Bioactive Phenolic Constituents from the Seeds of *Pharbitis nil*

Ki Hyun Kim,[a] Sang Keun Ha,[b,c] Sang Un Choi,[d] Sun Yeou Kim,[b,e] and Kang Ro Lee*,[a]

[a] *Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University; Suwon 440–746, Korea:* [b] *Graduate School of East-West Medical Science, Kyung Hee University; Yongin 449–701, Korea:* [c] *Korea Food Research Institute; Sungnam 463–746, Korea:* [d] *Korea Research Institute of Chemical Technology; Teajeon 305–600, Korea: and* [e] *East-West Integrated Medical Science Research Center, Kyung Hee University Global Campus; Yongin 446–701, Korea.*

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Two new lignans, termed pharsyringaresinol (1) and pharbilignoside (2), a new phenylethanoid glycoside, termed pharbiniloside (3), and 22 known compounds, were isolated from the ethanol extract of the seeds of *Pharbitis nil*. The structures of the new compounds (1—3) were determined on the basis of spectroscopic analyses, including 2D-NMR and circular dichroism (CD) spectroscopy studies. Among the isolates, compounds 2, 11, 12, and 24 exhibited significant cytotoxicity against human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) with IC_{50} values ranging from 8.07 to 28.30 μM. In addition, compounds 11, 12 and 24 potently inhibited nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells, a microglia cells with IC_{50} values ranging from 14.7 to 19.9 μM.

Key words *Pharbitis nil*; Convolvulaceae; phenolic; cytotoxicity; neuroinflammation

Pharbitidis Semen, the seeds of *Pharbitis nil* (Convolvulaceae), comes from the pharbitis (also known as the morning glory), an annual climbing herb found throughout Southeast Asia that many people use as an ornamental plant. Pharbitidis Semen and its resin have traditionally been used as a purgative drug in Korea, China, and Japan.[1] In Chinese traditional medicine, it has been used as a folk medicine for its analgesic effects against abdominal pain and for the treatment of a variety of digestive problems.[2] The seeds of *P. nil* are very well known for their diversified chemistry, particularly for the presence of resin glycosides and gibberellins.[3—6] Moreover, there have been several reports on the isolation of flavonoids,[7] chlorogenic acid derivatives,[7] anthocyanins,[8,9] a diterpenoid,[10] and triterpene saponins[11] from the seeds and flowers of this plant, but there has been no report on the chemical investigation of lignan derivatives in the seeds. Seeds of this plant have shown antitumor, anti-fungal, and gastroprokinetic effects.[2,12,13] As a part of our continuing

