Arch Pharm Res Vol 28, No 1, 34-38, 2005

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

A New Antipsychotic Effective Neolignan from *Firmiana simplex*

Yeon Kyoung Son[1], Ming Hong Lee[1], and Yong Nam Han[1,2]

[1]Natural Products Research Institute, and [2]College of Pharmacy, Seoul National University, Seoul 110-460, Korea

(Received August 16, 2004)

A new neolignan, named simplidin was isolated from the the n-butanol extract of stem of *Firmiana simplex*, together with six known compounds, scopoletin (**1**), syrigaresinol (**2**), aquillochin (**3**), nitidanin (**4**), tamarixetin 3-rhamnoside (**6**), and quercitrin (**7**). On the basis of spectral and chemical evidence, simplidin (**5**) was determined to be rel-(7*R*,8*R*)-4,5,9,9'-tetrahydroxy-3,3'-dimethoxy-7-*O*-5',8-*O*-4'-neolignan. All the six compounds were also isolated for the first time from this plant.

Key words: *Firmiana simplex*, Sterculiaceae, Benzodioxane lignan, Neolignan, Antipsychotic effect

INTRODUCTION

Firmiana simplex (L.) W. F. Wight (Sterculiaceae) is a deciduous tree having large stem and leaves, and is called Chinese parasol tree. Its seeds have been used in a traditional medicine for diarrhea and stomach disorder (Hotta *et al.*, 1989; Bae, 2000). This tree is very common around, but the chemical constituents of this tree have not been studied until now. Now we describe the isolation and structural determination of simplidin (**5**), a new neolignan, from *F. simplex*. The known two lignans (**2**, **4**), two coumarins (**1**, **3**) and two flavonoid glycosides (**6**, **7**) were also isolated for the first time from this plant.

METERIALS AND METHODS

General

The melting point was determined with a Büchi B-540 apparatus and was uncorrected. The UV spectra were recorded on a Hewlett Packard HP V-550 UV-VIS spectrophotometer. The IR spectra were recorded on a JASCO FT/IR-5300 spectrometer. The EI-MS was recorded on Hewlett Packard HP 5985 mass spectrometer. FAB-HRMS was recorded on a High Resolution Tandem Mass Spectrometer JMS-HX110. NMR spectra and 2D sepctra were recorded on a Bruker Avance-400 and 600 MHz for ^{1}H- and 100 for ^{13}C-NMR, respectively. Optical rotations were determined on a Jasco P-1020 polarimeter.

Correspondence to: Yong Nam Han, Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea
Tel: 82-2-740-8928, Fax: 82-2-762-8322
E-mail: snake@snu.ac.kr

Plant materials

The stem of *F. simplex* was collected at the vicinity of Seoul in Korea. The stems were separated from foliage and air-dried at room temperature, and then finely ground (4 kg). Voucher specimens have been deposited at Herbarium of Natural Products Research Institute, Seoul National University, Korea.

Extraction and Isolation

The dried stem powder of *F. simplex* was percolated with MeOH (40L×3) three times for 3 weeks. The MeOH extract (270 g) was fractionated by its solubility in hexane, *n*-BuOH and water. The *n*-BuOH soluble part (68 g) was chromatographed on a silica gel column (850 g) in CH_2Cl_2-MeOH (10:1 3:1) to give six fractions (Fr. A Fr. F). Fr. A (8.2 g) was chromatographed on RP-18 eluting with 70% MeOH to afford five fractions (Fr. A1 Fr. A5). Compound **1** (10 mg) was isolated from Fr. A1 on silica gel column eluting $CHCl_3$ and MeOH (40:1). Fr. A3 was applied to silica gel column for chromatography with $CHCl_3$ and MeOH (10:1) to give two fractions, of which each was further purified by recrystallization with MeOH to afford compound **2** (5 mg) and compound **3** (30 mg). Compound **4** (15 mg) was isolated from Fr. A4 on silica gel column eluting $CHCl_3$ and MeOH (20:1). Chromatography of Fr. B on RP-18 eluting with from 50% MeOH to 70% MeOH yielded compound **5** (200 mg), which was further purified by recrystallization with MeOH. Fr. E (5 g) was chromatographed on a Sephadex LH-20 eluting with 70% MeOH to give compound **6** (10 mg) and compound **7** (3 g). Compound **5** (10 mg) was subjected to acetylation (Ac_2O 1 mL, pyridine 1 mL, 25 °C, 24 h) and compound

5a (5 mg) was separated by SiO_2 eluting with $CHCl_3$-EtOAc =6:1.

