

Cytotoxic and Antimutagenic Stilbenes from Seeds of *Paeonia lactiflora*

Hyo Jin Kim¹, Eun Ju Chang¹, Song Ja Bae², Sun Mi Shim², Heui Dong Park³, Chang Ho Rhee³, Jun Hong Park⁴, and Sang Won Choi¹

¹Department of Food Science and Nutrition, Catholic University of Daegu, Gyeongbuk 712-702, Korea, ²Department of Food Science and Nutrition, Silla University, Pusan 617-736, Korea, ³Department of Food Science and Technology, Kyungpook National University, Daegu 702-701, Korea and ⁴Uisong Medicinal Plant Experiment Station, Gyeongbuk Provincial A.T.A., Uisong, Gyeongbuk 769-800, Korea

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Cytotoxic and antimutagenic effects of a novel *cis-ε*-viniferin and five known stilbenes, *trans*-resveratrol, *trans-ε*-viniferin, gnetin H, suffruticosols A and B, isolated from the seeds of *Paeonia lactiflora* Pall. (Paeoniaceae) were determined against five different cancer cell lines, and mutagenicity of *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) in *Salmonella typhimurium* TA100, respectively. Six stilbenes showed cytotoxic activity in a dose-dependent manner, and especially did potent cytotoxic activity against C6 (mouse glioma) cancer cell with IC₅₀ values ranging from 8.2 to 20.5 μg/ml. *trans*-Resveratrol showed significant cytotoxic activity against HepG2 (liver hepatoma) and HT-29 (colon) human cancer cell lines with IC₅₀ values of 11.8 and 25.2 g/ml, respectively. In contrast, *trans-ε*-viniferin and *cis*-viniferin, and gnetin H exhibited marked cytotoxic activity against Hela (cervix) and MCF-7 (breast) human cancer cell lines with IC₅₀ values of 20.4, 21.5, and 12.9 μg/ml, respectively. However, suffruticosol A and B had less cytotoxic effect against all cancer cells except C6. Meanwhile, six stilbenes exerted antimutagenic activity in a dose-dependent fashion. Of them, *trans*-resveratrol exhibited the strongest antimutagenic effect against MNNG with IC₅₀ value of 27.0 μg/plate, while other five resveratrol oligomers also did moderate antimutagenic activity with IC₅₀ values ranging from 31.7 to 35.2 μg/plate.

Key words : *Paeonia lactiflora*, *trans*-Resveratrol, *trans-ε*-Viniferin, *cis-ε*-Viniferin, Gnetin H, Suffruticosol A, Suffruticosol B, Cytotoxicity, Antimutagenicity

INTRODUCTION

Resveratrol (*trans*-3,4',5-trihydroxystilbene), a naturally occurring phytoalexin which is found in many plant species including grapes (Langcake and Pryce, 1976; Vastano, 2000), has been reported to have a variety of biological and pharmacological activities (Fremont, 2000).

Meanwhile, many oligostilbenes have been isolated from only seven plant families, i.e. Dipterocarpaceae, Vitaceae, Cyperaceae, Gnetaceae, Welwitschiaceae, Umbelliferae and Leguminosae (Sotheeswaran and Pasupathy, 1993; Ono *et al.*, 1995), and various

physiological actions have been reported (Bokel *et al.*, 1988; Kitanaka *et al.*, 1990; Kawabata *et al.*, 1991; Oshima *et al.*, 1995). Thus, resveratrol and its oligomers are receiving much attention as potential therapeutic agents for several pathological diseases. For this reason, much extensive search for novel naturally occurring stilbene derivatives have been undertaken. However, phytochemical studies on antimutagenic and anticarcinogenic activities of resveratrol and its oligomers are still very limited.

