Cytotoxic and Antimutagenic Stilbenes from Seeds of Paeonia lactiflora

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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 μg/ml, respectively. In contrast, trans-ε-viniferin and cis-ε-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 Pall. (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
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 pentagonal hexa-galloylgucose, 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 antioxidant constituents from fifty-five different plant seeds available in Korea. A result, the methanic extract from the seeds of Paeonia lactiflora Pall. was found to have strong antioxidant, 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 antimitogenic 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 antimitogenic 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. \(^1\)H-NMR (500 MHz) and \(^13\)C-NMR (125 MHz) spectra were measured in CD\(_3\)OD on a Unity Plus 500 spectrometer (Varian, U.S.A) and chemical shifts are given as a \(\delta\) 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, Damstadt, 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 \(\mu\)m thickness, silica 60F_{254} gel-coated glass, Merck, Damstadt, Germany) with compounds revealed under UV light.

Plant materials

The seeds of Paeonia lactiflora Pall. 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-nitosoguanidine (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 \(\mu\)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% methanic 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 CHCl\(_3\)-MeOH (5:1, v/v) to give seven fractions (fr.1-7). 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 seperately on Sephadex LH-20 with MeOH to afford Compound 2 (78