Scopoletin (1)
Yellowish neddles, mp 202-204°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) 7.85 (1H, d, *J* = 9.3 Hz, H-4), 7.11 (1H, s, H-5), 6.77 (1H, s, H-8), 6.19 (1H, d, *J* = 9.3 Hz, H-3), 3.86 (3H, s, OCH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) 164. 9 (C-2), 153.7 (C-7), 152.2 (C-9), 147.9 (C-6), 146.9 (C-4), 113.4 (C-3, 10), 110.7 (C-5), 104.8 (C-8), 57.6 (OCH_3).

Syringaresinol (2)
Colorless needles, mp: 172-174°C; $[\alpha]_D^{22}$ 0 (c=0.01, $CHCl_3$); EI-MS: *m/z* (rel. int. %): 418 (M^+, 100), 210 (19), 193 (27), 181 (98), 167 (70); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.58 (4H, s, aromatic H), 5.52 (2H, brs, OH), 4.73 (2H, d, *J* = 7, 4.2 Hz, H-7), 4.28 (2H, dd, *J* = 9.0, 6.5 Hz, H-9, 9'), 3.91 (2H, dd, *J* = 9.0, 3.9 Hz, H-9,9'), 3.09 (2H, m, H-8, 8'), 3.90 (12H, s, OCH_3×4); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) 147.2 (C-3, 3', 5, 5'), 134.3 (C-4, 4'), 132.0 (C-1, 1'), 102.7 (C-2, 2', 6, 6'), 86.1 (C-7,7'), 71.8 (C-9, 9'), 56.4 (OCH_3), 54.3 (C-8, 8').

Aquillochin (3)
Amorphous powder, mp: 210°C (dec); $[\alpha]_D^{22}$ 0 (c=0.02, MeOH); IR (KBr) cm^{-1}: ν_{max} = 3416 (OH), 2920, 1720, 1612 (α-pyrone), 1572 (Ar); UV λ_{max} (MeOH) nm: 268, 325; EI-MS: *m/z* (rel. int. %): 416 (M^+, 19), 210 (100), 398 (7), 208 (73), 193 (23), 182 (29), 167 (72); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm) 7.96 (1H, d, *J* = 9.5 Hz, H-4), 6.92 (1H, s, H-5), 6.70 (2H, s, H-2', 6'), 6.24 (1H, d, *J* = 9.5 Hz, H-3), 4.96 (1H, d, *J* =7.9 Hz, H-7'), 4.36 (1H, m, H-8'), 3.64 (2H, m, H-9'); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) 160.1 (C-2), 147.9 (C-3', 5'), 145.3 (C-4), 144.9 (C-6), 138.1 (C-9), 137.1 (C-7), 136.2 (C-4'), 131.7 (C-8), 125.7 (C-1'), 117.2 (C-3), 115.5 (C-10), 105.6 (C-2', 6'), 100.8 (C-5), 77.8 (C-8'), 76.6 (C-7'), 59.9 (C-9'), 55.9 (OCH_3).

Nitidanin (4)
Amorphous powder, mp 240°C; $[\alpha]_D^{22}$ 0 (c=0.02, $CHCl_3$-MeOH 1:1); IR (KBr) cm^{-1}: ν_{max} = 3451 (OH); UV λ_{max} (MeOH) nm: 224, 272; EI-MS: *m/z* (rel. int. %): 404 $[M]^+$ (45), 210 (83), 167 (100); ^{1}H- and ^{13}C-NMR: see Table I.

