI-MS m/z: 244 [M-glc]$^+$, ^{1}H-NMR (CD_3OD, 300 MHz) δ: 3.34-3.79 (6H, m, sugar H), 4.50 (1H, d, *J* = 7.8 Hz, H-1''), 6.24 (1H, d, *J* = 2.8 Hz, H-6), 6.61 (1H, d, *J* = 2.8 Hz, H-4), 6.75 (2H, d, *J* = 6.7 Hz, H-3',5'), 6.91 (1H, d, *J* = 16.4 Hz, H-β), 7.44 (2H, dd, *J* = 1.9, 6.7 Hz, H-2',6'), 7.70 (1H, d, *J* = 16.4 Hz, H-α), ^{13}C-NMR (CD_3OD, 75 MHz); see Table II.

(*Z*)-2,3,5,4'-tetrahydroxystilbene-2-β-D-glucoside (4)

EI-MS m/z: 244 [M-glc]$^+$, ^{1}H-NMR (CD_3OD, 300 MHz) δ: 3.39-3.82 (6H, m, sugar H), 4.58 (1H, d, *J* = 7.6 Hz,

Table I. NMR spectral data for Compound **1**

Carbon	δ_H	δ_C	DEPT	HMBC correlations
1		133.4	C	
2		137.9	C	
3		152.1	C	
4	6.31 (d, *J* = 3.0 Hz)	103.5	CH	2,3,5,6
5		155.9	C	
6	6.68 (d, *J* = 3.0 Hz)	102.1	CH	α,2,4,5
1'		130.4	C	
2',6'	7.39 (d, *J* = 8.7 Hz)	128.8	CH	β,4'
3',5'	6.67 (d, *J* = 8.7 Hz)	116.4	CH	1'
4'		158.0	C	
α	7.80 (d, *J* = 16.8 Hz)	121.7	CH	2,6,1'
β	6.95 (d, *J* = 16.8 Hz)	129.6	CH	1,2',6'
1''	4.64 (d, *J* = 7.8 Hz)	107.9	CH	2
2''	3.69 (m)	75.5	CH	1''
3''	3.62 (m)	77.5	CH	2'',4''
4''	3.68 (m)	70.6	CH	3'',5''
5''	3.74 (m)	75.6	CH	1'',3''
6''	4.44, 4.57 (m)	63.9	CH_2	4'',5'', -COO-
1'''		121.7	C	
2''',6'''	7.23(s)	110.1	CH	1''',3''',4''',5''',-COO-
3''',5'''		146.1	C	
4'''		138.9	C	
-COO-		166.9	C	

Table II. ^{13}C NMR spectra of stilbenes **2-3**

	2*	3#
1	132.5	133.8
2	135.2	138.0
3	151.6	152.1
4	103.2	103.7
5	155.0	156.0
6	102.5	102.8
1'	129.1	130.9
2',6'	128.4	129.3
3',5'	116.0	116.5
4'	157.6	158.3
α	119.0	121.7
β	130.3	130.2
1''	103.2	108.2
2''	74.8	75.5
3''	75.2	78.0
4''	70.5	70.8
5''	77.2	78.2
6''	61.5	62.1
1'''	121.3	
2''',6'''	110.0	
3''',5'''	145.5	
4'''	138.4	
-COO-	165.8	

*: acetone-d_6, #: methanol-d_4

H-1"), 6.15 (1H, d, J = 2.8Hz, H-6), 6.24 (1H, d, J = 2.8 Hz, H-4), 6.50 (1H, d, J = 12.2 Hz, H-α), 6.62 (2H, d, J = 8.5 Hz, H-3',5'), 6.73 (1H, d, J = 12.2 Hz, H-β), 7.08 (2H, d, J = 8.5 Hz, H-2',6'),^{13}C-NMR (CD_3OD, 75 MHz) δ: 61.1 (C-6"), 70.8 (C-4"), 75.5 (C-2"), 78.0 (C-3"), 78.2 (C-5"), 103.8 (C-6), 107.7 (C-1"), 108.2 (C-4), 115.9 (C-3',5'), 125.7 (C-α), 129.3 (C-1'), 131.3 (C-β), 131.4 (C-2',6'), 133.8 (C-1), 137.8 (C-2), 151.5 (C-3), 155.2 (C-5), 157.5 (C-4').

