Arch Pharm Res Vol 29, No 12, 1109-1113, 2006

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

A New Furofuran Lignan from *Geranium thunbergii* Sieb. et Zucc.

Qing-He Liu, Ji-Eun Jeong, Eun Jin Choi[1], Young Hee Moon, and Eun-Rhan Woo

College of Pharmacy, Chosun University, Gwangju 501-759, Korea and [1]Department of Biomaterials, Chosun University, Gwangju 501-759, Korea

(Received August 11, 2006)

A new furofuran lignan, 4-hydroxykobusin (**3**), together with known lignans, kobusin (**1**), and 7,7'-dihydroxybursherenin (**2**), were isolated from the whole plant of *Geranium thunbergii* Sieb. et Zucc (Geraniaceae). The structures were determined based on the spectral data and a comparison with the published data. This is the first report of the presence of furofuran lignan in *Geranium* species.

Key words: *Geranium thunbergii* Sieb. et Zucc., Geraniaceae, Furofuran lignan, 4-Hydroxykobusin

INTRODUCTION

Geranium thunbergii Sieb. et Zucc. (Geraniaceae) is a perennial plant that is distributed throughout Korea, China, and Japan. The whole plant is used in oriental medicine as an antihemorrhage, sterilization, diarrhea, and astringent (Bae, 2000). Previous phytochemical studies on this species reported the extraction of tannins and flavonoids, such as geraniin, corilagin, ellagic acid, gallic acid, quercetin, kaempferol, and kaempferol-7-rhamnoside (Ito *et al.*, 1999; Okuda *et al.*, 1986).

As part of an ongoing investigation into biologically active compounds from natural products, the methanol extract of *Geranium thunbergii* Sieb. et Zucc. was investigated. A new furofuran lignan, 4-hydroxykobusin (**3**), along with kobusin (**1**), and 7,7'-dihydroxybursehernin (**2**), were isolated using repeated column chromatography on silica gel, Sephadex LH-20, LiChroprep RP-18. Two known compounds were isolated from this plant for the first time. 7,7'-dihydroxybursehernin (**2**) has been reported previously to be a product of chemical synthesis but the complete NMR data has not been published. This paper reports the isolation and structural characterization of these compounds.

Correspondence to: Eun-Rhan Woo, College of Pharmacy, Chosun University, 375 Seosuk-dong, Dong-ku, Gwang-ju 501-759, Republic of Korea
Tel: 82-62-230-6369, Fax: 82-62-222-5414
E-mail: wooer@chosun.ac.kr

MATERIALS AND METHODS

General procedure

The melting point was obtained with a Fisher Scientific melting point apparatus and was uncorrected. The UV spectra were obtained on a Shimadzu UV/Visible Spectrophotometer. The IR spectra were recorded on an IMS 85 (Bruker). The NMR spectra were recorded on a Varian Unity Inova 500 (500 MHz) spectrometer. The ^1H-^1H COSY, DEPT, HMQC, and HMBC NMR spectra were obtained using the usual pulse sequences. The HR-EIMS was determined on a JMS 700 (JEOL). TLC and column chromatography were carried out on precoated Si Gel F_{254} plates (Merck, art. 5715), RP-18 F_{254} plates (Merck, art. 15423), and Si gel 60 (Merck, 230-400 mesh).

Plant material

The whole plant of *Geranium thunbergii* Sieb. et Zucc. (Geraniaceae) was collected from the Herbarium of College of Pharmacy, Chosun University, Korea, in May 2003. A voucher specimen was deposited in the Herbarium of College of Pharmacy, Chosun University (CSU-1019-17).