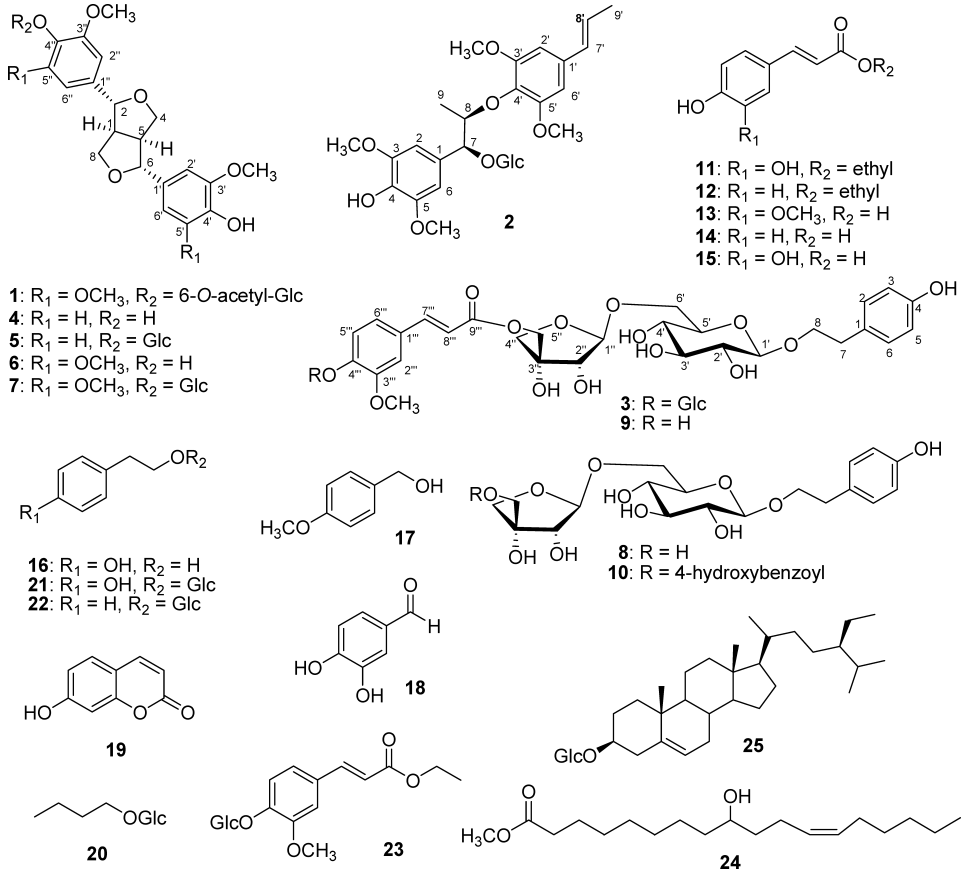

Fig. 1. The Chemical Structures of Compounds **1**—**25** from *Pharbitis nil*

* To whom correspondence should be addressed. e-mail: krlee@skku.ac.kr

study of the seeds of *P. nil*,[14,15] our interest in the further research of active metabolites from this source led us to investigate the seeds and allowed the isolation and identification of 25 constituents, including two new lignans, a new phenylethanoid glycoside, and 22 known compounds (Fig. 1).

Results and Discussion

Pharsyringaresinol (**1**) was isolated as a colorless gum and possessed a molecular formula of $C_{30}H_{38}O_{14}$ (12 of unsaturation) as deduced from the positive mode high resolution-electrospray ionization-mass spectrometry (HR-ESI-MS) data at *m/z* 623.2342 $[M+H]^+$ (Calcd for $C_{30}H_{39}O_{14}$, 623.2340). The UV and IR spectra of **1** were similar to those of **7**. Moreover, the ^{1}H- and ^{13}C-NMR spectra (Table 1) of **1** were almost identical to those of **7**,[16] except for the presence of signals for an additional acetyl group (δ_H 1.94; δ_C 172.8, 20.8). The lignan skeleton of **1** was confirmed by analysis of 2D-NMR data (Fig. 2). The heteronuclear multiple bond correlation (HMBC) correlation of H-1‴ (δ_H 4.81)/C-4″ (δ_C 135.4) indicated that the glucosyl unit was linked to the oxygen at C-4″. The downfield shift of the signal at C-6‴ ($\Delta\delta_C$ +2.0) in **1**, compared to the corresponding signal of **7**, suggested that the acetyl group should be linked to C-6‴ of the glucose unit. This deduction was confirmed by the HMBC correlation between H-6‴ (δ_H 4.28, 4.24) and a carbonyl carbon (δ_C 172.8). Thus, the structure of **1** was determined to be (−)-syringaresinol 4-*O*-(6‴-*O*-acetyl)-*β*-D-glucopyranoside.

Pharbilignoside (**2**), a colorless gum, exhibited a molecular ion peak at *m/z* 567.2440 $[M+H]^+$ (Calcd for $C_{28}H_{39}O_{12}$, 567.2442) in a positive HR-ESI-MS analysis, corresponding to a molecular formula of $C_{28}H_{38}O_{12}$ (10 of unsaturation). The ^{1}H- and ^{13}C-NMR spectroscopic data (Table 1) of **2** were similar to those of *rel*-(7*S*,8*R*)-Δ^7-4,7-dihydroxy-3,5,3′,5′-tetramethoxy-8-*O*-4′-neolignan,[17] except for the presence of signals for a glucopyranosyl moiety (δ_H 4.13, 3.87—3.10; δ_C 100.4, 78.0, 78.0, 75.2, 71.9, 63.0). In the ^{1}H-NMR spectrum, the anomeric proton at δ_H 4.13 (1H, d, *J*=7.5 Hz) suggested the presence of a *β*-glucopyranosyl unit. Based on analysis of the 2D-NMR spectra, assignments for all proton and carbon signals were achieved (Table 1), and the position of the glucosyl group was confirmed to be C-7 by the HMBC correlation of H-1″ (δ_H 4.13)/C-7 (δ_C 82.2) (Fig. 2). Acidic hydrolysis of **2** afforded its aglycone **2a** and D-glucose. Its aglycone was identified by ^{1}H-NMR and MS data.[17] In the ^{1}H-NMR spectrum of its aglycone **2a**, a small coupling constant of H-7 (*J*=4.5) was observed. This was in good agreement with data of the *erythro*-derivatives, and indicated that the relative configuration of C-7 and C-8 of **2** was in the *erythro*-form.[17,18] Furthermore, the circular dichroism (CD)