Simplidin (5)
Amorphous powder, mp 194°C; $[\alpha]_D^{22}$ 0 (c=0.02, $CHCl_3$-MeOH 1:1); IR (KBr) cm^{-1}: ν_{max} = 3491 (OH), 1599, 1512, 1464, 964; UV λ_{max} (MeOH) nm: 224, 272. HRFAB-MS *m/z*: 413.1216 (Calcd for $C_{20}H_{22}O_8Na$). EI-MS: *m/z* (rel. int. %): 390 (M^+, 2.5), 196 (54), 153 (66), 66 (100); ^{1}H- and ^{13}C-NMR: see Table I.

Simplidin tetraacetate (5a)
Amorphous powder, [α] 0 (c=0.02, $CHCl_3$-MeOH 1:1); EI-MS: *m/z* (rel. int. %): 558 (M^+, 25), 238 (100); ^{1}H- and ^{13}C-NMR: see Table I.

Tamarixetin 3-*O*-rhamnoside (6)
Yellowish powder, ^{1}H-NMR (600 MHz, CD_3OD) δ (ppm) 7.44 (1H, d, *J* = 1.9 Hz, H-2'), 7.41 (1H, dd, *J* = 8.3, 1.9 Hz, H-6'), 6.95 (1H, d, *J* = 8.3 Hz, H-5'), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.38 (1H, d, *J* = 1.6 Hz, rha H-1"), 4.19-3.35 (4H, m, rha H-2", 3", 4", 5"), 3.95 (3H, s, OCH_3), 0.90 (3H, d, *J* = 5.6 Hz, rha CH_3); ^{13}C-NMR (150 MHz, CD_3OD) δ 179.8 (C-4), 166.1 (C-7), 163.4 (C-5), 159.4 (C-2), 158.7 (C-9), 151.1 (C-3'), 149.0 (C-4'), 136.3 (C-3), 124.4 (C-6'), 123.0 (C-1'), 116.7 (C-5'), 113.7 (C-2'), 106.0 (C-10), 103.6 (rha C-1), 72.3 (rha C-2), 72.2 (rha C-3), 72.0 (rha C-5), 17.8 (rha CH_3).

Quecitrin (7)
Yellowish powder, ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm) 7.29 (1H, d, *J* = 2.1, H-2'), 7.24 (1H, dd, *J* =8.4, 2.1 Hz, H-6'), 6.85 (1H, dd, *J* = 8.4, 2.1 Hz, H-5'), 6.38 (1H, d, *J* = 2.1, H-8), 6.19 (1H, d, *J* = 2.1, H-6), 5.24 (1H, d, *J* = 1.2, rha H-1"), 3.10-3.40 (4H, m, rha H-2", 3", 4", 5"), 0.80 (3H, d, *J* = 5.7, rha CH_3); ^{13}C-NMR (75 MHz, CD_3OD) δ (ppm) 178.0 (C-4), 164.6 (C-7), 161.6 (C-5), 157.6 (C-2), 156.7 (C-9), 148.7 (C-4'), 145.5 (3'), 134.5 (C-3), 121.4 (C-6'), 121.0 (C-1'), 115.9 (C-5'), 115.8 (C-2'), 104.3 (C-10), 102.1 (rha C-1), 99.0 (C-6), 93.9 (C-8), 71.5 (rha C-4), 70.9 (rha C-2), 70.7 (rha C-3), 70.3 (rha C-4), 17.8 (rha CH_3).

RESULTS AND DISCUSSION

Compound **1** (scopoletin, Prescott *et al.*, 2002), compound **2** (syringaresinol, Bermes *et al.*, 1991) and compound **7** (quercitrin, Lee *et al.*, 2004) were characterized by comparing their physical and spectroscopic data with those of the reported literatures.