Paeoniae Radix (Korean name: Jakyak), the root of *Paeonia lactiflora* Pallas (*Paeoniaceae*), is widely used in Chinese traditional medicine for treatment of abdominal pain and syndromes such as stiffness of abdominal muscles. It has been reported that the roots contain various biologically active compounds, such as paeoniflorin, benzoylpaeoniflorin and albinoflorin, which exhibit antiallergic, anticonvulsive, anti-inflammatory, and

Correspondence to : Sang-Won Choi, Department of Food Science and Nutrition, Catholic University of Daegu, Hayang, Gyeongbuk 712-702, Korea
E-mail: swchoi@cataegu.ac.kr

antispasmodic actions (Hattori *et al.*, 1985; Kang *et al.*, 1993; Takeda *et al.*, 1995; Goto *et al.*, 1996; Okubo *et al.*, 2000). In addition, hydrolyzable tannins including *penta-* and *hexa-galloyl*glucose, proanthocyanidins, and phenolic compounds have been found to have some important physiological actions (Tanaka *et al.*, 1997, 2000). Thus, although much extensive works on the chemistry and pharmacology of paeony root have been performed, few studies on the screening of biologically active compounds from paeony seed are available.

Recently, we have screened novel antioxidative constituents from fifty-five different plant seeds available in Korea. As a result, the methanolic extract from the seeds of *Paeonia lactiflora* Pall. was found to have strong antioxidative, anti-inflammatory and anticarcinogenic activities. *trans*-Resveratrol and its oligomers, and some flavonoids were shown to be active principles for biological activity of paeony seeds (Kim *et al.*, 1998, Choi *et al.*, 1998; Kim *et al.*, 2001). In addition, Sarker *et al.* (1999) reported that the seeds of *Paeonia suffruticosa* Andrews (Paeoniaceae), analogous to *Paeonia lactiflora* Pall., contained resveratrol and its trimers, named suffuticosol A, B and C with ecdysteroid antagonist activity. Thus, paeony seed, now unused plant seed, is receiving much attention as potential source of important medicinal crude drugs. However, systematic studies on identification of resveratrol and its oligomers from the seeds of *Paeonia lactiflora* Pall., and on their cytotoxic and antimutagenic effects have not yet been reported. In the present study, we fully isolated and identified resveratrol and its oligomers from the seeds of *Paeonia lactiflora* Pall., and further determined their cytotoxic and antimutagenic effects against five different cancer cell lines, and *Salmonella typhimurium* TA98, respectively.

MATERIALS AND METHODS

General experimental procedures

Melting points and optical rotations were measured on a Yanaco 595-030G apparatus (Kyoto, Japan) and JASCO DIP-1000 polarimeter (Tokyo, Japan), respectively. UV and IR spectra were obtained with a photodiode array Sinco UV-vis spectrophotometer (Seoul, Korea) and an IFS 120 HR FT-IR spectrometer (Bruker, Germany), respectively. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectra were measured in CD₃OD on a Unity Plus 500 spectrometer (Varian, U.S.A) and chemical shifts are given as a δ value with tetramethylsilane (TMS) as an internal standard. Fast-Atom Bombardment Mass Spectrometry (FABMS) was recorded on a JEOL JMS-700 mass spectrometer (ion source, Xe atom beam; accelerating voltage, 10 kV) with glycerol as a mounting

matrix. Silica gel (70-230 mesh, Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) were used for column chromatography. All fractions were screened on precoated silica gel thin-layer chromatography (TLC) plates (200 μ m thickness, silica 60F₂₅₄ gel-coated glass, Merck, Darmstadt, Germany) with compounds revealed under UV light.

Plant materials

The seeds of *Paeonia lactiflora* Pallas were directly harvested on the late of August in the herb garden of Uisong Medicinal Plant Experiment Station, Gyeongbuk, Korea. A voucher specimen has been retained in the Herbarium of Uisong Medicinal Plant Experiment Station.

Chemicals

Culture supplies such as flasks and 96-well plates (Primaria™, flat-bottom) were obtained from Falcon (Franklin, NJ, USA). Dulbecco's modified Eagle medium (DMEM), minimum essential medium (MEM), RPMI 1640 medium, fetal bovine serum (FBS), penicillin-streptomycin, trypsin-EDTA, phosphate buffered saline (PBS) and other tissue culture reagents were purchased from Gibco Life Technol. Laboratories (Gaithersburg, MD, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents and solvents used were sterilized by passing through sterile membrane filters (Nylon, 0.2 μ m pore size, Nalgene™). All containers were thoroughly cleaned, rinsed with triple-distilled water, and sterilized by autoclaving at 121°C for 15 min. All other reagents used for this study were of analytical and HPLC grades.

