Arch Pharm Res Vol 26, No 8, 620-630, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

Proliferative Effects of Flavan-3-ols and Propelargonidins from Rhizomes of *Drynaria fortunei* on MCF-7 and Osteoblastic Cells

Eun Ju Chang, Won Jung Lee[1], Sung Hee Cho, and Sang Won Choi

Department of Food Science and Nutrition, Catholic University of Daegu, Hayang, Gyeongbuk 712-702, Korea and [1]Department of Physiology, School of Medicine, Kyungpook National University, Daegu 700-422, Korea

(Received May 9, 2003)

The proliferative effects of thirty Oriental medicinal herbs on MCF-7 (estrogen-sensitive breast cancer cell line) and ROS 17/2.8 osteoblast-like cells were determined using the MTT assay. Methanol extracts from several herbs was found to show proliferative activity on the above two cell lines in the range of 5 to 100 μg/mL. Among these active herbs, the methanol extract from the rhizomes of *Drynaria fortunei* showed the most potent proliferative activity, and the cell proliferations were significantly increase by 136 and 158% in the MCF-7 and ROS 17/2.8 cells, respectively, when treated with 100 μg/mL. Through a bioassay-guided separation, eight flavonoids, including four new flavan-3-ols and two propelargonidins, together with the known (-)-epiafzelechin and naringin, were isolated. Their chemical structures were characterized as (-)-epiafzelechin (**1**), (-)-epiafzelechin-3-*O*-β-D-allopyranoside (**2**), (-)-epiafzelechin-3-*O*-(6"-*O*-acetyl)-β-D-allopyranoside (**3**), 4β-carboxymethyl-(-)-epiafzelechin methyl ester (**4**), 4β-carboxymethyl-(-)-epiafzelechin sodium salt (**5**), naringin (**6**), (-)-epiafzelechin-(4β→8)-4β-carboxymethylepiafzelechin methyl ester (**7**) and (-)-epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin-(4β→8)-epiafzelechin (**8**) by extensive 1D and 2D NMR spectroscopy. Most of these flavonoids, in the range of 10^{-15}~10^{-6} M, accelerated the proliferation of MCF-7 cell, with compounds **7** and **8**, in the range of 10^{-15}~10^{-12} M, showing especially potent proliferation effects. Meanwhile, seven flavonoids, with the exception of compound **4**, stimulated the proliferation of ROS 17/2.8 cells in the range of 10^{-15}~10^{-6} M, with compounds **5-8** especially accelerating the proliferation, in dose-dependent manners (10^{-15}~10^{-9} M), and their proliferative effect was much stronger than that of E_2 and genistein. These results suggest that propelargonidin dimers and trimers isolated from the rhizomes of *Drynaria fortunei* may be useful as potential phytoestrogens, which play important physiological roles in the prevention of postmenopausal osteoporosis.

Key words: *Drynaria fortunei*, Phytoestrogen, Flavan-3-ols, Propelargonidins, MCF-7 and ROS 17/2.8 osteoblastic cell lines

INTRODUCTION

Estrogen deficiency is known to cause several physical disorders in postmenopausal women, such as osteoporosis, blood cholesterol elevation, and menopausal symptoms. Particularly, postmenopausal osteoporosis induced by estrogen deficiency is the most common cause of age-related bone loss. Estrogen replacement therapy (ERT) has helped to relieve postmenopausal osteoporosis (Genant *et al.*, 1989; Lindsay, 1993), but has a low acceptance rate due to the possible incidence of breast and endometrial cancers with long-term use (Persson *et al.*, 1999). Phytoestrogens including isoflavones, lignans, and coumestans, which are widely distributed in oil seeds, vegetables, and soybeans, exhibit estrogen-like activities (Phipps *et al.*, 1993; Markaverich *et al.*, 1995; Tham *et al.*, 1998). Phytoestrogens are reported to prevent bone resorption, and maintain, or increase bone density (Anderson and Garner, 1997; Kim *et al.*, 2002). The development of safe and efficacious novel phytoestrogens, as alternative therapies to ERT, for the treatments of postmenopausal osteoporosis are, therefore, required.

Our research has recently focused on the screened new phytoestrogens from many Oriental medicinal herbs, which are widely used for the treatments of bone disorders such as bone fracture, arthritis and rheumatism (Lee,

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

1981), in an *in vitro* cell proliferation assay using MCF-7 (estrogen-sensitive breast cancer cell line) and ROS 17/2.8 osteoblast-like cell lines. As a result, the methanol extracts from several of these herbs have been found to have strong proliferative activities in the range of 5 to 100 μg/ml. The extract of the rhizomes of *Drynaria fortunei* have shown particularly potent proliferative effects against the above two cell lines. Through a bioassay-guided separation, eight flavonoids, including four new flavan-3-ols and two propelargonidins, together with the known (-)-epiafzelechin and naringin, were isolated. Rhizomes of *Drynaria fortunei* (kunze) J. Sm (Polypodiaceae, Korean name; Golsoebo, GSB), including the *Polypodiaceae* and *Davalliaceae* families, are commonly employed in traditional Chinese medicine as a bone fracture therapy (Sun *et al.*, 2002). Several condensed tannins, such as flavan-3-ols and oligomeric proanthocyanidins have been reported from the rhizomes of several ferns (Nonaka *et al.*, 1983; Hwang *et al.* 1989; 1990; Kashiwada *et al.*, 1990; Tanaka *et al.* 1991; Cui *et al.*, 1992). Particularly, Hwang *et al.* (1989, 1990) have isolated several flavan-3-ols and proanthocyanidin allosides, together with oligomeric procyanidins possessing a carboxymethyl group at the C-4 position from the rhizomes of the subtropical fern, *Davallia divaricata* Blume (Davalliaceae), which is used as a crude drug in China and Taiwan for the relief of joint pain. Many studies have been reported on the therapeutic effects of the rhizomes of GSB on osteoporosis and bone fracture (Du *et al.*, 1993; Ma *et al.*, 1995; Wang *et al.*, 2001; Sun *et al.*, 2002). However, few phytochemical studies have been conducted on the active constituents for the bone healing effects of these rhizomes. This paper reports on the isolation of phytoestrogens from the rhizomes of *Drynaria fortunei*, and on the structure elucidation of several flavan-3-ols and propelargonidins.

MATERIALS AND METHODS

General experimental procedures

The optical rotations were measured on a JASCO DIP-1000 polarimeter (Tokyo, Japan). The UV and IR spectra were obtained with a V-530 JASCO spectrophotometer (Tokyo, Japan) and an IFS 120 HR FT-IR spectrometer (Bruker, Germany), respectively. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were measured in CD_3OD on a Unity Plus 500 spectrometer (Varian, U.S.A) and the chemical shifts are given as δ value relative to tetramethylsilane (TMS) as an internal standard. The Fast-Atom Bombardment Mass Spectrometry (FABMS) was performed with a JEOL JMS-700 mass spectrometer (ion source, Xe atom beam; accelerating voltage, 10 kV), with glycerol as a mounting matrix. Silica gel 60 (70-230 mesh, Merck, Damstadt, Germany), Polyamide C-200 (75~150 μm, Wako Pure Chem. Ind. Ltd., Japan) and Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) were used as column chromatography stationary phases. All fractions were screened on precoated silica gel thin-layer chromatography (TLC) plates (200 μm thickness, silica $60F_{254}$ gel-coated glass, Merck, Damstadt, Germany) with compounds visualized under UV light and with H_2SO_4 spray reagent after heating.