Compound **3**, mp 210°C, $C_{21}H_{20}O_9$, showed a molecular ion at *m/z* 416 and had no optical rotation. The UV absorption at 325 nm and 268 nm, a δ-lactone absorption band at 1720 and 1612 cm^{-1} in the IR spectrum and two characteristic doublets (AB pattern) at δ 6.24 (H-3) and 7.96 (*J* = 9.5 Hz, H-4) indicated the existence of coumarin nucleus. The signals of δ 4.96 (a mono-oxybenzylic proton, H-7'), δ 4.36 (an oxy methin proton, H-8') and δ 6.70 (two aryl protons) in the ^{1}H-NMR spectrum were found to be consistent with the following bonding unit, Ar-CH(O)-CH(O)-CH_2O: The existence of the C_6-C_3 unit as C_6H_2 3',5'-OCH_3 (4'-OH) was indicated by the ^{1}H-NMR spectrum showing two equivalent aromatic protons (δ 6.70, s, H-2',6') and two vicinal aliphatic oxymethines (δ 4.96, d, *J* =

7.9 Hz) linked to the phenyl group and to a CH_2OH group (δ 4.26, m) and the presence of the most abundant retro-Diels-Alder fragment ion at *m/z* 210 in the MS of compound **3**. This was also supported by the fragment at *m/z* 167 due to a cation $C_9H_{11}O_3$ in the MS of compound **3**. The coupling constant between the H-7' and H-8' signals was 7.9 Hz, demonstrating the two hydrogens are *trans*-conformation. Upon analysis of above data and with comparison of reported literature values, compound **3** was identified as aquillochin (Bhandari *et al.*, 1982).

Compound **5**, mp 194°C, was obtained as white amorphous powder. The FAB-HRMS spectrum showed a $[M+Na]^+$ ion at *m/z* 413.1216, consistent with the molecular formula of $C_{20}H_{22}O_8$ (calculated mass; 413.1212). The ^{1}H-NMR spectrum of **5** showed the presence of two sets of two meta-coupled doublets at δ 6.66 and 6.55 (J = 1.6 Hz); 6.51 and 6.47 (J = 1.7 Hz) on two phenyl rings with two methoxy groups at δ 3.76 and 3.72 (Table I). HMBC connectivities and NOESY correlation (Fig. 2) of **5** indicated that the appearance of the methoxy group at δ 3.76 and the meta-coupled doublets at δ 6.66 and δ 6.55 (J = 1.6 Hz) could be assigned to an unsymmetical 3-methoxy-4,5-dioxygenated phenyl group. The substituent could be expanded to a 5-hydroxyconiferyl alcohol unit by the following signal sequence: δ 4.07 (2H, d, J = 5.2 Hz, H-9'), δ 6.20 (1H, dt, J = 15.9, 5.2 Hz, H-8') and δ 6.38 (1H, d, J = 15.9 Hz, H-7'). Since acetylation of **5** with acetic anhydride-prydine yielded a tetraacetate, **5a** ($C_{28}H_{30}O_{12}$ M^+ 558), compound **5** contains two apliphatic hydroxy and two phenyl hydroxy groups. Since the signal for the H-9' showed a downfield shift of δ 0.59 after acetylation, this group must bear the hydroxyl group. The methoxy group at δ 3.72, the meta-coupled doublets at δ 6.51 and δ 6.47 (J = 1.7 Hz) indicated the presence of an unsymmetrical 4,5-dihydroxy-3-methoxy phenyl group substituent (Fig. 2). The three carbon sequence, Ar-CH(O)CH(O)CH_2OH,

Table I. ^{1}H-NMR and ^{13}C-NMR (DMSO-d_6, ^{13}C: 100 MHz; ^{1}H: 400 MHz) spectrum of compound **4**, **5**, and **5a**