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1** and **2** in CD_3OD[a]

Position	1		Position	2	
	δ_H	δ_C		δ_H	δ_C
1	3.14 br m	55.6	1		129.6
2	4.78 d (4.0)	87.3	2/6	6.81 s	107.5
4	4.30 dd (15.0, 9.0)	73.0	3/5		148.9
	3.92 dd (9.0, 4.0)				
5	3.14 br m	55.8	4		136.1[b]
6	4.74 d (4.0)	87.7	7	5.00 d (4.5)	82.2[c]
8	4.30 dd (15.0, 9.0)	72.9	8	4.55 m	82.3[c]
	3.92 dd (9.0, 4.0)				
1′		133.2	9	1.18 d (6.5)	16.0
2′/6′	6.67 s	104.71	1′		133.0
3′/5′		149.5	2′/6′	6.60 s	104.5
4′		136.3	3′/5′		154.8
1″		139.7	4′		136.2[b]
2″/6″	6.72 s	104.78	7′	6.31 d (16.0)	132.2
3″/5″		154.7	8′	6.17 dd (16.0, 6.5)	126.4
4″		135.4	9′	1.85 d (6.5)	18.6
3′/5′-OCH_3	3.85 s	57.0	3/5-OCH_3	3.83 s	56.9
3″/5″-OCH_3	3.86 s	57.1	3′/5′-OCH_3	3.76 s	56.7
Glc			Glc		
1‴	4.81 d (7.5)	105.1	1″	4.13 d (7.5)	100.4
2‴	3.36 m	75.6	2″	3.35 m	75.2
3‴	3.42 m	77.8	3″	3.26 m	78.0
4‴	3.35 m	71.8	4″	3.27 m	71.9
5‴	3.50 m	75.6	5″	3.10 m	78.0
6‴	4.28 dd (12.0, 2.5)	64.6	6″	3.87 br d (11.5)	63.0
	4.24 dd (12.0, 5.5)			3.68 dd (11.5, 4.0)	
OAc	1.94 s	20.8			
		172.8			

a) ^{1}H- and ^{13}C-NMR data were recorded at 500 and 125 MHz, respectively. Coupling constants (in Hz) are given in parentheses. *b*, *c*) May be interchanged.

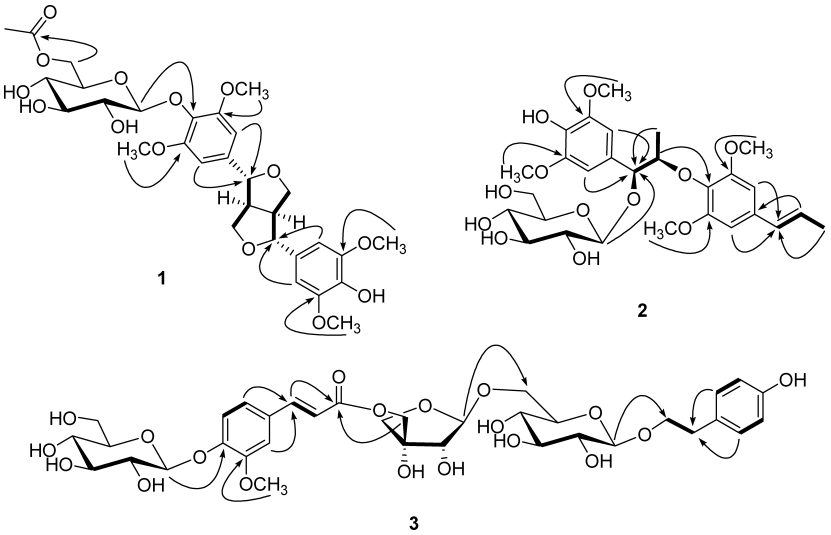

Fig. 2. Key ^{1}H–^{1}H COSY (——) and HMBC (⟶) Correlations of **1**—**3**

Table 2. ^{1}H- and ^{13}C-NMR Data of Compound **3** in CD_3OD[a)]