Plant materials

Thirty Oriental medicinal drugs including rhizomes of *Drynaria fortunei* (Kunze) J. Smith, which were imported from China, were purchased in the herbal medicine market in Daegu, Korea. A voucher specimen has been retained in the Department of Food Science and Nutrition.

Chemicals

Culture supplies such as flasks and 96-well plates (Primaria™, flat-bottom) were obtained from Falcon (Franklin, NJ, USA). The Dulbecco's Modified Eagles Medium (high glucose, DMEM), phenol red-free DMEM, Minimum Essential Medium Eagle (alpha modification, α-MEM) and penicillin-streptomycin were purchased from GIBCO Co. (Gaithersburg, MD, USA). The fetal bovine serum (FBS) and charcaol-dextran treated FBS (CDT-FBS) were obtained from Hyclone Lab. (Logan, UT, USA). The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolin bromide (MTT), trypsin-EDTA and genistein were purchased from Sigma Chem. Co. (St. Louis, MO., USA). The 17β-estradiol (E_2) was purchased from Aldrich Chem. Co. (Inc., Milwaukee, WI, USA). All reagents and solvents were used after sterilization by passing through sterile membrane filters (Nylon, 0.2 μm 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

Dried rhizomes (6 kg) of *Drynaria fortunei* were extracted continuously with MeOH at a room temperature, filtered and evaporated under reduced pressure. The methanolic extract (544 g) was further solubilized in 80% MeOH, and allowed to stand overnight in a refrigerator before filtration. The filtrate was evaporated and successively partitioned with Et_2O, EtOAc and *n*-BuOH. The EtOAc fraction (39.1 g) underwent chromatography on silica gel, with $CHCl_3$-MeOH (3:1, v/v) as the eluent, to give seven fractions (fr.1-fr.7). The third (0.36 g) fraction was subjected a Polyamide column (3×20 cm) and eluted stepwise with one litre each of 20, 50, 80 and 100% MeOH. The 80% MeOH fr. underwent further chromatography on a Sephadex LH-20, with 90% MeOH as eluent, to separate Compound **3** (11.8 mg) and Compound **5** (12.5 mg). Moreover, the fourth

(1.31 g) fraction also underwent chromatography on a Polyamide column to give 20 and 50% MeOH fractions, which underwent further chromatography on a Sephadex LH-20, with 90% MeOH as the eluent, to separate Compound **6** (15.9 mg) and Compound **7** (26.1 mg), respectively. The fifth (2.64 g) fraction underwent chromatography on a Polyamide column to give 50 and 100% MeOH fractions, which were subjected to further chromatography on a Sephadex LH-20, with 90% MeOH as the eluent, to separate Compound **1** (94 mg), Compound **4** (71.1 mg) and Compound **8** (28.9 mg). Finally, the sixth fraction (3.52 g) was subjected to the same Polyamide and Sephadex LH-20 column purification procedures, which afforded pure Compound **2** (16.1 mg).

Chemical structures of 1-8

Compound **1** [(-)-Epiafzelechin)]: pale yellow amorphous; $[\alpha]_D$ -50.6° (c=0.1, MeOH); UV λ_{max} (MeOH): 219 and 268 nm; IR ν_{max} (KBr): 3410 (phenolic OH), 1600 & 1510 (aromatic C=C), 1241 (aromatic C-O) cm^{-1}; Positive FABMS (glycerol): 275 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.31 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.77 (2H, d, *J* = 8.5 Hz, H-3', 5'), 5.94 (1H, d, *J* = 2.4 Hz, H-8), 5.91 (1H, d, *J* = 2.4 Hz, H-6), 4.86 (1H, s, H-2), 4.17 (1H, m, H-3), 2.87, 2.73 (1H, dd, *J* = 3, 17 Hz, H-4); ^{13}C-NMR (125 MHz, CD_3OD) see the Table I.

Compound **2** [(-)-Epiafzelechin 3-*O*-β-D-allopyranoside]: pale brown crystal; $[\alpha]_D$ -37.0° (c=0.1, MeOH); UV λ_{max} (MeOH): 218 and 270 nm; IR ν_{max} (KBr): 3400 (phenolic OH), 1600 & 1512 (aromatic C=C), 1145 (aromatic C-O) cm^{-1}; positive FABMS (glycerol): 437 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ: 7.34 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.72 (2H, d, *J* = 8.5 Hz, H-3', 5'), 5.93 (1H, d, *J* = 2.4 Hz, H-8), 5.89 (1H, d, *J* = 2.4 Hz, H-6), 5.14 (1H, s, H-2), 4.77 (1H, d, *J* = 7.5 Hz, allose H-1), 4.47 (1H, m, H-3), 2.77 (2H, m, H-4), 3.3-4.0 (allose H-2H-6); ^{13}C NMR (125 MHz, CD_3OD) see the Table I.

Compound **3** [(-)-Epiafzelechin-3-*O*-(6''-acetyl)-β-D-allopyranoside)]: white amorphous; $[\alpha]_D$ -12.0° (c=0.1, MeOH); UV λ_{max} (MeOH): 218 and 270 nm; IR ν_{max} (KBr): 3410 (phenolic OH), 1721 (carboxyl group, R-O-CO-R'), 1600 & 1510 (aromatic C=C), 1156 (aromatic C-O) cm^{-1}; positive FABMS (glycerol): 579 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ: 7.30 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.69 (2H, d, *J* = 8.5 Hz, H-3', 5'), 5.94 (1H, d, *J* = 2.4 Hz, H-8), 5.91 (1H, d, *J* = 2.4 Hz, H-6), 5.14 (1H, d, *J* = 1.8 Hz, H-2), 4.77 (1H, d, *J* = 7.0 Hz, allose H-1), 4.38 (1H, m, H-3), 2.80 & 2.69 (1H, dd, *J* = 4.6, 13.7 Hz, H-4), 4.32 & 4.11 (allose H-6), 4.02 (allose H-3), 3.83 (allose H-5), 3.46 (allose H-4), 3.27 (allose H-2), 2.05 (3H, s, $-COCH_3$); ^{13}C-NMR (125 MHz, CD_3OD) see the Table I.

Compound **4** [(4β-Carboxymethyl-(-)-epiafzelechin methyl ester)]: pale brown amorphous; $[\alpha]_D$ -32.4° (c=0.1, MeOH); UV λ_{max} (MeOH): 220 and 270 nm; IR ν_{max} (KBr): 3400 (phenolic OH), 1716 (ester group, R-COO-R'), 1600 & 1510 (aromatic C=C), 1241 (aromatic C-O) cm^{-1}; positive FABMS (glycerol): 681 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ: 7.32 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.80 (2H, d, *J* = 8.5 Hz, H-3', 5'), 5.96 (1H, d, *J* = 2.4 Hz, H-8), 5.92 (1H, d, *J* = 2.4 Hz, H-6), 4.94 (overlap, H-2), 3.88 (1H, s, H-3), 3.72 (3H, s, $-COOCH_3$), 3.40 (1H, dd, *J* = 4, 11 Hz, H-4), 3.06 (1H, dd, *J* = 4, 16 Hz, $-CH_2$), 2.41 (1H, dd, *J* = 11, 16 Hz, $-CH_2$); ^{13}C-NMR (125 MHz, CD_3OD) see the Table I.