Extraction and Isolation

Ground seeds (500 g) of *Paeonia lactiflora* were extracted continuously with MeOH at a room temperature, and then filtered, evaporated under reduced pressure. The methanolic extract (62.6 g) was further solubilized in 80% MeOH, and then defatted twice with *n*-hexane. The concentrated 80% methanolic extract (42.4 g) was further suspended in 10% MeOH, and then partitioned with ethylether. The ether soluble fraction (12.3 g) was chromatographed on silica gel with CHCl₃-MeOH (5:1, v/v) to give seven fractions (fr.1fr.7). The second (1.41 g) and third (0.24 g) fractions were combined and further chromatographed repeatedly on a Sephadex LH-20 with MeOH to separate Compound **1** (97 mg), and some flavonoids (0.12 g). Moreover, each fourth (1.1 g) and fifth (0.26 g) fraction was also chromatographed separately on Sephadex LH-20 with MeOH to afford Compound **2** (78

mg), and Compound **3** (26 mg) and Compound **4** (0.45 g), respectively. Finally, the sixth fraction (7.9 g) was subjected to the same purification procedure on Sephadex LH-20 column which afforded pure Compound **5** (2.5 g) and Compound **6** (3.4 g). In the procedure of isolation and purification, all extracts were constantly protected from light to avoid photochemical isomerization of *trans*-stilbenes to *cis*-stilbenes.

Chemical structures of 1-6

Compound 1 (trans-Resveratrol): pale yellow crystal; $[\alpha]_D +4.6^\circ$ (c=0.52, MeOH); mp. 259-262 ; UV λ_{max} log ϵ (MeOH): 219 (4.28), 308 (4.02), 320 (3.34) nm; IR ν_{max}

(KBr): 3306 (OH), 1630 (C=C), 1600, 1586 & 1512 (aromatic C=C), 1262 (aromatic C-O), 1173 (phenolic OH), 965 (*trans* double bond) cm^{-1} ; Positive FABMS (glycerol): 229 [M+H]⁺; ¹H-NMR (500 MHz, CD₃OD) δ : 7.33 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.94 (1H, d, *J*=16.5 Hz, H-7'), 6.79 (1H, d, *J*=16.5 Hz, H-8'), 6.75 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.43 (2H, d, *J*=2.5 Hz, H-2, 6), 6.14 (1H, t, *J*=2.5 Hz, H-4); ¹³C-NMR (125 MHz, CD₃OD) see the Table 1.

Compound 2 (trans- ϵ -Viniferin): pale yellow amorphous; $[\alpha]_D +64.4^\circ$ (c=0.52, MeOH); mp. 158-160 ; UV λ_{max} log ϵ (MeOH): 218 (4.52), 312 (4.40), 324 (4.48) nm; IR ν_{max} (KBr): 3430 (OH), 1607 & 1416 (aromatic C=C), 1175 (aromatic C-O), 1024 (dihydrofuran -O-), 962

Table 1. ¹³C-NMR spectral data of six compounds 1-6 from the seeds of *Paeonia lactiflora*