Position	3	
	δ_H	δ_C
1		130.8
2/6	7.05 d (8.5)	131.0
3/5	6.69 d (8.5)	116.2
6		156.9
7	2.82 t (7.5)	36.5
8	3.98 dd (9.5, 7.5)	72.3
	3.69 dd (9.5, 7.5)	
Glc 1′	4.28 d (8.0)	104.5
2′	3.18 m	75.2
3′	3.33 m	78.1
4′	3.29 m	71.8
5′	3.39 m	77.0
6′	4.01 dd (11.5, 3.0)	68.8
	3.62 dd (11.5, 5.5)	
Api 1″	5.04 d (2.0)	110.7
2″	3.98 m	78.4
3‴		80.6
4″	4.05 d (10.0)	74.9
	3.84 d (10.0)	
5″	4.26 d (11.5)	67.7
	4.24 d (11.5)	
1‴		130.6
2‴	7.25 d (2.0)	112.6
3‴		151.1
4‴		150.2
5‴	7.14 d (8.0)	117.5
6‴	7.17 d (8.0, 2.0)	124.3
7‴	7.66 d (16.5)	146.2
8‴	6.45 d (16.5)	117.2
9‴		169.5
3‴-OCH_3	3.90 s	56.9
Glc 1⁗	4.96 d (7.5)	102.4
2⁗	3.35 m	75.2
3⁗	3.25 m	78.1
4⁗	3.27 m	71.4
5⁗	3.18 m	78.0
6⁗	3.91 dd (11.5, 3.5)	62.6
	3.75 dd (11.5, 5.5)	

a) ^{1}H- and ^{13}C-NMR data were recorded at 500 and 125 MHz, respectively. Coupling constants (in Hz) are given in parentheses.

spectrum ($[\theta]_{237}$ −6120, $[\theta]_{275}$ −670) of **2a** established the absolute configuration of **2** to be 7*S*,8*R* as shown in Fig. 1 ($[\theta]_{242}$ −14870, $[\theta]_{276}$ −2870 for **2**).[18)] According to the above analysis, the structure of **2** was elucidated as shown in Fig. 1.

Pharbiniloside (**3**) was isolated as a colorless gum. It had the molecular formula $C_{35}H_{46}O_{19}$ with 13 of unsaturation, as confirmed by HR-ESI-MS *m/z* 771.2716 $[M+H]^+$ (Calcd for $C_{35}H_{47}O_{19}$, 771.2712). The UV spectrum of **3** showed absorption maxima at 220, 285, and 314 nm, indicating the presence of a feruloyl moiety in **3**.[19)] The ^{1}H- and ^{13}C-NMR spectral data (Table 2) of **3** were similar to those of **9**,[19)] except for the presence of signals for an additional glucopyranosyl moiety (δ_H 4.96, 3.91—3.18; δ_C 102.4, 78.1, 78.0, 75.2, 71.4, 62.6). The assignments of all the proton and carbon signals were established on the basis of analysis of the 2D-NMR data (Table 2). The glucose moieties were determined to have a β-configuration at C-1′ and C-1⁗ due to a large coupling constant for the anomeric proton of the sugar units at δ_H 4.28 (1H, d, J=8.0 Hz) and 4.96 (1H, d, J=7.5 Hz), respectively and the apiose unit was also determined to have a β-configuration at C-1″ due to a coupling constant of H-1″ (J=2.0 Hz) and the chemical shift of its anomeric carbon signal in the ^{13}C-NMR at δ_C 110.7.[19)] Furthermore, the D-glucose and D-apiose were identified by GC analysis of their chiral derivatives in the acidic hydrolysate.[20,21)] The HMBC spectrum showed the correlation of the apiosyl anomeric proton H-1″ (δ_H 5.04) with the glucosyl C-6′ carbon (δ_C 68.8) and correlation of the apiosyl H_2-5″ (δ_H 4.26, 4.24) with C-9‴ (δ_C 169.5) of the *trans*-feruloyl group, respectively (Fig. 2). In addition, a correlation between the other glucosyl anomeric proton H-1⁗ (δ_H 4.96) and C-4‴ (δ_C 150.2) was observed in the HMBC spectrum, indicating that the glucosyl unit was attached to C-4‴ of the *trans*-feruloyl group in **3** (Fig. 2). Thus, the structure of **3** was determined as depicted in Fig. 1.