C No.	Compound **4**		Compound **5**		Compound **5a**	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1		126.6		127.3		134. 5
2	6.69(s)	105.3	6.51(d, J=1.7 Hz)	103.6	7.18(d, J = 1.5 Hz)	109. 7
3		147.9		148.8		152.2
4		135.9		134.7		131.8
5		147.9		145.8		143.1
6	6.69(s)	105.3	6.47(d, J = 1.7 Hz)	108.7	6.97(d, J = 1.5 Hz)	114.6
7	4.84(d, J=7.3 Hz)	76.0	4.75(d, J = 7.6 Hz)	76.3	5.08(d, J = 7.5 Hz)	75..3
8	4.09(m)	77.9	3.99(m)	78.4	4.52(m)	74..3
9	3.55(d, J=11.4 Hz)	60.1	3.52(dd, J = 12.3, 2.4 Hz)	60.6	4.17(dd, J = 12.5, 3.3 Hz)	62.3
	3.30(m)		3.32(dd, J = 12.3, 4.8 Hz)		3.99(dd, J = 12.5, 4.7 Hz)	
1'		129.1		129.7		128.9
2'	6.68(d, J=1.8 Hz)	102.3	6.66(d, J = 1.6 Hz)	102.8	6.80(d, J = 1.6 Hz)	102.9
3'		148.6		149.1		148.7
4'		132.5		132.7		132.2
5'		144.9		144.4		143.5
6'	6.58(d, J =1.8 Hz)	107.5	6.55(d, J = 1.6Hz)	108.7	6.71(d, J = 1.6 Hz)	108.1
7'	6.40(d, J =15.9 Hz)	128.5	6.38(d, J = 15.9 Hz)	129. 3	6.55(d, J =1 5.9 Hz)	133.1
8'	6.23(dt, J =15.9, 5.1 Hz)	129.3	6.20(dt, J = 15.9, 5.2 Hz)	129. 3	6.28(dt, J = 15.9, 6.3 Hz)	122.5
9'	4.07(d, J =4.5 Hz)	61.6	4.07(d, J = 5.2 Hz)	61.9	4.66(d, J = 6.0 Hz)	64.4
	3, 5-OMe 3.75(s)	56.1	**3-OMe** 3.72(s)	56.4	**3-OMe** 3.81(s)[a]	54.3[a]
	3'-OMe 3.79(s)	55.6	**3'-OMe** 3.76(s)	56.2	**3'-OMe** 3.82(s)[a]	55.3[a]
					Ar-OAc	Me (acetyl)
					2.29(s), 2.28(s)	20.0, 20.8,
					-OAc	20.3, 20.4
					2.06(s), 1.99(s)	C= (acetyl)
						167.5, 170.2
						168.2, 170.1

[a] Assignments may be interchanged.

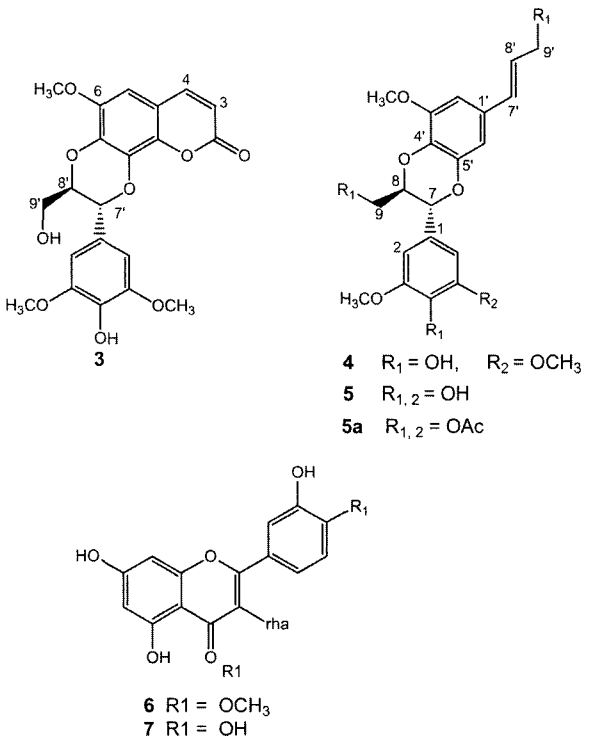

Fig. 1. Chemical structures of compounds from the stem of *Firmiana simplex*

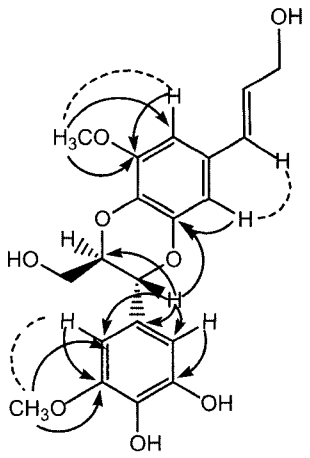

Fig. 2. Major HMBC connectivities (→) and NOE correlation (···) of compound **5**