Compound **5** [(4β-Carboxymethyl-(-)-epiafzelechin sodium salt)]: brown crystal $[\alpha]_D$ -6.4° (c=0.1, MeOH); UV λ_{max} (MeOH): 220 and 270 nm; IR ν_{max} (KBr): 3400 (phenolic OH), 1600 & 1400 (aromatic C=C), 1550 & 1400 (carboxylate group, R-COO^-), 1240 (aromatic C-O) cm^{-1}; positive FABMS (glycerol): 355 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ: 7.35 (2H, d, *J* = 8.5 Hz, H-2', 6'), 6.79 (2H, d, *J* = 8.5 Hz, H-3', 5'), 5.95 (1H, d, *J* = 2.0 Hz, H-8), 5.93 (1H, d, *J* = 2.0 Hz, H-6), 4.93 (overlap, H-2), 3.95 (1H, s, H-3), 3.38 (1H, dd, *J* = 4, 11 Hz, H-4), 2.84 (1H, dd, *J* = 4, 16 Hz, $-CH_2$), 2.32 (1H, dd, *J* = 11, 16 Hz, $-CH_2$); ^{13}C-NMR (125 MHz, CD_3OD) see the Table I.

Compound **6** (Naringin): pale yellow crystal; $[\alpha]_D$ -82.4° (c=0.1, MeOH); UV λ_{max} (MeOH): 219 and 275 nm; IR ν_{max} (KBr): 3400 (phenolic OH), 1641 (unsaturated C=O), 1515 & 1449 (aromatic C=C) cm^{-1}; positive FABMS (glycerol): 581 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ: 7.31 (2H, d, *J* = 8.1 Hz, H-2', 6'), 6.81 (2H, d, *J* = 8.1 Hz, H-3', 5'), 6.16 (1H, d, *J* = 3.3 Hz, H-6, 8), 5.35 (1H, d, *J* = 12.6 Hz, H-2), 5.25 (1H, s, rhamnose H-1), 5.09 (1H, t, *J* = 6.2 Hz, glucose H-1), 3.40-3.94 (rhamnose & glucose H-2~H-6), 1.29 (3H, rhamnose H-6); ^{13}C-NMR (125 MHz, CD_3OD) see the Table I.

Compound **7** [(-)-Epiafzelechin-(4β→8)-4β-carboxymethylepiafzelechin]: pale brown crystal; $[\alpha]_D$ +65.2° (c=0.1, MeOH); UV λ_{max} (MeOH): 220 and 270 nm; IR ν_{max} (KBr): 3400 (phenolic OH), 1716 (carboxyl group, R-COO-R'), 1600 & 1400 (aromatic C=C), 1241 (aromatic C-O) cm^{-1}; positive FABMS (glycerol): 619 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ: 7.32 (2H, d, *J* = 8.5 Hz, lower H-2', 6'), 7.25 (2H, d, *J* = 8.5 Hz, upper H-2', 6'), 6.80 (2H, d, *J* = 8.5 Hz, lower H-3', 5'), 6.76 (2H, d, *J* = 8.5 Hz, upper H-3', 5'), 6.10 (1H, s, upper H-8), 6.08 (1H, s, upper H-6), 6.03 (1H, s, lower H-6), 4.95 (1H, s, upper H-2), 4.90 (1H, s, lower H-2), 4.58 (1H, s, upper H-4), 4.01 (1H, s, lower H-3), 3.86 (1H, s, upper H-3), 3.63 (3H, s, $-COOCH_3$), 3.35 (1H, s, lower H-4), 2.84 (1H, dd, *J* = 4.1, 18 Hz, $-CH_2$), 2.47 (1H, dd, *J*=9.7, 18 Hz, $-CH_2$); ^{13}C-NMR (125 MHz, CD_3OD) see the Table I.

Table 1 ^{13}C-NMR spectral data of compounds **1-8** from the rhizomes of *Drynaria fortunei*.

Carbon	1	2	3	4	5	6	7	8
C-2 (upper)	79.85	79.20	79.08	75.67	75.84	80.62	77.44	100.05
C-3	67.42	73.16	73.91	71.51	71.56	44.13	70.89	67.43
C-4	29.41	24.61	24.69	37.21	37.17	198.23	37.74	29.67
C-5	157.48	157.68	157.68	158.26	157.95	164.71	159.60	154.10
C-6	95.77	96.32	95.61	95.92	95.96	97.71	95.94	98.36
C-7	157.73	157.68	157.79	158.53	158.50	166.28	156.30	156.88
C-8	96.27	95.72	96.40	97.23	97.35	96.61	96.55	96.60
C-9	157.84	157.68	157.05	158.26	157.95	164.36	155.10	158.32
C-10	99.90	99.89	100.35	104.30	104.18	102.37	103.18	104.73
C-1'	131.47	130.85	130.81	131.41	131.06	130.55	131.50	131.87
C-2' & C-6'	129.0	129.78	129.80	129.02	128.97	128.99	129.17	129.38
C-4'	157.21	156.85	157.91	157.92	156.44	158.83	157.95	151.77
C-3' & C-5'	115.59	115.25	115.32	115.84	115.89	116.18	115.78	115.73
C-2 (middle)							75.60	78.96
C-3							72.48	72.88
C-4							36.87	48.15
C-5							159.60	155.70
C-6							98.88	96.11
C-7							156.66	158.79
C-8							108.14	104.73
C-9							155.10	155.70
C-10							103.18	106.77
C-1'							131.32	131.87
C-2' & C-6'							129.17	129.38
C-4'							157.95	151.77
							115.78	115.73
C-2 (terminal)								80.22
C-3								67.26
C-4								28.95
C-5								155.87
C-6								96.11
C-7								157.90
C-8								106.30
C-9								151.77
C-10								108.90
C-1'								131.87
C-2' & C-6'								129.38
C-4'								157.90
C-3' & C-5'		100.15	100.56					115.73
C-2		72.20	72.08					
C-3		72.81	72.79					
C-4		68.92	68.98					
C-5		75.25	72.61					
C-6		63.22	65.20					
Glucose C-1						104.73		
C-2						77.97		

Table I. Continued

Carbon	1	2	3	4	5	6	7	8
C-3						79.05		
C-4						72.06		
C-5						78.85		
C-6						62.16		
Rhamnose C-1						99.20		
C-2						71.06		
C-3						71.06		
C-4						73.80		
C-5						69.91		
C-6						18.29		
-COR			172.97	180.87			174.69	
$-CH_2$				45.23	45.23		40.35	
$-RCH_3$			20.80	overlap			52.22	
-COONa					180.91			

Spectra were measured in CD_3OD. Assignments were confirmed by DEPT, ^1H-^1H COSY, HMQC, HMBC and ^1H-^1H NOESY spectra.