Carbon	1	2	3	4	5	6
C-1	141.31			133.54	128.41	130.90
C-2	105.76			128.05	128.04	130.53
C-3	159.65			116.34	113.92	116.50
C-4	102.64			158.26	156.47	159.12
C-5	159.65			116.34	113.92	116.50
C-6	108.20			128.05	128.04	130.53
C-7		133.93	133.84	94.68	89.07	91.12
C-8		128.27	128.50	58.77	47.47	49.85
C-9		116.34	116.20	147.42	139.37	142.38
C-10		158.48	157.82	107.37	124.47	122.86
C-11		116.34	116.20	159.85	152.47	157.16
C-12		128.27	128.50	102.12	99.46	104.94
C-13		94.86	94.91	159.85	154.19	158.40
C-14		58.32	57.73	107.37	103.48	103.67
C-1'	131.40	147.40	147.33	130.56	131.47	133.81
C-2'	129.39	107.53	107.24	128.60	128.30	133.07
C-3'	115.84	160.07	159.53	116.13	111.72	114.66
C-4'	158.37	102.25	101.82	158.05	152.00	156.11
C-5'	116.48	160.07	159.53	116.13	111.72	114.66
C-6'	128.79	107.53	107.24	128.60	128.30	133.07
C-7'	130.42	130.38	130.07	122.45	37.28	46.47
C-8'	127.02	128.81	131.13	130.36	47.13	47.80
C-9'		116.42	115.88	134.42	142.20	147.48
C-10'		158.48	158.39	120.21	114.81	118.49
C-11'		116.42	115.88	162.82	157.70	160.20
C-12'		128.81	131.13	91.53	93.80	96.24
C-13'		130.44	131.63	162.82	152.68	155.73
C-14'		123.75	126.63	120.21	120.56	123.56
C-1''		136.96	137.81	133.54	133.09	135.51
C-2'' & C-6''		120.10	120.37	128.05	128.26	129.47
C-3'' & C-5''		162.77	162.78	116.34	113.00	115.15
C-4''		96.89	96.58	158.26	154.02	156.05
C-7''		159.77	159.53	94.68	58.59	63.07
C-8''		104.41	108.91	58.77	52.08	56.87
C-9''				147.42	145.94	147.48
C-10''				107.37	104.40	107.34
C-11''				159.85	156.81	159.37
C-12''				102.12	98.93	101.45
C-13''				159.85	156.76	159.37
C-14''				107.37	104.40	107.34

Spectra were measured in CD₃OD. Assignments were confirmed by ¹H-¹H COSY, HMQC, HMBC, and ¹H-¹H NOESY spectra.

(*trans* double bond), 837 cm^{-1} ; positive FABMS (glycerol): 455 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (500 MHz, CD_3OD): 7.14 (d, $J=8.5$ Hz, H-2, 6), 7.03 (d, $J=8.5$ Hz, H-2', 6'), 6.82 (d, $J=16.0$ Hz, H-7'), 6.76 (d, $J=8.5$ Hz, H-3, 5), 6.65 (d, $J=8.5$ Hz, H-3', 5'), 6.63 (d, $J=2.0$ Hz, H-14'), 6.57 (d, $J=16.0$ Hz, H-8'), 6.25 (d, $J=2.0$ Hz, H-12'), 6.18 (t, $J=2.0$ Hz, H-12), 6.16 (d, $J=2.0$ Hz, H-10, H-14), 5.36 (d, $J=6.0$ Hz, H-7), 4.35 (d, $J=6.0$ Hz, H-8); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) see the Table 1.

Compound 3 (*cis*- ϵ -Viniferin): pale yellow amorphous; $[\alpha]_{\text{D}} -96.5^\circ$ ($c=0.24$, MeOH); mp. 160-163 ; UV $\lambda_{\text{max}} \log \epsilon$ (MeOH): 218 (4.52), 275 (4.31) nm; IR ν_{max} (KBr): 3431 (OH), 1617, 1511 & 1457 (aromatic C=C), 1158 (aromatic C-O), 1163 (phenolic OH), 1028 (dihydrofuran -O-), 834 cm^{-1} ; positive FABMS (glycerol): 455 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (500 MHz, CD_3OD): 6.94 (d, $J=8.5$ Hz, H-2, 6), 6.91 (d, $J=8.5$ Hz, H-2', 6'), 6.71 (d, $J=8.5$ Hz, H-3, 5), 6.57 (d, $J=8.5$ Hz, H-3', 5'), 6.23 (d, $J=2.0$ Hz, H-14'), 6.21 (d, $J=2.0$ Hz, H-12'), 6.19 (d, $J=12.0$ Hz, H-7'), 6.08 (t, $J=2.0$ Hz, H-12), 6.02 (d, $J=12.0$ Hz, H-8'), 5.92 (d, $J=2.0$ Hz, H-10, 14), 5.17 (d, $J=5.4$ Hz, H-7), 3.77 (d, $J=5.4$ Hz, H-8); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) see the Table 1.