The known compounds were identified as (−)-pinoresinol (**4**),[22)] (−)-pinoresinol 4-*O*-β-D-glucopyranoside (**5**),[23)] (−)-syringaresinol (**6**),[16)] (−)-syringaresinol 4-*O*-β-D-glucopyranoside (**7**),[16)] osmanthuside H (**8**),[19)] osmanthuside J (**9**),[19)] 2-(4-hydroxyphenyl)ethyl 1-*O*-β-D-[5-*O*-(4-hydroxybenzoyl)]-apiofuranosyl-(1→6)-β-D-glucopyranoside (**10**),[24)] (*E*)-ethyl caffeate (**11**),[25)] (*E*)-*p*-ethyl coumarate (**12**),[25)] (*E*)-ferulic acid (**13**),[25)] (*E*)-*p*-coumaric acid (**14**),[25)] (*E*)-caffeic acid (**15**),[25)] 2-(*p*-hydroxyphenyl)-ethanol (**16**),[19)] *p*-methoxybenzylalcohol (**17**),[26)] 3,4-dihydroxybenzaldehyde (**18**),[27)] umbelliferone (**19**),[28)] *n*-butyl β-D-glucopyranoside (**20**),[29)] 2-(*p*-hydroxyphenyl)-ethanol 1-*O*-β-D-glucopyranoside (**21**),[19)] 2-phenylethyl β-D-glucopyranoside (**22**),[30)] (*E*)-ethyl ferulate 4-*O*-β-D-glucopyranoside (**23**),[31)] isoricinoleic acid methyl ester (**24**),[32)] and sitosteryl β-D-glucoside (**25**),[33)] by comparison of their spectroscopic data with previously reported values. To the best of our knowledge, a group of lignans (**1**, **2**, **4**—**7**), including two new ones, was isolated for the first time from *P. nil* and compounds **8**—**12**, **16**, **17**, and **20**—**24** were isolated from this plant for the first time.

The cytotoxic activities of the isolated compounds **1**—**25** were evaluated by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) using the sulforhodamine B (SRB) bioassay.[34)] Compounds **2**, **11**, **12**, and **24** exhibited cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT15 cells with IC_{50} values ranging from 8.07 to 28.30 μM (Table 3), but the other compounds were inactive (IC_{50}: >30.0). Particularly, compound **24** showed significant cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines with IC_{50} values of 10.83, 16.36, 11.17, and 8.07 μM, respectively. It appears that the ethyl ester group in cinnamic acid derivatives is essential to cytotoxicity, as compounds **11** and **12** with the above function showed cytotoxicity, but other compounds **13**—**15** were inactive, though more related compounds need to be tested to confirm this hypothesis. But, the sugar unit in cinnamic acid derivatives seems to have a negative effect on cytotoxicity, since compound **23** with a glucose moiety was not active though the compound possessed an ethyl ester group.

Despite the fact that there are no known folkloric reports of use of this herb in the management or cure of neurodegenerative diseases, because of several reports on the anti-neuroinflammatory activity of phenolic compounds including lignan derivatives,[35,36)] we also evaluated the isolates (**1**—**25**) for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells, a microglial

Table 3. Cytotoxicity of Compounds **2**, **11**, **12**, and **24** against Four Cultured Human Tumor Cell Lines in the SRB Bioassay

Compound	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
2	27.12	>30.0	28.30	>30.0
11	>30.0	18.12	>30.0	23.55
12	27.93	15.84	19.27	16.07
24	10.83	16.36	11.17	8.07
Doxorubicin[b]	0.010	0.001	0.001	0.028

a) 50% inhibitory concentration; the concentration of the compound that caused a 50% inhibition of cell growth. *b*) Doxorubicin as a positive control.

Table 4. Inhibitory Activities of Isolated Compounds **1**, **6**, **11**, **12**, and **24** on the NO Production in LPS-Activated BV-2 Cells

Compounds	Inhibition (%)[a]	IC_{50} (μM)
1	21.4	<50
6	25.7	<50
11	65.3	14.7
12	53.3	17.9
24	50.2	19.9
L-NMMA	54.2	17.7

a) Values indicate the inhibition of NO production relative to the LPS control at 20 μM concentration of each compound (n=3).

cell line. Among the tested compounds, compounds **1**, **6**, **11**, **12**, and **24** showed significant inhibitory effects on LPS-stimulated NO production without cytotoxicity in BV-2 microglial cells at 20 μM (Table 4). Compound **11** was more potent than N^G-monomethyl-L-arginine (L-NMMA), an inducible NO synthase (iNOS) inhibitor, in inhibiting NO production with an IC_{50} of 14.7 μM. The rest of the compounds did not show any significant inhibitory effects on NO production in range from 1 to 20 μM concentration.