Extraction and isolation

The air-dried whole plant of *Geranium thunbergii* (0.46 Kg) was cut and extracted with MeOH (3 L×3) at 60°C for 4 h (×3). The MeOH extract (82.92 g) was suspended in water (1.0 L), and then partitioned sequentially with equal

volumes of dichloromethane, ethyl acetate, and *n*-butanol. Each fraction was evaporated *in vaccuo* to yield the residues of the CH_2Cl_2 (12.18 g), EtOAc (20.97 g), *n*-BuOH (12.43 g), and water (22.49 g) extracts. A portion of the CH_2Cl_2 soluble fraction (9.0 g) was subjected to column chromatography over a silica gel (400 g) eluting with a *n*-Hexane-EtOAc = 100:0 → 1:4, $CHCl_3$-MeOH = 1:0 → 1:1 in a gradient system. The fractions were combined based on their TLC pattern to yield the subfractions designated C1-C6. Subfraction C3 (1.67 g) was further purified by column chromatography over a silica gel (300 g) eluting with a $CHCl_3$-MeOH gradient system to afford four subfractions (C31-C34). Subfraction C32 (876 mg) was purified by repeated Sephadex LH-20 column chromatography (MeOH-H_2O = 3:2→1:0) and preparative TLC (RP-18 F_{254}s, 0.5 mm, MeOH-H_2O = 7:3, R_f = 0.35) to give compounds **1** (26.2 mg) and **2** (12.4 mg), respectively. Subfraction C33 (101.6 mg) was purified by repeated Sephadex LH-20 column chromatography (MeOH-H_2O = 3:2→1:0, 4:1→1:0), preparative TLC (Si Gel F_{254} plates, 0.5 mm, *n*-hexane-acetone = 1:1, R_f = 0.6) to give compound **3** (3.5 mg).

Kobusin (1)

Colorless oil, $[\alpha]_D^{24}$ +58.0° ($CHCl_3$; c 0.03); UV (MeOH) λ_{max} nm: 232, 284; IR ν_{max} (KBr) cm^{-1}: 1610, 1595, 1505, 1250; ^{1}H-NMR (500 MHz, CD_3OD) δ : 6.97(1H, br.s, H-2'), 6.92 (2H, s, H-5'/6'), 6.88 (1H, d, *J*=1.5 Hz, H-2"), 6.84 (1H, dd, *J*=2.0, 8.0 Hz, H-6"), 6.77 (1H, d, *J*=8.0 Hz, H-5"), 5.92 (2H, s, -OC<u>H</u>$_2$O-), 4.72 (2H, dd, *J*=5.0, 10.5 Hz, H-2/6), 4.23 (2H, dd, *J*=7.0, 9.0 Hz, H-4eq/8eq), 3.86 (2H, dd, *J*=4.0, 9.0 Hz, H-4ax/8ax), 3.83 (3H, s, -OC<u>H</u>$_3$), 3.81 (3H, s, -OC<u>H</u>$_3$), 3.11 (2H, m, H-1/5); ^{13}C-NMR (125 MHz, CD_3OD) δ : 150.81 (s, C-3'), 150.32 (s, C-4'), 149.56 (s, C-3"), 148.75 (s, C-4"), 136.68 (s, C-1"), 135.42 (s, C-1'), 120.78 (d, C-6"), 119.96 (d, C-6'), 113.07 (d, C-5'), 111.35 (d, C-2'), 109.14 (d, C-5"), 107.69 (d, C-2"), 102.54 (t, -O<u>C</u>H$_2$O-), 87.50 (d, C-2), 87.38 (d, C-6), 72.86 (t, C-4), 72.82 (t, C-8), 56.68 (q, -O<u>C</u>H$_3$), 56.65 (q, -O<u>C</u>H$_3$), 55.75 (d, C-5), 55.56 (d, C-1).

7,7-Dihydroxybursehernin (2)