Compound 4 (Gnetin H): pale brown amorphous; $[\alpha]_{\text{D}} +160.7^\circ$ ($c=0.52$, MeOH); mp. 184-186 ; UV $\lambda_{\text{max}} \log \epsilon$ (MeOH): 239 (4.50), 325 (2.32) nm; IR ν_{max} (KBr): 3412 (OH), 1607 & 1452 (aromatic C=C), 1231 (aromatic C-O), 1167 (phenolic OH), 1031 (dihydrofuran -O-), 960 (*trans* double bond), 838 cm^{-1} ; positive FABMS (glycerol): 681 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (500 MHz, CD_3OD): 7.22 [d, $J=8.5$ Hz, H-2(6), H-2''(6'')], 6.82 [d, $J=8.5$ Hz, H-3(5), H-3''(5'')], 6.74 (d, $J=8.5$ Hz, H-2, 6), 6.56 (d, $J=8.5$ Hz, H-3, 5), 6.48 (s, H-12), 6.43 (s, H-7, 8), 6.20 [s, H-10(10''), H-12(12''), H-14(14'')], 5.50 (d, $J=6.0$ Hz, H-7, 7''), 4.45 (d, $J=6.0$ Hz, H-8, 8''); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) see the Table 1.

Compound 5 (Suffruticosol A): pale brown amorphous $[\alpha]_{\text{D}} -31.2^\circ$ ($c=0.48$, MeOH); mp. 290-295 ; UV $\lambda_{\text{max}} \log \epsilon$ (MeOH): 225 (4.10), 284 (3.42) nm; IR ν_{max} (KBr): 3403 (OH), 1613, 1512 & 1447 (aromatic C=C), 1155 (aromatic C-O), 1009 (dihydrofuran -O-), 834 cm^{-1} ; positive FABMS (glycerol): 681 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (500 MHz, CD_3OD): 7.12 (d, $J=8.8$ Hz, H-2, 6), 6.97 (d, $J=8.5$ Hz, H-2'', 6''), 6.69 (d, $J=8.8$ Hz, H-3, 5), 6.49 (d, $J=8.5$, H-2', 6), 6.39 (d, $J=8.5$ Hz, H-3'', 5''), 6.27 (d, $J=2.0$ Hz, H-12), 6.21 (d, $J=1.0$, H-12'), 6.13 (d, $J=8.5$ Hz, H-3', 5'), 6.07 (t, $J=2.5$ Hz, H-12''), 6.00 (d, $J=2.5$ Hz, H-10'', 14''), 5.94 (d, $J=2.0$ Hz, H-14), 5.70 (d, $J=11.5$ Hz, H-7), 5.44 (d, $J=3.5$ Hz, H-7'), 4.76 (s, H-8''), 4.35 (d, $J=11.5$ Hz, H-8), 3.95 (m, H-8'), 3.70 (d, $J=7.5$ Hz, H-7''); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) see the Table 1.

Compound 6 (Suffruticosol B): pale brown amorphous; $[\alpha]_{\text{D}} +13.0^\circ$ ($c=0.48$, MeOH); mp. 295-300 ; UV $\lambda_{\text{max}} \log \epsilon$ (MeOH): 226 (4.10), 282 (3.45) nm; IR ν_{max} (KBr): 3410 (OH), 1610, 1514 & 1449 (aromatic C=C),

1153 (aromatic C-O), 1010 (dihydrofuran -O-), 835 cm^{-1} ; positive FABMS (glycerol): 681 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (500 MHz, CD_3OD): 7.58 (d, $J=8.8$ Hz, H-2, 6), 6.95 (brd, H-2', 6), 6.91 (d, $J=8.8$ Hz, H-3, 5), 6.50 (d, $J=8.5$ Hz, H-3', 5'), 6.28 (d, $J=8.5$ Hz, H-3'', 5''), 6.26 (d, $J=8.5$ Hz, H-2'', 6''), 6.22 (d, $J=2.0$ Hz, H-10'', 14''), 6.19 (s, H-12'), 6.17 (d, $J=2.0$ Hz, H-12), 6.16 (t, $J=2.0$ Hz, H-12''). 5.94 (d, $J=2.5$ Hz, H-14), 5.86 (d, $J=11.0$ Hz, H-7), 5.08 (d, $J=11.0$ Hz, H-8), 4.22 (d, $J=11.5$ Hz, H-7'), 4.11 (m, H-8'), 4.09 (s, H-8''), 3.81 (d, $J=6.5$ Hz, H-7''); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) see the Table 1.