Torachrysone-8-O-β-D-glucoside (7)

EI-MS m/z: 246 [M-glc]$^+$, ^{1}H-NMR (Acetone-d_6, 300 MHz) δ: 2.27 (3H, s, CH_3), 2.52 (3H, s, CH_3), 3.50 - 3.99 (sugar H), 3.87 (3H, s, OCH_3), 5.20 (1H, d, J = 7.0 Hz, anomeric H), 6.86 (1H, d, J = 2.1 Hz, H-5), 7.01 (1H, d, J = 2.1 Hz, H-7), 7.04 (1H, s, H-4), ^{13}C-NMR (Acetone-d_6, 75 MHz) δ: 19.5 (CH_3), 31.8 (CH_3), 55.2 (OCH_3), 61.8 (C-6'), 70.6 (C-4'), 74.1 (C-2'), 77.2 (C-3'), 77.9 (C-5'), 101.4 (C-1'), 103.3 (C-5), 103.4 (C-7), 109.4 (C-9), 119.2 (C-4), 123.5 (C-2), 134.6 (C-10), 137.8 (C-3), 152.5 (C-1), 156.2 (C-8), 159.1 (C-6), 204.1 (C=O).

Acetylcholinesterase assay

Acetylcholinesterase (AChE) activities were determined by the Amplex® Red acetylcholinesterase assay kit (Molecular Probes, Inc., Eugene, OR), which uses purified AChE from electric eel and a sensitive fluorogenic probe (10-acetyl-3,7-dihydroxyphenoxazine; Amplex® Red) for H_2O_2. In the assay, aliquots of AchE enzyme (0.1 U/mL), Amplex Red reagents (200 mM), choline oxidase (0.1 U/mL), horseradish peroxidase (1 U/mL), acetylcholine (50 mM), and appropriate concentrations of test compounds were added to 200 mL of 50 mM Tris-HCl buffer (pH 8.0). After 30 min incubation at room temperature, protected from light, the resorufin fluorescence was monitored on a fluorometric plate reader, Victor 1420 (Ex_{571nm}/Em_{585nm}). The inhibitory effects of compounds on enzyme activities were determined with 6~8 concentrations of the test compound run in duplicate tubes, and isotherms from two assays were calculated by nonlinear regression analysis (GraphPad Prism Program, San Diego, CA) to yield IC_{50} values (Kim et al., 2002).

RESULTS AND DISCUSSION

Extensive phytochemical investigation on the root extract of *P. multiflorum* (Polygonaceae), *i.e.*, the serial solvent partition of the extract with CH_2Cl_2, EtOAc, and *n*-BuOH, followed by purification of the resultant solvent fractions with repeated column chromatography using the silica gel, ODS, and Sephadex LH-20, resulted in the isolation of four stilbene glycosides (**1**~**4**), including a novel component **1**, as well as four anthraquinones (**5**~**8**) and a naphthalene gycoside (**9**) (Fig. 1).