Experimental

General Experimental Procedures Optical rotations were measured on a Jasco P-1020 polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. CD spectra were measured on a Jasco J-715 spectropolarimeter. UV spectra were recorded with a Shimadzu UV-1601 UV–visible spectrophotometer. ESI and HR-ESI mass spectra were recorded on a SI-2/LCQ DecaXP Liquid chromatography (LC)-mass spectrometer. NMR spectra, including ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and HMBC experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), with chemical shifts given in ppm (δ). Preparative high-performance liquid chromatography (HPLC) was performed using a Gilson 306 pump with a Shodex refractive index detector. Silica gel 60 (Merck, 230—400 mesh) and RP-C_{18} silica gel (Merck, 230—400 mesh) were used for column chromatography. Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates were used for thin layer chromatography (TLC). Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.).

Plant Materials The seeds of *P. nil* were purchased at Kyungdong herbal market, Seoul, Korea, in July 2006, and were identified by one of the authors (K.R.L.). A voucher specimen (SKKU 2006-7) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation The dried seeds (10 kg) of *P. nil* were extracted with 50% EtOH (3×4 l, on each of 3 d) at room temperature and filtered. The filtrate was evaporated *in vacuo* to obtain the EtOH extract (1.4 kg), which was suspended in distilled H_2O (7.2 l) and then successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, to yield 10, 7, 10, and 550 g of dried organic extracts, respectively. Extracts were tested for cytotoxic activity, and a bioactivity-guided fractionation method was used for isolation work. On the basis of the bioactivity of the extracts, the most active EtOAc-soluble fraction (10 g) was chromatographed on a silica gel (230—400 mesh, 300 g) column and eluted with $CHCl_3$/MeOH (10 : 1→1 : 1, gradient system) to yield six fractions (A—F). Fraction A (200 mg) was purified by semi-preparative reversed-phase HPLC, using a 250 mm×10 mm i.d., 10 μm, Econosil RP-18 column with a solvent system of 90% MeOH to obtain **4** (5 mg), **6** (6 mg), **24** (5 mg), and **25** (4 mg). Fraction B (2.5 g) was chromatographed further on an RP-C_{18} silica gel (230—400 mesh, 150 g) column and eluted with MeOH/H_2O (3 : 2→4 : 1, gradient system) to give seven subfractions (B1—B7). Fraction B1 (90 mg) was purified by semi-preparative normal-phase HPLC, using a 250 mm×10 mm i.d., 5 μm, Apollo Silica column with a solvent system of $CHCl_3$/MeOH (18 : 1) to give **16** (25 mg) and **18** (30 mg). Fraction B2 (200 mg) was subjected to low-pressure liquid chromatography (LPLC) on a 240 mm×10 mm i.d., 40—63 μm, LiChroprep Lobar-A RP-18 column using 25% MeCN, to give two subfractions (B21—B22). Fraction B21 (80 mg) was purified by semi-preparative normal-phase HPLC, using a solvent system of $CHCl_3$/MeOH (20 : 1) to give **13** (50 mg) and **14** (6 mg). Fraction B22 (20 mg) was also purified by semi-preparative normal-phase HPLC, using a solvent system of $CHCl_3$/MeOH (18 : 1) to obtain **1** (6 mg). Fraction B3 (30 mg) was purified by semi-preparative reversed-phase HPLC, using 28% MeCN to afford **19** (5 mg). Compound **11** (65 mg) was purified from fraction B5 (700 mg) by semi-preparative reversed-phase HPLC (40% MeCN) and compound **12** (8 mg) was obtained from fraction B6 (40 mg) by semi-preparative reversed-phase HPLC (45% MeCN). Fraction C (2.7 g) was chromatographed on an RP-C_{18} silica gel (230—400 mesh, 150 g) column and eluted with MeOH/H_2O (3 : 2→1 : 0, gradient system) to furnish eight subfractions (C1—C8). Fraction C1 (250 mg) was subject to LPLC on a LiChroprep Lobar-A RP-18 column (20% MeCN), and further purified by semi-preparative normal-phase HPLC ($CHCl_3$/MeOH, 12 : 1) to give **17** (7 mg). Fraction D (2.5 g) was applied to an RP-C_{18} silica gel column chromatography (60% MeOH) to yield six subfractions (D1—D6). Fraction D1 (250 mg) was subject to LPLC on a LiChroprep Lobar-A RP-18 column (23% MeCN), and further purified by semi-preparative reversed-phase HPLC (30% MeCN) to afford **7** (5 mg) and **15** (10 mg). Fraction D2 (340 mg) was passed through a Sephadex LH-20 column eluted with 100% MeOH, and further purified by semi-preparative reversed-phase HPLC (30% MeCN) to give **23** (7 mg). Fraction D3 (40 mg) was purified by semi-preparative reversed-phase HPLC (30% MeCN) to give **20** (5 mg). Fraction D6 (300 mg) was passed through a Sephadex LH-20 column (100% MeOH), and further purified by semi-preparative reversed-phase HPLC (50% MeCN) to obtain **2** (4 mg). Fraction E (3.0 g) was chromatographed further on an RP-C_{18} silica gel (230—400 mesh, 150 g) column and eluted with MeOH/H_2O (1 : 1→1 : 0, gradient system) to give 11 subfractions (E1—E11). Fraction E1 (250 mg) was passed through a Sephadex LH-20 column (80% MeOH), and further purified by semi-preparative reversed-phase HPLC (20% MeCN) to yield **21** (5 mg). Fraction E2 (200 mg) was subject to LPLC on a LiChroprep Lobar-A RP-18 column (20% MeCN), and further purified by semi-preparative reversed-phase HPLC (25% MeCN) to give **5** (14 mg) and **10** (20 mg). Fraction E3 (50 mg) was subject to LPLC on a LiChroprep Lobar-A RP-18 column (25% MeCN), and further purified by semi-preparative normal-phase HPLC ($CHCl_3$/MeOH, 9 : 1) to afford **22** (15 mg). Fraction E4 (150 mg) was passed through a Waters Sep-Pak Vac 6 cc (Waters, Milford, MA, U.S.A.) (30% MeCN), and then, was subject to LPLC on a LiChroprep Lobar-A RP-18 column (30% MeCN), and further purified by semi-preparative normal-phase HPLC ($CHCl_3$/MeOH, 7 : 1) to give **9** (40 mg). Fraction F (3.6 g) was passed through a Sephadex LH-20 column and eluted with CH_2Cl_2/MeOH (1 : 1) to obtain four subfractions (F1—F4). Fraction F2 (1.6 g) was chromatographed further on an RP-C_{18} silica gel (230—400 mesh, 150 g) column and eluted with MeOH/H_2O (2 : 3→3 : 2, gradient system) to give five subfractions (F21—F25). Finally, fraction F24 (150 mg) was purified by semi-preparative reversed-phase HPLC (20% MeOH) to give **3** (6 mg) and **8** (10 mg).