Cell culture

The five cancer cell lines, HepG2 human hepatocellular carcinoma cell, MCF-7 human breast adenocarcinoma cell, Hela human cervix adenocarcinoma cell, C6 mouse glioma cancer cell and HT-29 human colon adenocarcinoma cell, as well as a normal human lung cell, L-132, used in this study were obtained from the Korea Cell Line Bank (Seoul, Korea). HepG2 and C6, and Hela and MCF-7, and HT-29 were cultured in MEM supplemented with 10% FBS and 1% antibiotic-antimycotic mixture, DMEM with 10% FBS, and RPMI 1640 medium with 10% FBS, respectively. All cells were maintained at 37 , 5% CO_2 in a humidified atmosphere incubator.

Cytotoxicity

Cytotoxic effect of samples against cancer cells was determined according to slightly modified MTT colorimetric assay (Scudiero *et al.*, 1988). Cells were dissociated with 0.05% trypsin-0.02% EDTA, and then seeded into 96-well microtiter plates (1×10^4 cells/well) with various concentrations of the sample (20 μl). After culture plates were incubated for 70 h, 20 μl of MTT solution (2.5 mg MTT/ml PBS) was added and further incubated at 37°C for 4 h. The purple formazan product was solubilized in 150 μl of dimethyl sulfoxide (DMSO) for 5~10 min at room temperature. The optical density was measured at 540 nm in an Eliza reader. Four replicate wells were used to obtain all data points, and all of the reported experiments were performed at least twice. A curve plotting concentration against percentage inhibition was used to calculate half maximal inhibition concentration (IC_{50}).

Antimutagenic test

Anti-mutagenic activity of sample was determined according to a pre-incubation method of Maron and Ames (1983) using the *Salmonella typhimurium* strains TA100 (*hisG46*, *rfa*, Δ *uvrB*). The genotype of the tester strain was checked routinely for their histidine requirement,

deep rough (*rfa*) character, UV sensitivity (*uvr B* mutation) and the presence of R factor. The bacterial strain was cultured at 37°C for 36 h with shaking at a speed of 150 rpm in an MRS nutrient broth. MNNG was used as mutagen in distilled water, and the final concentration of MNNG used on *S. typhimurium* TA100 was 5 µg per plate. For the anti-mutagenic activity test, 100 µl of each sample being tested, 50 µl of each mutagen solution, 100 µl of an overnight culture of *S. typhimurium*, and 0.5 ml of a 0.2 M sodium phosphate buffer (pH 7.0) for MNNG was mixed in glass cap tubes. The mixture was then pre-incubated at 37°C for 30 min with agitation in a shaking incubator. Following incubation, 3 ml of a molten top agar solution containing histidine and biotin was added, and the resulting mixtures were plated on a minimal glucose agar medium. After the plates were incubated at 37°C for 2 days in the dark, the number of His⁺ revertants per plate was counted. The anti-mutagenic activity was expressed as the percentage inhibition of mutagenesis; anti-mutagenic ratio (%) = 100 [(A - B) / (A - C)], where A = number of His⁺ revertants induced by a mutagen in the absence of a sample (positive control), B = number of His⁺ revertants induced by a mutagen in the presence of a sample, and C = number of spontaneous His⁺ revertants in the absence of a mutagen (negative control).

RESULTS AND DISCUSSION

Six compounds 1-6 were first isolated from *P. lactiflora* seed and their structures (Fig. 1) were characterized on the basis of comprehensive analysis of their 1D- and 2D-NMR spectra and, in the case of the known compounds, by comparison of their UV, IR, MS and NMR data with those reported in the literature.