Compound **8** [(-)-Epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin-(4β→8)-epiafzelechin]: pale gray amorphous; $[\alpha]_D$ +12.8° (c=0.1, MeOH); UV λ_{max} (MeOH): 219 and 270 nm; IR ν_{max} (KBr): 3412 & 1229 (phenolic OH), 1615, 1517 (aromatic C=C) cm^{-1}; positive FABMS (glycerol): 817 $[M+H]^+$; ^1H-NMR (500 MHz, CD_3OD): δ: 7.55 (2H, d, *J*=8.6 Hz, middle H-2', 6'), 7.22 (2H, d, *J* = 8.6 Hz, upper H-2', 6'), 7.10 (2H, d, *J* = 8.6 Hz, terminal H-2', 6'), 6.76 (2H, d, *J* = 8.6 Hz, middle H-3', 5'), 6.72 (2H, d, *J* = 8.6 Hz, upper H-3', 5'), 6.62 (2H, d, *J* = 8.6 Hz, terminal H-3', 5'), 6.0 (1H, s, terminal H-6), 5.91 (1H, d, *J* = 2.3 Hz, upper H-8), 5.87 (1H, d, *J* = 2.3 Hz, upper H-6), 5.71 (1H, br, s, middle H-6), 5.68 (2H, br, s, middle H-2), 4.48 (1H, br, s, middle H-4), 4.37 (1H, s, terminal H-2), 3.94 (1H, d, *J* = 3.4 Hz, upper H-4), 3.93 (1H, s, middle H-3), 3.76 (1H, m, terminal H-3), 3.02 (1H, d, *J* = 3.4 Hz, upper H-3), 3.02 & 2.75 (terminal H-4); ^{13}C-NMR (125 MHz, CD_3OD) see the Table I.

Cell culture

The MCF-7 (an estrogen-sensitive breast cancer cell) cell line was obtained from Korea Cell Line Bank (KCLB), and the ROS 17/2.8 cell (rat osteosarcoma cell) line was kindly provided by Dr. Lee W. J. of the Department of physiology, School of Medicine, Kyungpook National University, Korea. The MCF-7 and ROS 17/2.8 cells were cultured with DMEM (high glucose) and α-MEM, respectively, containing 10% FBS and 1% antibiotic-antimycotic at 37 °C in a humidified 5% CO_2 incubator. For the experiments, phenol red-free DMEM was used to rule out the possibility of estrogen binding to the phenol red in the culture medium.

Cell proliferation assay

The proliferation effect of the samples against the MCF-7 and ROS 17/2.8 cell lines was determined according to a slightly modified MTT colorimetric assay (Scudiero *et al.*, 1988). The cells were trypsinized (0.05% trypsin-0.02 % EDTA) and seeded into 96-well tissue culture plates at a concentrations of 4×10^3 cells/well. The cells were allowed to attach for 24 h. The seeding medium was removed and replaced by the experimental medium (5% CDT-FBS in phenol red-free DMEM) containing various concentrations of each sample. 17β-Estradiol (E_2) and genistein, soybean isoflavone, were used as positive controls. After 48 h incubation, 50 μL of MTT reagent (2.5 mg/mL) was added to each well. The culture was then incubated for 4 h to allow for the MTT metabolism to formazan. After this, the supernatant was aspirated and 150 μL of DMSO was added to dissolve the formazan. The plates were agitated on a plate shaker to ensure a homogeneous solution, and the optical densities read on an automated spectrophotometric plate reader (Bio-Rad model 550, Japan) at 570 nm.

Statistical analysis

All data are represented as the mean±S.E.M. The statistical significance of the differences was assessed using an analysis of variance (ANOVA) and the Duncan multiple range test. A probability of less than 5% was deemed significant.

RESULTS AND DISCUSSION

Proliferative effects of medicinal herbs on MCF-7 and ROS 17/2.8 cells

The proliferative effects of thirty medicinal herbs were

Table II. Proliferative effects of the methanol extracts from thirty different Oriental herbs on MCF-7 cells

Scientific Name	Concentration (μg/mL)			
	5	10	50	100
Glycyrrhiza uralensis Fisch.	94±10.4[a]	121±9.0*	103±7.4	90±2.2
Dryn aria fortunei (Kunze) J. Sm.	96±1.2	93±3.0	114±2.3*	136±9.9*
Cilb otiu n baromts J. Sm.	90±4.6	92±5.5	108±3.7*	131±4.8*
Plat rcodon grandiflorum (Jacq.) A. DC	100±6.2	114±4.6	89±11.8	61±4.5
Euc ommia ulmoides Oliv.	128±2.4*	108±2.4*	120±4.1*	121±2.9*
Atra:tylodes japonica Koidz.	96±1.9	117±6.5*	110±8.5	117±1.7*
Dol chos lablab L.	105±3.2	110±5.7	119±6.7*	123±13.2*
Por a cocos Wolf.	54±4.7	66±3.4	35±7.1	19±5.8
Rut us coreanus Miq.	137±0.9*	126±7.5*	122±2.6*	137±6.2*
Tori is japonica DC.	106±7.4	83±3.3	70±2.2	47±1.1
Cra aegus pinnatifida Bunge	103±5.8	120±12.2	89±7.7	87±9.0
Cor nus officinalis Sieb. Et Zucc.	103±3.2	118±2.6*	111±4.4*	87±1.7
Dio scorea batatas Decne.	87±5.3	86±6.0	97±12.9	111±9.8
Lor nthu s parasiticus Merr.	93±1.3	88±5.3	49±1.0	3±0.5
Pun ica granatum L.	93±8.0	107±10.8	100±10.8	98±12.3
Phl omis umbrosa Turcz.	82±5.4	123±3.4*	120±4.3*	114±3.3*
Cyn omorium songaricum Rupr.	106±4.9	99±2.3	115±0.9*	151±4.2*
Mag nol ia liliflora Desr.	96±5.5	86±1.7	86±8.4	39±7.6
Acc nth opanax sessiliflorus Rupr. et Maxim.	116±2.9*	130±1.2*	126±3.2*	135±0.6*
Sch isa ndra chinensis Baill.	88±7.7	87±6.7	100±12.0	109±7.7
Ach yran thes japonica Nakai.	84±2.8	104±3.7	115±2.8*	121±1.2*
Cis anche deserticola Y.C. Ma.	106±5.2	110±4.6	108±4.4	103±12.0
Epi medium koreanum Nakai.	109±2.0	85±4.9	105±0.5	117±6.5*
Alo nia oxyhylla Miq.	105±11.6	118±7.5	94±8.2	73±5.9
Pan ax ginseng C.A. Mey.	91±1.5	96±2.1	105±8.8	77±6.1
Ane marrhena asphodeloides Bunge	68±7.0	94±2.6	99±3.7	99±2.8
Cro ton tigium L.	101±2.6	93±6.8	116±7.6*	97±1.9
Ast ragalus membranaceus Bunge.	107±3.4*	96±1.8	118±1.2*	123±3.8*
Pol ygonatum siliricum Redt.	80±4.0	103±5.4	99±8.3	103±2.2
Mag nolia officinalis Rehd. Et. Wils	118±9.4*	139±1.6*	115±5.8*	95±5.2

All values are mean±S.E.M. (n=5).

[a] Values represent percentages relative to the control value (100%).

* Significantly different from control group.

tested on the MCF-7 cell line. Table II shows the MCF-7 proliferative activities of the methanol extract from thirty orier tal herbs. The extracts of *Drynaria fortunei, Cilbotium baromts, Dolicho lablab, Cynomorium songaricum, Acanthoo anax sessiliflorus* and *Astragalus membranaceus* stimulated the proliferation of the MCF-7 cells in dose-depe ndcant manners in the range of 5~100 μg/mL. The extracts of *Eucommia ulmoides* and *Rubus coreanus* particularly exhibited considerable proliferative activities at a co ncentration as low as 5 μg/mL.