was deduced by two vicinal aliphatic oxymethines at δ 4.75 (1H, d, J = 7.6 Hz, H-7) and δ 3.99 (1H, m, H-8) linked to a phenyl and to a CH_2OH (H-9) group, respectively, and by two germinally coupled methylene at δ 3.52 and δ 3.32 (J = 12.3 Hz). Since the signals for the H-9 protons showed downfield shift of δ 0.65 and 0.67 after acetylation, these protons must bear the hydroxyl group. The 7,8-*trans* conformation was inferred from the relatively large coupling constant of 7.6 Hz (Fang *et al.*, 1992) and two methoxy groups linked to a phenyl group were confirmed by NOESY experiment (Fig. 2). The HMBC spectrum of **5** showed by the correlation of H-7 at δ 4.75 with C-5' at δ 144.4 and C-1 at δ 127.3 (Fig. 1 and Table I) allowed to conclude the 4,5-dihydroxy-3-methoxy phenyl group is attached to the C-7. These data exhibited that compound **5** has the structure of benzodioxane-type neolignan from cross-coupling of two 5-hydroxyconiferyl alcohols. This was confirmed by the presence retro-Diels-Alder fragment ion peak at *m/z* 196 ($C_{10}H_{12}O_4$; 5-hydroxyconiferyl alcohol) in the EI-MS. Compound **5** was fully assigned as rel-(7*R*,8*R*)-4,5,9,9'-tetahydroxy-3,3'-dimethoxy- 7-*O*-5',8-*O*-4'-neolignan, named simplidin.

Compound **4**, mp 240°C, $C_{21}H_{24}O_8$ showed a molecular ion peak at *m/z* 404 in the EI-MS and had the similar pattern of IR, UV, ^1H and ^{13}C with those of **5**. The major differences between the ^1H-NMR spectrum of **5** in relation with that of **4** were the presence of a 2H singlet at δ 6.69 instead of two meta-coupled doublets of **5** and a 6H singlet two methoxy groups at δ 3.75. The ^1H-NMR data showed the presence of symmetrical 4-hydroxy-3,5-dimethoxyphenyl substituent. In addition, ^{13}C-NMR, ^1H-^1H COSY, HETCOR and HMBC spectra exhibited that **4** is a benzodioxane-type neolignan from cross-coupling of sinapyl alcohol with 5-hydroxyconiferyl alcohol. Compound **4** was assigned as rel-(7*R*,8*R*)-4,9,9'-trihydroxy-3,5,3'-trimethoxy-7-*O*-5',8-*O*-4'-neolignan, nitidanin isolated from *Xanthoxylum nitidum* (Ishikawa *et al.*, 1995). These benzodioxane lignans of **4** and **5** skeletons were recently found in *Chamaecyparis fomosensis* (Lin *et al.*, 1999) and *Pedicularis verticillata* (Su *et al.*, 1997), and biosynthesis of benzodioxane lignans and its pathway have been reported by advance in genetic engineering (Marita *et al.*, 2003; Fournand *et al.*, 2003; Ralph *et al.*, 2001).

Compound **6** was obtainded as yellow powder. The ^1H-NMR spectrum of **6** exhibited typical signals for quercetin 4'-methyl ether glycoside compared with that of quercitrin (**7**). The ^1H-NMR spectrum displayed three proton signals at δ 7.44 (1H, d, J = 1.9 Hz), 7.41 (1H, dd, J = 8.3, 1.9 Hz) and 6.95 (J = 8.3 Hz) attributable to H-2', H-6' and H-5', respectively, and two meta-coupled doublets at δ 6.39 and 6.21 (J = 2.0 Hz) for H-8 and H-6. Acid hydrolysis of **6** afforded rhamnose as a sugar component, identified by HPLC comparison with an authentic sample. In HMBC spectrum, the proton signal at δ 3.95 was shown to be correlated with the C-4' (δ 136.2), indicating that methoxy group is attached to the C-4' position. In addition, the anomeric proton signal (δ 5.38) was correlated with C-3 (δ 117.2), indicating that rhanmose is attached to the C-3 position. By assistance of the HMBC and HMQC spectrum, compound **6** was determined as tamarixetin 3-rhamnoside and was isolated from *Flemingia stricta* (Rao *et al.* 1983).