Compound 1 and 2 were identified as *trans*-resveratrol and *trans-ε*-viniferin, respectively, which have already been found in several plants (Langcake and Pryce, 1976; Sotheeswaran and Pasupathy, 1993). In particular, ¹H- & ¹³C-NMR spectra of 2 were very similar to those of (-)-*ε*-viniferin, a well-known phytoalexin from grapevines (Langcake and Pryce, 1977), but its optical rotation was opposite to that of (-)-*ε*-viniferin. Hence, the absolute configuration of 2 was determined to be 7S, 8S, which was coincided well with that of *trans*-(+)-*ε*-viniferin isolated from *Vitis heyneana* (Li *et al.*, 1996). Compound 3 (*cis-ε*-viniferin) was first isolated from the seeds of *P. lactiflora*, although its glycoside (Baderschneider and Winterhalter, 2000), and some resveratrol oligomers containing its moiety have already been reported previously in other plants (Ono *et al.*, 1995; Oshima *et al.*, 1995). Herein, we identified completely the structure of 3 by FAB-MS, 1D- and 2D-NMR spectroscopy.

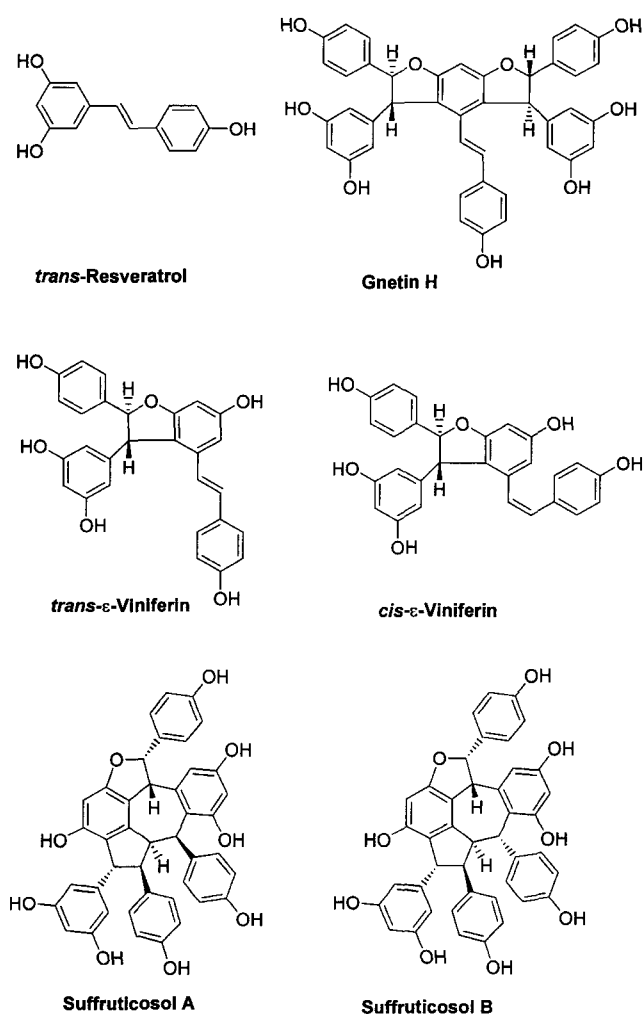


Fig. 1. Chemical structures of compounds 1-6 isolated from seeds of *Paeonia lactiflora*.

Compound 3 gave a molecular ion peak at *m/z* 455 [M+H]⁺ in the positive FAB-MS spectrum. The ¹H-NMR spectra showed two sets of *ortho*-coupled aromatic proton signals [6.71, 6.94 (2H each, *d*, *J*=8.5 Hz, H-3(5) & H-2(6)) and 6.57, 6.91 (2H each, *d*, *J*=8.5 Hz, H-3'(5') & H-2(6)), a set of AX₂-type three aromatic proton signals [5.92 (2H, *d*, *J*=2.0 Hz, H-10 & 14) and 6.08 (1H, *t*, *J*=2.0 Hz, H-12)], and a set of *meta*-coupled aromatic protons [6.21 (1H, *d*, *J*=2.0 Hz, H-12), 6.23 (1H, *d*, *J*=2.0 Hz, H-14)], as well as a pair of doublets of 3.77 and 5.17, which are assignable to H-8 and H-7 of dihydrobenzofuran ring (Kawabata *et al.*, 1989). Thus, the ¹H- and ¹³C-NMR spectra were closely analogous to those of *trans-ε*-viniferin, except for the appearance of *cis*-coupled olefinic protons at 6.19 and 6.02 protons (*d*, *J*=12 Hz), which are correlated with C-7 (131.63) and C-8 (126.63) carbon signals in the HMQC spectrum. Hence, these H-7' and H-8' are olefinic protons and their configuration should be *cis*. The NOESY data