Colorless oil, $[\alpha]_D^{24}$ +30.0° (MeOH; c 0.50); UV (MeOH) λ_{max} nm : 234, 281; IR ν_{max} (KBr) cm^{-1}: 3410, 1751, 1600, 1502; ^{1}H-NMR (500 MHz, CD_3OD) δ : 6.96 (2H, d, *J*=1.5 Hz, H-2/2'), 6.93 (1H, dd, *J*=1.5, 8.5 Hz, H-6), 6.88 (2H, dd, *J*=1.5, 8.5 Hz, H-5/6'), 6.79 (1H, d, *J*=8.5 Hz, H-5'), 5.93 (2H, s, -OC<u>H</u>$_2$O-), 5.41 (1H, d, *J*=4.0 Hz, H-7), 5.21 (1H, d, *J*=3.5 Hz, H-7'), 4.30 (1H, dd, *J*=7.0, 9.5 Hz, H-9'a), 4.02 (1H, dd, *J*=4.5, 9.5 Hz, H-9'b), 3.84 (3H, s, -OC<u>H</u>$_3$), 3.82 (3H, s, -OC<u>H</u>$_3$), 3.60 (1H, dd, *J*=4.0, 9.5 Hz, H-8), 3.38 (1H, m, H-8'); ^{13}C-NMR (125 MHz, CD_3OD) δ : 179.49 (s, C-9), 151.05 (s, C-3/4), 149.04 (s, C-3'), 148.74 (s, C-4'), 136.00 (s, C-1'), 133.87 (s, C-1), 120.42 (d, C-6'), 119.67 (d, C-6), 113.18 (d, C-5), 110.96 (d, C-2), 109.23 (d, C-5'), 107.38 (d, C-2'), 102.54 (t, -O<u>C</u>H$_2$O-), 86.79 (d, C-7), 85.05 (d, C-7'), 74.00 (t, C-9'), 56.73 (q, -O<u>C</u>H$_3$), 56.65 (q, -O<u>C</u>H$_3$), 54.68 (d, C-8), 51.10 (d, C-8').

4-Hydroxykobusin (3)

Colorless oil, $[\alpha]_D^{24}$ -42.0° (MeOH; c 0.63); UV (MeOH) λ_{max} nm : 232, 284; IR ν_{max} (KBr) cm^{-1}: 3400, 1610, 1595, 1505, 1250; EI-MS *m/z* (rel. int.): 386 ([M]$^+$, 100), 339 (20.0), 267 (12), 177 (60); HR-EIMS *m/z*: 386.1363 (calcd. for $C_{21}H_{22}O_7$: 386.1366); ^{1}H-NMR (500 MHz, CD_3OD) δ : 7.21 (1H, d, *J*=2.0 Hz, H-2'), 6.98 (1H, dd, *J*=2.0, 8.0 Hz, H-6'), 6.91 (1H, d, *J*=8.0 Hz, H-5'), 6.88 (1H, d, *J*=2.0 Hz, H-2"), 6.85 (1H, dd, *J*=2.0, 8.0 Hz, H-6"), 6.79 (1H, d, *J*=8.0 Hz, H-5"), 5.94 (2H, s, -OC<u>H</u>$_2$O-), 5.50 (1H, d, *J*=1.0 Hz, H-4), 4.90 (1H, d, *J*=7.0 Hz, H-2), 4.84 (1H, d, *J*=7.0, H-6), 4.21 (1H, dd, *J*=6.0, 9.0 Hz, H-8eq), 4.02 (1H, dd, *J*=2.5, 9.0 Hz, H-8ax), 3.85 (3H, s, -OC<u>H</u>$_3$), 3.82 (3H, s, -OC<u>H</u>$_3$), 3.14 (1H, m, H-1), 2.84 (1H, br.t, *J*=7.5 Hz, H-5); ^{13}C-NMR (125 MHz, CD_3OD) δ : 150.80 (s, C-4'), 150.19 (s, C-3'), 149.65 (s, C-4"), 148.83 (s, C-3"), 137.25 (s, C-1"), 137.04 (s, C-1'), 120.55 (d, C-6"), 120.12 (d, C-6'), 112.81 (d, C-5'), 111.55 (d, C-2'), 109.97 (d, C-5"), 107.37 (d, C-2"), 102.97 (d, C-4), 102.58 (t, -O<u>C</u>H$_2$O), 88.69 (d, C-2), 85.07 (d, C-6), 73.24 (t, C-8), 63.79 (d, C-5), 56.69 (q, -O<u>C</u>H$_3$), 56.57 (q, -O<u>C</u>H$_3$), 55.09 (d, C-1).