The molecular formula of a novel stilbene **1** was determined as $C_{27}H_{26}O_{13}$ by MALDI-TOF/MS *m/z*: 581 ($C_{27}H_{26}O_{13}Na$) and FAB-MS spectrum (*m/z* 558). The ^{1}H-NMR spectrum of **1** exhibited a singlet equivalent of two protons at δ 7.23 that implicated the presence of a galloyl group. It also exhibited a pair of aromatic A_2B_2-type signals (δ 6.67, 7.39, J = 8.7 Hz), a pair of meta-coupled doublets (δ 6.31, 6.68, J = 3.0 Hz), two proton signals coupled by a trans-olefinic coupling pattern (δ 6.95, 7.80, J = 16.8 Hz) and sugar proton signals between δ 3.5~δ 4.0, which suggested that **1** was a galloyl ester of (*E*)-2,3,4',5-tetrahydroxystilbene-2-β-D-glucoside (**3**). Comparing the ^{1}H-NMR spectrum of **1** with that of **3** indicated that a pair of methylene proton signals of **1** were shifted downfield to δ 4.44 and δ 4.57, whereas the corresponding proton signals of **3** were observed between δ 3.5~δ 4.0. These observations strongly suggested that the galloyl group of **1** was attached to the sugar moeity and not to the hydroxystilbene scaffold. Thus, all proton signals and carbon signals were completely identified with various two-dimensional NMR experiments such as COSY, DEPT, HMQC, and HMBC to determine the connectivity between each atom in the structure (Table I). In particular, the correlations between the methylene protons (δ 4.44 and 4.57) and the carboxy carbon (δ 166.9) observed in HMBC spectra showed the linkage point of gallic acid unambiguously to be 6-position of glucose (Fig. 2). Therefore, the structure of compound **1** was assigned as (*E*)-2,3,4',5-tetrahydroxystilbene-2-β-D-(6"-galloyl)-glucopyranoside.

Two kinds of galloyl esters of **3**, (*E*)-2,3,4',5-tetrahydroxystilbene-2-β-D-(3"-galloyl)-glucopyranoside and (*E*)-2,3,4',5-tetrahydroxystilbene-2-β-D-(2"-β-galloyl)-glucopyranoside (**2**), had been isolated from this species (Nonaka et al., 1982). However, this is the first report on the isolation of compound **1** from this species or from any other plant species.

Other isolated components (**2**~**9**) from the extract were identified as (*E*)-2,3,4',5-tetrahydroxystilbene-2-β-D-(2"-galloyl)-glucoside (**2**) (Nonaka et al., 1982), (*E*)-2,3,4',5-tetrahydroxystilbene-2-β-D-glucoside (**3**) (Chen et al., 1999, Yao et al., 2006), (*Z*)-2,3,4',5-tetrahydroxystilbene-2-β-D-glucoside (**4**) (Xu et al., 2006), physcion (**5**) (Kalidhar, 1989), emodin (**6**) (Francis et al., 1998), torachrysone-8-O-β-D-glucoside (**7**) (Masahiko et al., 1977, Yi et al., 2005), emodin-8-O-β-D-glucoside (**8**) (Takeshi and Yutaka, 1987), and physcion-8-O-β-D-glucoside (**9**) (Takeshi and Yutaka, 1987) by comparison of spectral data with the literature.

Both **1** and **2** dose-dependently inhibited purified acetylcholinesterase (AChE) from electric eel *in vitro*, whereas **3** showed weaker inhibition. Compounds **4**~**9** did not