Pharsyringaresinol (**1**): Colorless gum; $[\alpha]_D^{25}$ −18.0 (c=0.10, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.82), 280 (3.40) nm; IR (KBr) ν_{max} 3385, 2949, 2835, 1743, 1660, 1615, 1451, 1354, 1032, 670 cm^{-1}; ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data, see Table 1; ESI-MS (positive-ion mode) m/z: 623 $[M+H]^+$. HR-ESI-MS (positive-ion mode) m/z: 623.2342 $[M+H]^+$ (Calcd for $C_{30}H_{39}O_{14}$, 623.2340).

Pharbilignoside (**2**): Colorless gum; $[\alpha]_D^{25}$ −13.2 (c=0.10, MeOH); UV (MeOH) λ_{max} (log ε) 233 (3.98), 265 (3.25) nm; CD (MeOH) λ_{max} $[\theta]_{242}$ −14870, $[\theta]_{276}$ −2870; IR (KBr) ν_{max} 3356, 2947, 2833, 1608, 1451, 1354, 1033, 669 cm^{-1}; ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data, see Table 1; ESI-MS (positive-ion mode) m/z: 567 $[M+H]^+$. HR-ESI-MS (positive-ion

mode) *m/z*: 567.2440 $[M+H]^+$ (Calcd for $C_{28}H_{39}O_{12}$, 567.2442).