Bioassay of human interleukin (hIL)-6

The HIL-6 bioassay was carried out using a slight modification of an established method (Kim *et al.*, 2003). Briefly, 500 μL of the MG-63 cells (3×10^4 cells/mL) in DMEM containing 10% FBS were dispensed into a 24 well plate. The culture was incubated for 24 h at 37°C. 5 μL of TNF-α (15 ng/mL) and 5 μL of the DMSO with or without the compounds were then added. The medium was incubated at 37°C in an atmosphere containing 5% CO_2 for 24 h, and stored at -20°C until needed. The medium was used to determine the hIL-6 content using an ELISA procedure. 96 well plates were coated with 100 μL of the purified rat anti-human IL-6 monoclonal antibody in 0.1M $NaHCO_3$ (pH 9.6) by an overnight incubation at 4°C. The wells were blocked with 200 μL of 3% BSA in PBS for 2 h at RT, and incubated with 100 μL of the specific antibody for 2 h at RT. 100 μL of HRP conjugated rabbit anti-goat IgG (1:1000 dilution) was added to the well and incubated for 2 h at RT. 100 μL of a TMB(3, 3', 5, 5'-tetramethyl-benzidine) substrate solution was added and incubated for 10 min at RT. The color reaction was quenched with 50 μL of 0.4N HCl, and the optical density was read at 450 nm using a Microplate Reader (Molecular Devices Co., Ltd., U.S.A.).

RESULTS AND DISCUSSION

The CH_2Cl_2 soluble fraction of the MeOH extract of *G. thunbergii* was chromatographed on silica gel, Sephadex LH-20, reversed phase C-18 columns, which was followed by prep. TLC to afford three compounds (**1-3**) (Fig. 1).

Compound **3** was obtained as a colorless oil. This compound was determined to be a new 4-hydroxy derivative of the known furofuran lignan kobusin according to its MS, ^{1}H- and ^{13}C-NMR data. Its molecular formula was found to be $C_{21}H_{22}O_7$ by HR-EI mass spectrometry ([M]$^+$ found 386.1363). The UV spectrum showed absorption maxima at 232 and 284 nm, which corresponded to the furofuran type of lignan (Matsushita *et al.*, 1991). The IR spectrum showed absorption bands due to hydroxyl (3400 cm^{-1}) and aromatic rings (1610, 1505 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectral data of compound **3** were similar to that of kobusin except for the 4-hydroxyl group of the furofuran ring moiety. The ^{13}C- and HSQC NMR spectra of compound **3** showed 21 carbons including one hemiacetal carbon (δ 102.97), methylenedioxy carbon (δ 102.58), four trisubstituted carbons (δ 88.69, 85.07, 63.79, 55.09), one oxymethylene carbon (δ 73.24), two methoxy carbons (δ 56.69, 56.57), and two aromatic rings (δ 150.80, 150.19, 149.65, 148.83, 137.25, 137.04, 120.55, 120.12, 112.81, 111.55, 109.97, 107.37). There were downfield shifts of C-4 and C-5 in compound **3** compared with the chemical shifts of C-4 and C-5 in kobusin, which strongly suggested that the hydroxyl group was located at C-4. The ^{1}H-NMR spectrum revealed two ABX type coupling patterns, and the COSY spectrum indicating the presence of 1,3,4-trisubstituted phenyl groups [δ 7.21 (1H, d, *J*=2.0 Hz), 6.98 (1H, dd, *J*=2.0, 8.0 Hz), 6.91 (1H, d, *J*=8.0 Hz), 6.88 (1H, d, *J*=2.0 Hz), 6.85 (1H, dd, *J*=2.0, 8.0 Hz), 6.79 (1H, d, *J*=8.0 Hz)], two benzylic oxymethine protons at δ 4.90 (1H, d, *J*=7.0 Hz), 4.84 (1H, *J*=7.0 Hz), two methine protons at δ 3.14(1H, m), 2.84 (1H, br.t, *J*=7.5 Hz), one acetal proton at δ 5.50 (1H, d, *J*=1.0 Hz), one oxymethylene proton at δ 4.21 (1H, dd, *J*=6.0, 9.0 Hz), 4.02 (1H, dd, *J*=2.5, 9.0 Hz), one methylenedioxy proton at δ 5.94 (2H, s, -OC<u>H$_2$</u>O-), two methoxy protons at δ 3.85 (3H, s, -OCH$_3$), 3.82 (3H, s, -OCH$_3$). In the HMBC spectrum of compound **3**, the carbon signals at δ 88.69 (C-2) and 85.07 (C-6) correlated with the proton signal at δ 4.21 and 4.02 (H-8), and the carbon signals at δ 85.07 (C-6) and 88.69 (C-2) correlated with the proton signals at δ 5.50 (H-4), respectively (Fig. 2). The stereochemistry of the hydroxyl group at C-4 was assigned to an α-face because the NOE difference spectra showed a NOE effect between H-4 (δ 5.50) and H-2 (δ 4.90)/H-6 (δ 4.84) (Abe and Yamaguchi, 1988). Furthermore, the H-4 peak appeared as a broad singlet at δ 5.50 confirming the equatorial orientation of the hydroxyl group at C-4 (Malarz *et al.*, 2005; Hou *et al.*, 2003; Tene *et al.*, 2004; Min *et al.*, 2005). Therefore, compound **3** was characterized as 4-hydroxykobusin based on the above spectroscopic evidence.