Mear while, the proliferative effects of the sixteen medicinal herbs were also examined on the ROS 17/2.8 osteoblast-like cells. Table III shows the ROS 17/2.8 proliferative activities of the thirty oriental herb methanol extracts. The extracts of *Drynaria fortunei, Atractylodes japonica* and *Rubus coreanus* stimulated the proliferation of the ROS 17/2.8 cells in dose-dependant manners in the range of 5~100 μg/mL. The extracts of *Drynaria fortunei* and *Rubus coreanus* particularly exhibited significant proliferative activities at 100 μg/mL. Thus, the methanol extract from rhizomes of *Drynaria fortunei* exhibited reproducible and potent proliferative activities on both the MCF-7 and ROS 17/2.8 cell lines.

Bioassay-guided separation of the constituents from *Drynaria fortunei*

The methanol extract from the rhizomes of *Drynaria*

Table III. Proliferative effects of the methanol extracts from sixteen different Oriental herbs on ROS 17/2.8 cells

Scientific Name	Concentration (μg/mL)			
	5	10	50	100
Drynaria fortunei (Kunze) J. Sm.	100±4.4[a]	117±4.1*	137±3.0*	158±2.2*
Cilbotium baromts J. Sm.	73±0.5	84±4.5	69±2.3	91±2.0
Eucommia ulmoides Oliv.	75±3.6	78±2.2	77±7.2	76±0.8
Atractylodes japonica Koidz.	84±1.5	97±0.3	111±2.6*	131±9.4*
Dolichos lablab L.	84±4.1	74±1.5	87±8.7	82±3.2
Rubus coreanus Miq.	105±1.7	114±1.3*	142±7.6*	152±1.1*
Dioscorea batatas Decne.	79±2.0	82±4.9	80±0.5	84±6.5
Phlomis umbrosa Turcz.	73±9.3	94±2.7	71±4.2	81±10.4
Cynomorium songaricum Rupr.	109±4.7*	113±2.5*	119±2.4*	114±4.9*
Acanthooanax sessiliflorus Rupr. et Maxim.	72±3.5	93±4.2	84±5.6	100±1.1
Schisandra chinensis Baill.	83±1.4**	90±5.6**	88±2.5**	97±6.4
Achyranthes japonica Nakai.	80±6.4	92±2.6	77±2.4	90±3.5
Cistanche deserticola Y.C. Ma.	115±0.3*	107±2.4*	104±3.8*	97±2.7
Epimedium koreanum Nakai.	71±3.4	77±1.2	73±0.6	78±2.7
Astragalus membranaceus Bunge	82±3.8	86±3.4	88±2.0	83±4.3
Polyganatum siliricum Redt.	90±3.1	86±6.8	91±3.5	70±1.8

All values are mean±S.E.M. (n=5).
[a]Values represent percentages relative to the control value (100%).
*Significantly different from control group.

fortunei was separated into ether-, ethyl acetate- and *n*-butanol-souble fractions, and the proliferative activities of three fractions was further determined on the MCF-7 and ROS 17/2.8 cells (Table IV). Of the three different solvent fractions, the ethyl acetate-souble fraction stimulated the proliferation of the MCF-7 and ROS 17/2.8 cells in dose-dependant manners in the range of 25~200 μg/mL. The proliferative effect of the ethyl acetate fraction was particularly stronger on the MCF-7 cells than the ROS 17/2.8 cells. Furthermore, repeated chromatography of the ethyl acetate-souble fraction on Polyamide and Sephadex LH-20 columns, afforded four flavan-3-ols and two propelargonidins, together with the known (-)-epiafzelechin and naringin. Eight flavonoids were characterized on the basis of comprehensive analyses of their 1D and 2D NMR (^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY) spectra and, in the case of the known compounds, by comparison on their NMR data with those reported in the literature.

Seven compounds, with the exception of naringin (**6**), exhibited UV absorption maxima at around 220 and 270 nm, as well as a dark-blue color with $FeCl_3$ reagent and an orange-red color with vanillin-HCl reagent, all of which are characteristic of flavan-3-ols and proanthocyanidins (Furuichi *et al.*, 1986). Compounds **1** and **6** were identified as (-)-epiafzelechin (Kashiwada *et al.*, 1990) and naringin (Markham and Chari, 1982; Markham and Geiger, 1994), respectively, which have already been reported in several plants. The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to

Table IV. Proliferative effects of three different solvent fractions from the methanol extracts of rhizomes of *Drynaria fortunei* on MCF-7 and ROS 17/2.8 cells

Fractions	Concentration (μg/mL)			
	25	50	100	200
Ether-souble fraction	104±3.8[a](101±1.2)[b]	128±3.2*(118±2.3*)	101±2.8*(110±3.1)	71±3.1(96±1.5)
Ethyl acetate-souble fraction	132±1.2*(115±2.4*)	162±2.4*(119±1.6*)	195±1.8*(135±2.8*)	254±2.1*(137±2.9*)
n-Butanol-souble fraction	107±2.1(95±3.2)	102±1.7(97±2.6)	114±4.3*(112±3.5)	139±2.1*(143±2.9*)

All values are mean±S.E.M. (n=5).
[a]Values (against MCF-7) represent percentages relative to the control value (100%).
[b]Values (against ROS 17/2.8) represent percentages relative to the control value (100%).
*Significantly different from control group.