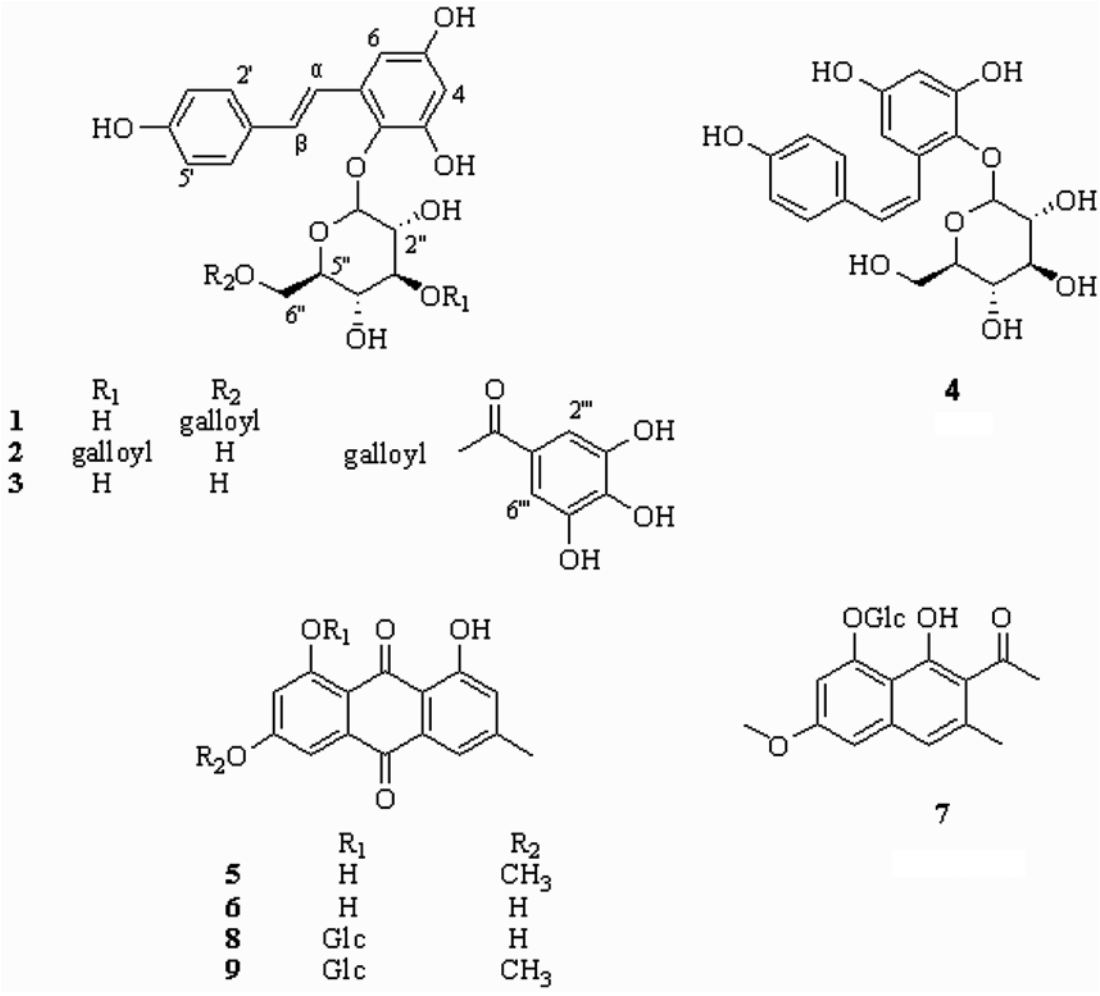

Fig. 1. Structures of compounds (**1-9**) isolated from *P. multiflorum*

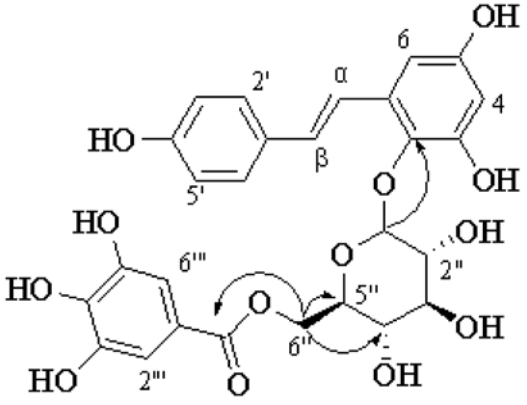

Fig. 2. Selected HMBC correlation of compound **1**

inhibit AChE up to 100 μM. The IC_{50} values of **1**~**3** for AChE inhibition were 17.2, 18.5, and 98.3 μM, relative to THA (tetrahydroaminoacridine; tacrine®), a positive control of 0.3 μM (Kim et al., 2002).

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