Pharbiniloside (**3**): Colorless gum; $[\alpha]_D^{25}$ −30.6 (c=0.15, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.24), 285 (3.98), 314 (3.76) nm; IR (KBr) ν_{max} 3360, 2948, 2843, 1720, 1629, 1595, 1452, 1378, 1273, 1033, 669 cm^{-1}; ^1H-(500 MHz) and ^{13}C- (125 MHz) NMR data, see Table 2; ESI-MS (positive-ion mode) *m/z*: 771 $[M+H]^+$. HR-ESI-MS (positive-ion mode) *m/z*: 771.2716 $[M+H]^+$ (Calcd for $C_{35}H_{47}O_{19}$, 771.2712).

Acid Hydrolysis of 1—3 and Sugar Analysis Solutions of **1** (3 mg) and **2** (2 mg) in 2 N HCl were heated (90 °C) for 2 h. After cooling, each reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer in each case was evaporated to dryness, and the residue was individually chromatographed by a silica gel Waters Sep-Pak Vac 6 cc ($CHCl_3$/MeOH, 30 : 1) to give each aglycone part, (−)-syringaresinol (**1a**) and *rel*-(7*S*,8*R*)-Δ^7-4,7-dihydroxy-3,5,3′,5′-tetramethoxy-8-*O*-4′-neolignan (**2a**), which was identified by comparison with ^1H-NMR, MS, and $[\alpha]_D$ in the literature.[16,17] **2a**: CD (MeOH) λ_{max} $[\theta]_{237}$ −6120, $[\theta]_{275}$ −670. Compound **3** (3 mg) was hydrolyzed by 1 N HCl (dioxane/H_2O, 1 : 1, 5 ml) under reflux conditions for 3 h. After cooling, the reaction mixture was diluted with H_2O and extracted with $CHCl_3$. A sample of the aqueous layer from each (**1**—**3**) was neutralized by passage through an Amberlite IRA-67 column and was repeatedly evaporated under reduced pressure to give each sugar fraction. The sugars in the fraction were analyzed by silica gel TLC by comparison with authentic samples. The solvent system was $CHCl_3$/MeOH/H_2O (8 : 5 : 1). Spots were visualized by spraying with 95% EtOH/H_2SO_4/anisaldehyde (9 : 0.5 : 0.5), then heated at 120 °C for 3 min. The *Rf* of glucose and apiose were 0.30 and 0.45, respectively for sugars of **1**—**3**. For GC analysis, each sugar fraction was dissolved in anhydrous pyridine (100 μl), and 0.1 M L-cysteine methyl ester hydrochloride in anhydrous pyridine (200 μl) was added.[20,21] The mixture was stirred at 60 °C for 1 h. Then 150 μl of HMDS/TMCS (hexamethyldisilazane/trimethychlorosilane/pyridine, 3 : 1 : 10) was added, and the mixture was stirred at 60 °C for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under an N_2 stream. The residue was partitioned between *n*-hexane and H_2O (0.1 ml each), and the hexane layer (1 μl) was analyzed by GC experiment. D-Glucose and D-apiose were detected by co-injection of the hydrolysate with standard silylated samples (D-glucose: 11.38 min; L-glucose: 12.62 min; D-apiose: 5.08; L-apiose: 5.65). The retention times of sugars obtained by acid hydrolysis were D-glucose (11.36 min) for **1**, D-glucose (11.32 min) for **2**, and D-glucose (11.41 min) and D-apiose (5.05 min) for **3**.

Cytotoxicity Assay A sulforhodamine B (SRB) bioassay was used to determine the cytotoxicity of each compound isolated against four cultured human tumor cell lines.[34] The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). Doxorubicin was used as a positive control. The cytotoxicities of doxorubicin against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines were IC_{50} 0.010, 0.001, 0.001, and 0.028 μM, respectively.

Measurement of NO Production and Cell Viability Inhibition of NO production was evaluated in lipopolysaccharide (LPS)-activated murine microglia BV-2 cells. Cells were stimulated with 100 ng/ml of LPS in the presence or absence of samples for 24 h. Nitrite in the culture media, a soluble oxidation product of NO, was determined using the Griess reaction.[35] Cell viability was measured using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.[37] N^G-monomethyl-L-arginine (L-NMMA, Sigma), a NOS inhibitor, was tested as a positive control.

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