those of (-)-epicatechin 3-O-β-D-allopyranoside (Murakami *et al.*, 1985), differing only in the observation of A_2B_2-type aromatic signals instead of ABX-type signals, suggesting the presence of an epiafzelechin unit. Thus, **2** was readily established as (-)-epiafzelechin 3-*O*-β-D-allopyranoside, a new natural flavan-3-ol. The ^{1}H- and ^{13}C-NMR spectra of **3** were closely analogous to those of **2**, except for the appearance of acetyl group at δ 20.8 and δ 172.97 carbons, which correlated with the proton signals (δ 4.11 & 4.32) of allose H-6 in the HMBC spectrum. Hence, **3** was first characterized as (-)-epiafzelechin 3-*O*-(6"-*O*-acetyl)-β-D-allopyranoside. Compounds **4** and **5** gave ^{1}H- and ^{13}C-NMR spectra similar to those of 4β-carboxymethyl-(-)-epicatechin methyl ester and 4β-carboxymethyl-(-)-epicatechin sodium salt, respectively, which have already been isolated from the rhizomes of *Davallia divaricata* Blume (Davalliaceae) (Hwang *et al.*, 1990), except for the presence of epiafzelechin skeleton. Thus, **4** and **5** were elucidated as 4β-carboxymethyl-(-)-epiafzelechin methyl ester and the sodium salt of 4β-carboxymethyl-(-)-epiafzelechin, respectively. The ^{1}H- and ^{13}C-NMR spectra of **7** closely resembled those of epicatechin-(4β→8)-4β-carboxymethylepicatechin (Hwang *et al.*, 1990), except for the presence of a epiafzelechin skeleton and a carbomethoxyl signal at δ 3.63 (Table I). The positive FABMS (*m/z* 756 $[M+H]^+$) was consistent with the methyl ester of (-)-epiafzelechin-(4β→8)-4β-carboxymethylepiafzelechin. Hence, **7** was characterized as (-)-epiafzelechin-(4β → 8)-4β-carboxymethylepiafzelechin methyl ester. Finally, the ^{1}H- and ^{13}C-NMR spectra of **8** correlated with those of the trimeric proanthocyanidins, with epiafzelechin nuclei, from *Dicranopteris pedata* (Kashiwada *et al.*, 1990) and *Selliguea feei* (Baek *et al.*, 1993). The ^{1}H-NMR spectrum showed the presence of a pair of one-proton doublets (δ 5.87 and 5.91) with *meta* coupling (J = 2.3), three pairs of two-proton doublets (δ 6.72 and 7.22, δ 6.76 and 7.55, and δ 6.62 and 7.10) with *ortho* coupling (J = 8.6) and two one-proton singlet (δ 5.71 and 6.0). The ^{13}C-NMR spectrum exhibited the presence of two flavan C-2 signals at δ 78.96 (middle) and 80.22 (terminal), and a ketal carbon at δ 100.05 (upper), indicating that **8** was a trimeric proanthocyanidin with an intramolecularly doubly-linked unit. The DEPT spectrum showed the absence of a CH_3 group, with the presence of one methylene, 20 methines and 22 quaternary carbon atoms. The molecular weight of **8** was determined by FABMS as 816, which is 18 mass units less than expected had this compound been a singly-linked trimeric proanthocyanidin. Thus, **8** was a trimeric compound composed of three 5,7,4'-trihydroxyflavan-3-ol units, possessing a doubly-linked proanthocyanidin-A-type unit (Kashiwada *et al*, 1990, 1990; Baek *et al.*, 1993). Additionally, the coupling patterns of H-3 (upper) [δ 3.02 (d, J = 3.4)], H-2 (middle) [δ 5.68 (br, s)] and H-3 (middle) (δ 3.93 s), as well

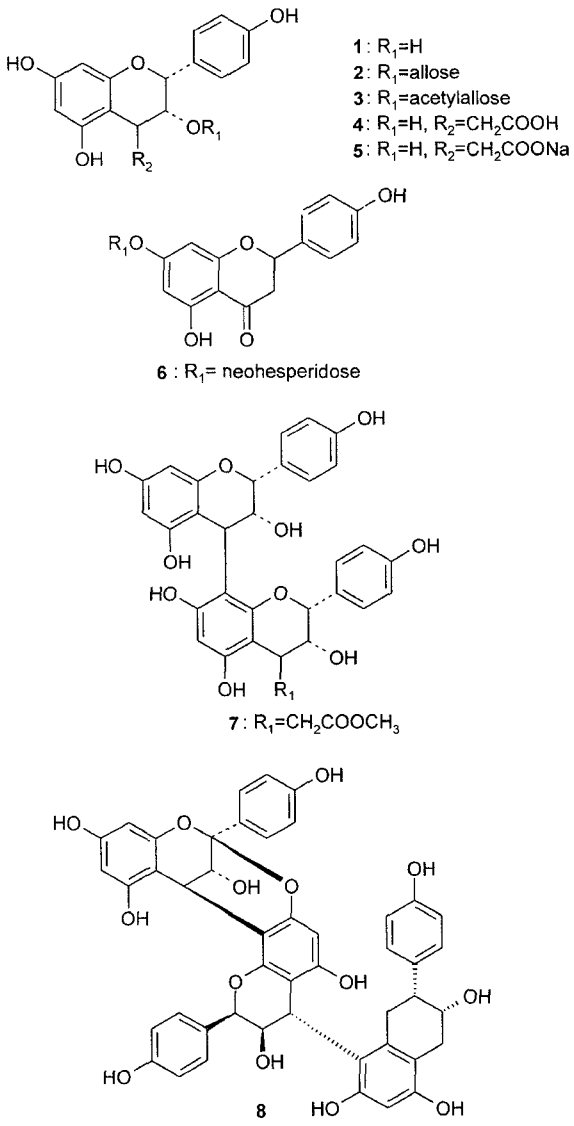

Fig. 1. *Chemical structures of compounds* **1-8** *isolated from the rhizomes of Drynaria fortunei*

as H-2 (terminal) (δ 4.37 s) and H-3 (terminal) (δ 3.76 m) in the ^{1}H-NMR spectrum of **8** suggested that the upper, middle and terminal flavan-3-ol units of the molecule possessed a 2,3-*cis* stereostructure (Tanaka *et al.*, 1991). Particularly, a NOESY experiment showed NOE the H-4(U)/H-2(T), H-3(U), H-6(U)/H-2'(M), H-6'(M), as well as H-4(M)/H-2(T), H-3(T) correlations of the molecule, which indicated the locations and configuration of the interflavonoid linkages of **8** were 4β(U)→8(M), 2β(U)→*O*7(M), and 4β(M)→8(T). This fact was confirmed by the HMBC cross peak between H-4(U) and C-8(M), H-2(U) and C-7 (M), and H-4(M) and C-8(T). On the base of these results, **8** was established as (-)-epiafzelechin-(4β→8, 2β→*O*7)-epiafzelechin-(4β→8)-epiafzelechin, which was first isolated from ferns. Thus, this is first report on the isolation and