ACKNOWLEDGEMENT

This study was supported by a grant 01-PJ2-PG6-01NA01-0002 funded by the Ministry of Health and Welfare, Korea.

REFERENCES

Bae, K. H., Medicinal plants of Korea, KyoHak Publishing Co., Ltd. Seoul, pp. 336, (2000).

Bermes, B., Seligmann, O., and Wagner, H., Synthesis of biologically active tetrahydro-furofuranlignan-(syringin, pinoresinol)-mono- and bis-glucosides. *Phytochem.*, 30, 3087-3089 (1991).

Bhandari, P, Pant, P., and Rastogi, R. P., Aquillochin, a coumarinolignan from *Aquilaria agallocha*. *Phytochem.*, 21, 2147-2149 (1982).

Fang, J. M., Lee, C. K., and Cheng, Y. S., Lignans from leaves of *Juniperus chinensis*. *Phytochem.*, 31, 3659-3661 (1992).

Fournand, D., Cathala, B., and Lapierre, C., Initial steps of the peroxidase-catalyzed polymerization of coniferyl alcohol and/or sinapyl aldehyde: capillary zone electrophoresis study of pH effect. *Phytochem.*, 62, 139-1496 (2003).

Hotta, M., Ogata, K., Nitta, A., Hosikawa, K., Yanagi, M., and Yamazaki K., Useful plant of the world, Heibonsha, Tokyo, pp. 461, (1989).

Ishikawa, T., Seki, M., Nishigaya, K., and Miura, Y., Studies on the chemical constituents of *Xanthoxylum nididum* (ROXB.) D. C. (*Fagara nidida* ROXB.). III. The chemical constituents of the wood. *Chem. Pharm. Bull.*, 43, 2014-2018 (1995).

Lee, J. H., Ku, C. H., Baek, N. I., Kim, S. H., Park, H. W., and Kim, D. K., Phytochemical constituents from *Diodia teres*. *Phytochem. Arch. Pharm.* Res., 27, 40-43 (2004).

Lin, T. C., Fang, J. M., and Cheng, Y. S., Terpenes and lignans from leaves of *Chamaecyparis formosensis*. *Phytochem.*, 51, 793-801 (1999).

Marita, J. M., Ralph, J., Hatafield, R. D., Guo, D., Chen, F., and Dixon, R. A., Structural and compositional modifications in lignin of transgenic alfalfa down-regulated in caffeic acid 3-*O*-methyltransferase and caffeoyl coenzyme A 3-*O*-methyltransferase. *Phytochem.*, 62, 53-65 (2003).

Prescott, G. P., Stamford, N. P. J., Wheeler, G., and Firmin, J. L., *In vitro* properties of a recombinant flavonol synthase from *Arabidopsis thaliana*. *Phytochem.*, 60, 589-593 (2002).

Ralph, J., Lapierre, C., Lu, F., Marita, M. M., Pilate, G., Doorsselaere, J. V., Boerjan, W., and Jouanin, L., NMR evidence for benzodioxane structures resulting from incorporation of 5-hydoxyconiferyl alcohol into lignins of *O*-methyltransferase-deficient poplars. *J. Agric. Food Chem.*, 49, 86-91 (2001).

Rao, C. P., Hanumaiah, T., Venuri, V. S. S., and Rao, K. V. J., Flavonol 3-glycosides from the leaves of *Flemingia stricta*. *Phytochem.*, 22, 621-622 (1983).

Su, G. N., Yang, L., and Jia, Z. J., Neolignan, phenylpropanoid and iridoid glycosides from *Pedicularis verticillata*. *Phytochem.*, 45, 1271-1273 (1997).