Compound **2** was obtained as a colorless oil. The UV spectrum exhibited absorption maxima at 234 and 281 nm, which are characteristic absorption bands of a butyrolactone-type lignan (Kim *et al.*, 2004). The IR spectrum showed the signals for a hydroxyl (3410 cm^{-1}), unsaturated carbonyl (1751 cm^{-1}), and aromatic ring (1600 and 1502 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra showed a typical pattern of methylenedioxygenated dibenzylbutyrolactone-type lignan, and the structure of compound **2** was similar to that of bursehernin (Enders *et al.*, 2002). Downfield shifts of H-7, 8, 7' and 8' were observed in compound **2** compared with the chemical shifts of H-7, 8, 7' and 8' in bursehernin, which strongly suggested that the hydroxyl group was located at C-7 and 7'. In the HMBC spectrum of compound **2**, the carbon signals at δ 149.04 (C-3') and

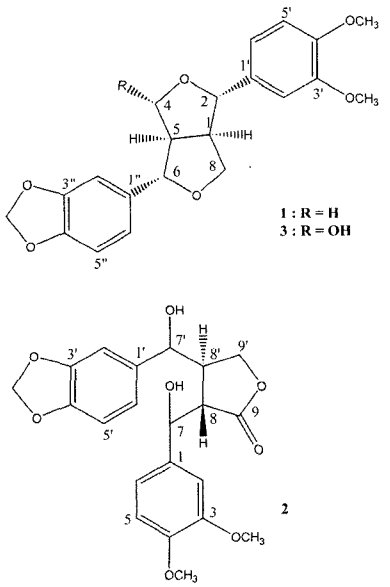

Fig. 1. Chemical structures of compounds **1-3** isolated from *G. thunbergii*

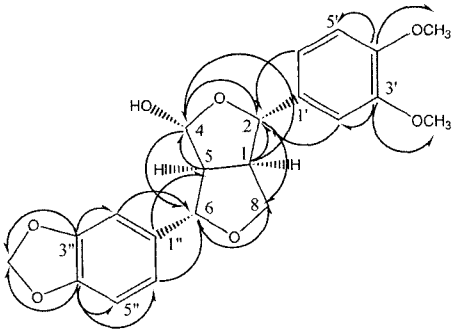

Fig. 2. Selected ^{1}H-^{13}C long-range correlations in the HMBC spectrum of compound **3**

148.74 (C-4') correlated with the proton signal at δ 5.93 ($OC\underline{H_2}O$), and the carbon signals at δ 133.87 (C-1) and 136.00 (C-1') correlated with the proton signals at δ 5.41 (H-7) and 5.21 (H-7'), respectively. Furthermore, the carbon signals at δ 54.68 (C-8) and 51.10 (C-8') correlated with the proton signals at δ 4.30 and 4.02 (methylene protons, H_2-9'). This indicated that the skeleton of compound **2** was a methylenedioxygenated dibenzylbutyrolactone-type lignan (Kwon *et al.*, 1999; Kim *et al.*, 2004). The *trans* junction of a γ-lactone group was deduced from the coupling constant ($J_{8\text{-}8'}$ = 9.0 Hz) and the reported data of ligans with the *trans* junction of the γ-lactone group (Tanoguchi *et al.*, 1991). Based on these observations, compound **2** was determined to be 7,7'-dihydroxybursehernin. Although, Ogiku *et al* previously reported the chemical synthesis of compound **2** (Ogiku *et al*, 1995), this is the first report of the naturally occurring 7,7'-dihydroxybursehernin from a plant.