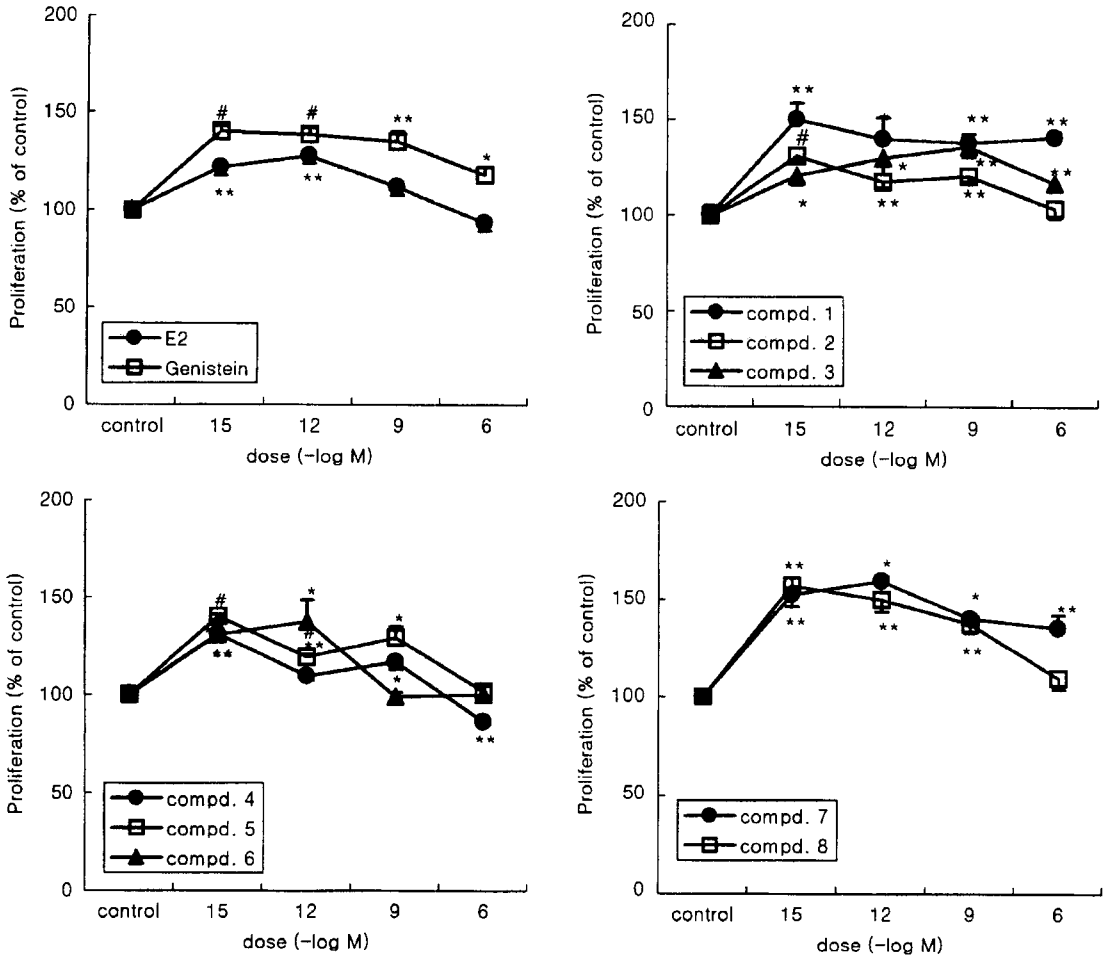

Fig. 2. Dose-dependent effects of the flavan-3-ols and propelargonidins, isolated from the rhizomes of *Drynaria fortunei*, 17β-estradiol (E_2) and genistein on the proliferation of MCF-7 cells. The cell proliferation was determined by the MTT assay, as described in Materials and Methods. The results are expressed as the percentage of the control, and values represent the mean±S.E.M. from 6 separate experiments. *p<0.05, **p<0.01, #p<0.001. vs. control.

identification of flavan-3-ols and propelargonidins, with the exception of (-)-epiafzelechin and naringin, from *Drynaria fortunei*, and the *Polypodiaceae* and *Davalliaceae* families.

Proliferative effects of eight flavonoids from rhizomes of *Drynaria fortunei*

Fig. 2 shows the proliferation effects of flavan-3-ols and propelargonidins, from rhizomes of *Drynaria fortunei*, on MCF-7 cells. The E_2 and genistein, as positive controls, stimulated proliferation of the MCF-7 cells in the range of 10^{-15}~10^{-12} M, but inhibited the proliferation at high concentrations. Of the eight flavonoids, only compound **3** stimulated the proliferation of MCF-7 in a dose-dependent manner, in the concentration range of 10^{-15}~10^{-6} M, and compounds **6** and **7** showed significant proliferation in the concentration range of 10^{-15}~10^{-12} M, However, the other compounds accelerated the cell proliferation at very low concentrations of 10^{-15} M, but their MCF-7 proliferation effects were decreased above concentrations >10^{-15} M. Meanwhile, Fig. 3 shows the ROS 17/2.8 proliferation effects of the eight flavonoids from the rhizomes. Of the eight compounds, compounds **5-8** stimulated the proliferation of the ROS 17/2.8 cells in dose-dependent manners, in the concentration range of 10^{-15}~10^{-9} M, with compounds **1-3** exhibiting somewhat moderate proliferative activities. However, compound **4** had a lesser proliferative effect in the range of 10^{-15}~10^{-6} M. Thus, these results suggest that the flavan-3-ols and propelargonidins could be mainly responsible for strong proliferation effects of the methanol extract from the rhizomes of *Drynaria fortunei* against the MCF-7 and ROS 17/2.8 cell lines. Additionally, flavonoids with epiafzelechin units are considered as potential phytoestrogens, which may play important roles in the prevention of the postmenopausal osteoporosis induced by estrogen deficiency. Further study is needed to determine the estrogenic effects of flavan-3-ols and propelargonidins *in vivo*.

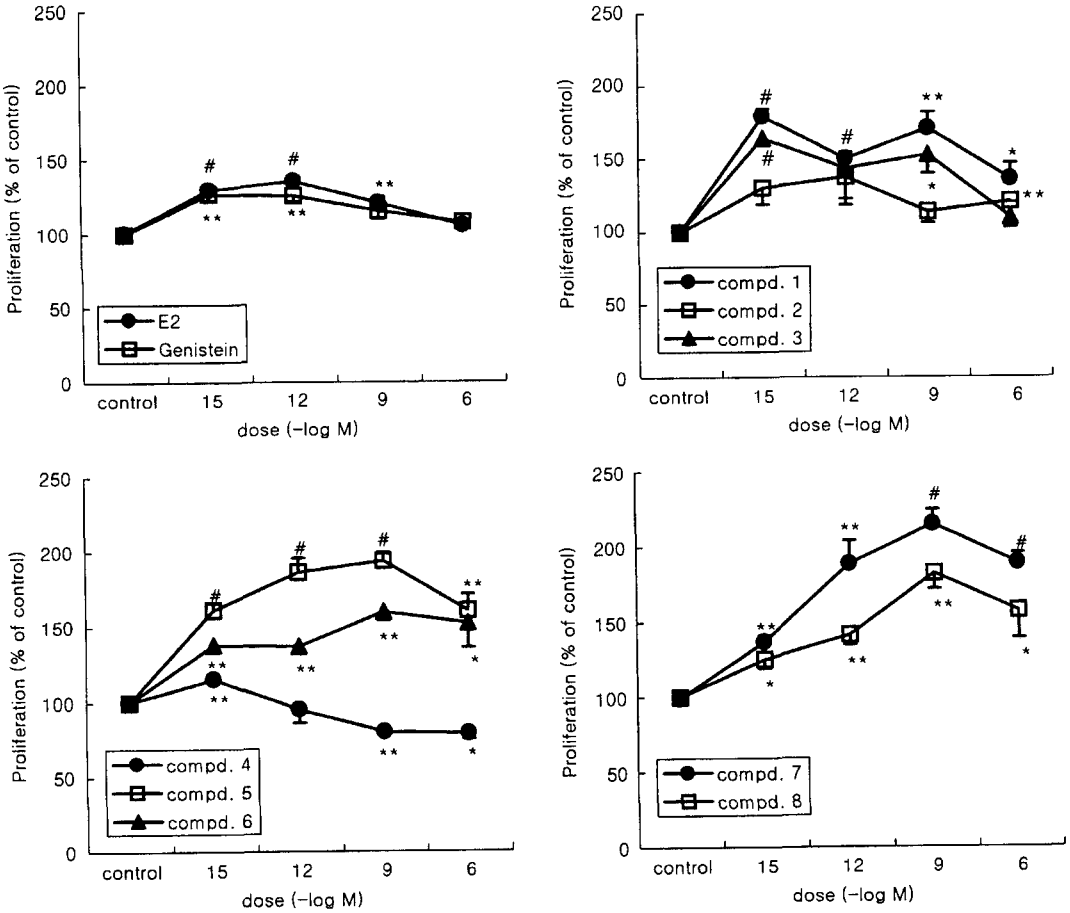

Fig. 3. Dose-dependent effects of the flavan-3-ols and propelargonidins, isolated from rhizomes of *Drynaria fortunei*, 17β-estradiol (E_2) and genistein on the proliferation of ROS 17/2.8 osteoblast-like cells. The cell proliferation was determined by the MTT assay, as described in Materials and Methods. The results are expressed as the percentage of the control, and values represent the mean±S.E.M. from 6 separate experiments. *p<0.05, **p<0.01, #

ACKNOWLEDGMENT

We are grateful to Dr. Sung H. Kim and Ms. Shin A Choi of the Korean Basic Science Institute, Daegu, Korea, for the FABMS and NMR measurements.