Table 2. Cytotoxic activity of six compounds 1-6 on five different cancer cell lines

compound	IC ₅₀ (µg/ml)*				
	C6	HepG2	Hela	MCF-7	HT-29
1	8.2	11.8	49.2	52.7	25.2
2	18.4	74.3	20.4	44.8	88.4
3	20.1	76.2	21.5	47.2	90.2
4	12.7	48.2	61.7	12.9	30.4
5	18.7	97.4	>100	>100	91.4
6	20.5	98.6	>100	>100	93.5

*IC₅₀ represents the concentration of a compound required for 50% inhibition of growth of cancer cell.

showed NOEs between 6.02 (H-8) and 6.21 (H-12) and between 6.19 (H-7) and 6.91 (H-2 & H-6), which indicated the protons of 6.02 and 6.19 are assignable to H-8' and H-7', respectively. In addition, the relative and absolute configuration of H-7 and H-8 of **3** were determined to be *trans* and 7aR, 8aR, respectively, as compared to NOESY spectra of previous report (Kurihara *et al.*, 1991). The residual protons and carbons can be easily assigned by ¹H-¹H COSY, HMQC and HMBC spectral data. On the base of these results, the structure of Comp. **3** was established as *cis*-viniferin.

Meanwhile, compound **4** was isolated for the first time from paeony seeds, although compound **4**, **5** & **6** have already been isolated from the wood of *Welwitschia mirabilis* (Lins *et al.*, 1986), and the seeds of *Paeonia suffruticosa* Andrew, analogous to *Paeonia lactiflora* Pall. (Sarker *et al.*, 1999), respectively. Thus, this is first report on isolation and identification of four stilbenes except compound **5** and **6** from paeony plants.

Cytotoxic activity of resveratrol and its oligomers isolated from the seeds against five different cancer cell lines, HepG2, Hela, MCF-7, HT-29 and C6 is shown in Table 2. Six stilbenes showed cytotoxic activity, in a dose-dependent manner. In particular, six stilbenes exhibited potent cytotoxic activity against C6 (mouse glioma) cancer cell with IC₅₀ values ranging from 8.2 to 20.5 µg/ml. *trans*-Resveratrol showed significant cytotoxic activity against HepG2 (liver hepatoma) and HT-29 (colon) human cancer cell lines with IC₅₀ values of 11.8 and 25.2 µg/ml, respectively. In addition, *trans*- ϵ -viniferin and *cis*- ϵ -viniferin, and gnetin H also exhibited marked cytotoxic activity against Hela (cervix) and MCF-7 (breast) human cancer cell lines with IC₅₀ values of 20.4, 21.5, and 12.9 µg/ml, respectively. However, suffruticosol A and B were less cytotoxic activity against all cancer cell lines except C6 cell, and especially accelerated somewhat the proliferation of MCF-7 cell in the range of 60~100 µg/ml (data not shown).

On the other hand, antimutagenic activity of resveratrol

Table 3. Antimutagenic activity of six compounds 1-6 on *Salmonella typhimurium* TA100

Compound	IC ₅₀ (g/plate) [†]
1	27.0
2	34.2
3	35.2
4	34.9
5	32.1
6	31.7

[†]IC₅₀ represents the concentration of a compound required for 50% inhibition of growth of cancer cell.

and its oligomers on MNNG in *S. typhimurium* TA100 is given in Table 3. In a concentration-dependent fashion, all stilbenes exerted strong antimutagenic activity. In particular, *trans*-resveratrol exhibited the strongest antimutagenic effect with IC₅₀ value of 27.0 µg/plate against MNNG. Other resveratrol dimers and trimers also showed considerable antimutagenic activity with IC₅₀ values ranging from 31.7 to 35.2 µg/plate. Thus, these results suggest that resveratrol and its oligomers could be mainly responsible for strong cytotoxic and antimutagenic effects of the MeOH extracts from the seeds of *P. lactiflora*.

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