Compound **1** was identified as a kobusin (Hua *et al.*, 2004; Ahmed *et al.*, 2002; Latip *et al.*, 1999; Lim *et al.*, 1999; Idida *et al.*, 1982) by comparing the NMR spectral data with those reported in the literature.

Interleukin-6 (IL-6) is a cytokine originally identified as a T-cell-derived factor regulating B-cell growth and differentiation (Hirano *et al.*, 1986). Human IL-6 is an important component of the inflammatory cascade. In particular, the dysregulation of IL-6 production has been implicated in a variety of inflammatory/autoimmune diseases including rheumatoid arthritis, cardiac myxoma, Castleman's disease, and mesangial proliferative glomerulonephritis (Hirano *et al.*, 1990). The proinflammatory cytokines IL-1 and tumor necrosis factor-α (TNF-α) markedly stimulate IL-6 production (Van Damme *et al.*, 1987).

The inhibitory activity of hIL-6 production in TNF-α stimulated MG-63 cell was examined. Among the compounds isolated, compounds **1** and **3** showed weak activity against hIL-6 production, while compound **2** showed potent inhibitory activity. The inhibitory activity of compounds **1**-**3** against hIL-6 production in TNF-α stimulated MG-63 cell was 17.1±6.33%, 60.5±7.76%, and 39.3±9.74%, respectively, at a concentration of 50 μg/mL.

REFERENCES

Abe, F. and Yamauchi, T., 9α-hydroxypinoresinol, 9α-hydroxymedioresinol and related lignans from *Allamanda neriifolia*. *Phytochemistry*, 27, 575-577 (1988).

Ahmed, A. A., Mahmoud, A. A., Ali, E. T., Tzakou, O., Couladis, M., Mabry, T. J., Gáti, T., and Tóth, G., Two highly oxygenated eudesmanes and 10 lignans from *Achillea holostericea*. *Phytochemistry*, 59, 851-856 (2002).

Bae, K. W., The medicinal plants of Korea. Kyo-Hak Publishing Co., Seoul, pp (2000).

Enders, D., Lausberg, V., Signore, G. D., and Berner, O. M., A general approach to the asymmetric synthesis of lignans: (-)-methyl piperitol, (-)-sesamin, (-)-aschantin, (+)-yatein, (+)-dihydroclusin, (+)-bursheran, and (-)-isostegane. *Synthesis*, 4, 515-522 (2002).

Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamatsu, A., Tsunsawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T., and Kishimoto, T., Complementary DNA for a novel human interleukin (BSF-2) that induced lymphocytes to produce immunoglobulin. *Nature*, 324, 73-76 (1986).

Hirano, T., Akira, S., Taga, T., and Kishimoto, T., Biological and clinical aspects of interleukin 6. *Immunol. Today*, 11, 443-449 (1990).

Hou, C.-C., Lin, S.-J., Cheng, J.-T., and Hsu, F.-L., Antidiabetic dimeric guianolides and a lignan glycosides from *Lactuca indica*. *J. Nat. Prod.*, 66, 625-629 (2003).

Hua, X. G., Kim, J. A., Park, S. H., Son, A. R., Chang, T. S., Chang, H. W., Chung, S. R., and Lee, S. H., Isolation of melanin biosynthetic inhibitory compounds from the flowers of *Magnolia denudata*. *Kor. J. Pharmacogn.*, 35, 152-156 (2004).

Kim, B. H., Chung, E. Y., Ryu, J.-C., Jung, S.-H., Min, K. R., and Kim, Y., Anti-inflammatory mode of isoflavone glycosides sophoricoside by inhibition of interleukin-6 and cyclooxygenase-2 in inflammatory response. *Arch. Pharm. Res.*, 26, 306-311 (2003).