REFERENCES

Anderson, J. J. B. and Garner, S. C., The effects of phytoestrogens on bone. *Nutr. Res.*, 17, 1617-1632 (1997).

Baek, N. I., Chung, M. S., Shamon, L., Kardono, L. B. S., Tsauri, S. Padmawwinata, K., Pezzuto, J. M., Soejarto, D. D., and Kinghorn, A. D., Selligueain A, a novel highly sweet pro-anthocyanidin form the rhizomes of *Selliguea feei*. *J. Nat. Prod.*, 56, 1532-1538 (1993).

Cui, C. B., Tezuka ,Y., Kikuchi, T., Nakano, H., Tamaoke, T., and Park, J. H., Davallin, a new tetrameric proanthocyanidin from the rhizomes of *Davallia mariesii*. *Chem. Pharm. Bull.*, 39, 2179-2181 (1992).

Drewes S. E., Taylor, C. W. Cunningham, A. B., Ferreira, D., Steenkamp, J. A., and Mouton, C. H. L., Epiafzelechin-(4β→8, 2β→O→7)-ENT-afzelechin from *Cassipourea gerrardii*. *Phytochemistry*, 31, 2491-2494 (1992).

Drewes, S. E., Taylor, C. W., and Cunningham, A. B., (+)-Afzelechin 3-rhamnoside from *Cassipourea gerrardii*. *Phytochemistry*, 31, 1073-1075 (1992).

Du, Z., Xu, G., and Xu, B., Pharmacological studies on *Rhizoma Drynairae*. *Chinese Traditional and Herbal Drugs*, 24, 379-380 (1993).

Furuichi, E. E., Nonaka, G., Nishioka, I., and Hayashi, K., Isolation and structures of procyanidins (condensed tannins) from *Rhaphiolepis umbellata*. *Agric. Biol. Chem.*, 50, 2061-2067 (1986).

Genant, H. K., Baylink, D. J., and Gallagher, J. C., Estrogens in the prevention of osteoporosis in postmenopausal women. *Am J. Obstet. Gynecol.*, 161, 1842-1846 (1989).

Hwang, T. H., Kashiwada, Y., Nonaka, G. I., and Nishioka, I., Flavan-3-ol and proanthocyanidin allosides from *Davallia divaricata*. *Phytochemistry*, 28, 891-896 (1989).

Hwang, T. H., Kashiwada, Y., Nonaka, G. I., and Nishioka, I., 4-

Carboxymethyl flavan-3-ols and procyanidins from *Davallia divaricata*. *Phytochemistry*, 29, 279-282 (1990).

Kashiwada, Y., Morita, M., Conaka, G. I., and Nishioka, I., Tannins and related compounds. XCI. Isolation and characterization of proanthocyanidins with an intramolecularly doubly-linked unit from the fern, *Dicranopteris pedata* Houtt. *Chem. Pharm. Bull.*, 38, 856-860 (1990).

Kashiwada, Y., Iizuka, H., Yoshioka, K., Chen, R-F., Nonaka, G. I., and Nishioka, I., Tannins and related compounds. XCIII. Occurrence of enantiomeric proanthocyanidins in the Leguminosae plants *Cassia fistula T.* and *C. javanica* L. *Chem. Pharm. Bull.*, 38, 888-893 (1990).

Kim, H. J., Bae, Y. C., Park, R. W., Choi, S. W., Cho, S. H., Choi, Y. S., and Lee, W. J., Bone-protecting effect of safflower seeds in ovariectomized rats. *Calci. Tissue Int.*, 71, 88-94 (2002).

Lee, S. I., Bonchohak, Susewon Press, Seoul, Korea, 1981.

Lindsay R., Hormone replacement therapy for prevention and treatment of osteoporosis. *Am. J. Med.*, 95, 37-43s (1993).

Ma, K. C., Zhu, T. Y., and Liu, W. Z., Promoting effect of Rhizoma Drynariae on the calcification of cultivated chick embryo bone primordium. *China J. Chinese Materia Medica*, 20, 178-180 (1995).

Markaverich, B. M., Webb, B., Donamore, C. L., and Gregory R. R., Effects of coumestrol on estrogen receptor function and uterine growth in ovariectomized rats. *Environ. Health Perspect.*, 103, 574-581 (1995).

Markham, K. R. and Chari, V. M., Carbon-13 NMR spectroscopy of flavonoids. In: *The flavonoids*: advances in research, Harborne J. B. and Mabry, T. J. (eds.), Chapman and Hall, London, 1982.

Markham, K. R. and Geiger, H., ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In: *The flavonoids*, Harborne J. B. (ed.), Chapman and Hall, London, 1994.

Murakami, T., Wada, H., Tanaka, N., Kuraishi, T., Saiki, Y., and Chen, C. M., Chemical and chemotaxonomical studies on Filices. LVI. Studies on the constituents of the Davalliaceous ferns (1). *Yakugaku Zasshi*, 105, 649-654 (1985).

Nonaka, G .I., Norimoto, S., and Nishioka, I., Tannins and related compounds. Part 13. Isolation and structures of trimeric, tetrameric, and pentameric proanthocyanidins from cinnamon. *J. Chem. Soc. Perkin Trans.* I, 2139-2145 (1983).

Persson, I., Weiderpass, E., Bergkvist, L., Bergstrom, R., and Schairer, C., Risks of breast and endometrial cancer after estrogen and estrogen-progestin replacement. *Cancer Causes Control*, 10, 253-260 (1999).

Phipps, W. R., Martini, M. C., Lampe, J. W., Slavin, J. L., and Kurzer, M. S., Effect of flax seed ingestion on the menstrual cycle. *J. Clin. Endocrinol. Metab.*, 77, 1215-1219 (1993).

Sun, J. S., Lin, C. Y., Dong, G. C., Sheu, S. Y., Lin, F. H., Chen, L. T., and Wang, Y. J., The effect of Gu-Sui-Bu (*Drynaria fortunei* J. Sm) on bone cell activities. *Biomaterials*, 23, 3377-3385 (2002).

Tanaka, N., Orii, R., Ogasa, K., Wada, H., Murakami, T., Sakik, Y., and Chen, C. M., Chemical and chemotaxonomica studies of ferns. LXXX.[1] Proanthocyanidins of *Arachniodes sporadosora* Nakaike and *A. exilis* Ching. *Chem. Pharm. Bull.*, 39, 55-59 (1991).

Tham, D. M., Gardner, C. D., and Haskell, W. L., Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. *J. Clin. Endocr. Metab.*, 83, 2223-2235 (1998).

Wang, D., Li, F., Gao, X., and Jinag, Z., Effect of *Drynaria baronii* rhizome extracts on the proliferation in osteoblast-like UMR 106 cells. *Pharmaceu. Biology*, 39, 259-262 (2001).