Iida, T., Nakano, M., and Ito, K., Hydroperoxysesquiterpene and lignan constituents of *Magnolia kobus*. *Phytochemistry*, 21, 673-675 (1982).

Ito, H., Hatano, T., Namba, O., Shirono, T., Okuda, T., and Yoshida, T., Constituents of *Geranium thunbergii* Sieb. Et Zucc. XV. Modified dehydroellagitannins, geraniinic acids B and C, and phyllanthusiin F. *Chem. Pharm. Bull.*, 47, 1148-1151 (1999).

Kim, M.-R., Moon, H.-I., Chung, J. H., Moon, Y. H., Hahm, K.-S., and Woo, E.-R., Matrix matalloproteinase-1 inhibitor from the stem bark of *Styrax japonica*. *Chem. Pharm. Bull.*, 52, 1466-1469 (2004).

Kwon, H. C., Choi, S. U., Lee, J. O., Bae, K. H., Zee, O. P., and Lee, K. R., Two new lignans from *Lindera obtusiloba* Blume. *Arch. Pharm., Res.*, 22, 417-422 (1999).

Latip, J., Hartley, T. G., and Waterman, P. G., Lignans and coumarins metabolites from *Melicope hayesii*. *Phytochemistry*, 51, 107-110 (1999).

Lim, Y.-H., Leem, M.-J., Shin, D.-H., Chang, H.-B., Hong, S.-W., Moon, E-Y., Lee, D.-K., Yoon, S.-J., and Woo, W.-S., Cytotoxic constituents from the roots of *Anthriscus sylvestris*. *Arch. Pharm. Res.*, 22, 208-212 (1999).

Malarz, J., Stojakowska, A., Szneler, E., and Kisiel, W., Furofuran lignans from a callus culture of *Cichorium intybus*. *Plant Cell. Rep.*, 24, 246-249 (2005).

Matsushita, H., Miyase, T., and Ueno, A., Lignan and terpene glycosides from *Epimedium sagittarium*. *Phytochemistry*, 30, 2025-2027 (1991).

Min, B.-S., Cui, H. S., Lee, H.-K., Sok, D.-E., and Kim, M. R., A new furofuran lignan with antioxidant and antiseizure activities from the leaves of *Petasites japonica*. *Arch. Pharm. Res.*, 28, 1023-1026 (2005).

Ogiku, T., Yoshida, S., Ohimizu, H., and Iwasaki, T., Stereocontrolled synthesis of diequatorial and axial-equatorial furofuran lignans. *J. Org. Chem.*, 60, 1148-1153 (1995).

Okuda, T., Yoshida, T., Hatano, T., Ikeda, Y., Shingu, T., and Inoue, T., Constituents of *Geranium thunbergii* Sieb. Et Zucc. XIII. Isolation of water-soluble tannins by centrifugal partition chromatography, a biomimetic synthesis of elaeocarpusin. *Chem. Pharm. Bull.*, 34, 4075-4082 (1986).

Tanoguchi, M., Hosono, E., Kitaoka, M., Arimoto, M., and Yamaguchi, H., Studies on the constituents of the seeds of *Hernandia ovigera* L. IX. Identification of two dibenzylbutyrolactone-type lignans and an attempt of conversion into phenyltetralin-type lignan. *Chem. Pharm. Bull.*, 39, 1873-1876 (1991).

Tene, M., Tane, P., Sondengam, B. L., and Connolly, J. D., Lignans from the roots of *Echinops giganteus*. *Phytochemistry*, 65, 2101-2105 (2004).

Van Damme, J., Opdenakker, G., Simpson, R. J., Rubira, M. R., Cayphas, S., Vink, A., Billiau, A., and Van Snick, J., Identification of the human 26-kD protein, interferon beta 2(IFN-beta 2), as a B cell hybridoma/plasmacytoma growth factor induced in interleukin 1 and tumor necrosis factor. *J. Exp. Med.*, 165, 914-919 (1987).