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Bioactive lignan derivatives from the stems of *Firmiana simplex*

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ABSTRACT

The $CHCl_3$ soluble fraction of the 80% MeOH extract of the stems of *Firmiana simplex* strongly inhibited nitric oxide production in lipopolysaccharide-activated BV-2 cells. A bioactivity-guided column chromatographic separation yielded two new lignans, firmianols A and B (**1**–**2**) together with seventeen known lignans (**3**–**19**). The structural elucidation of the new compounds was determined by spectroscopic methods, including 1D, 2D NMR and HR-FAB-MS. All isolated lignans were evaluated for their antineuroinflammatory effects on nitric oxide (NO) production in lipopolysaccharides (LPS)-activated murine microglia BV2 cells. Among the isolated, compounds **14** and **15** showed potent inhibitory activity against NO production (IC_{50} 1.05 and 0.929 μM, respectively) without cell toxicity in murine microglia BV-2 cells. Compounds **11**–**13** and **17** also exhibited strong inhibitory effects on NO production, with IC_{50} values ranging from 7.07 to 15.28 μM.

Neuroinflammation plays a central role in most neurodegenerative diseases such as Parkinson's disease, Alzheimer disease, multiple sclerosis, and stroke, and is mediated by microglial activation.[1] Microglia cells exist in the CNS and are the major targets of microgliosis following neurodegeneration.[2] Following microglial activation due to injury in the brain, excessive NO is produced, which initiates a cascade of neuroinflammatory responses.[3] Therefore, discovering neuroprotective drugs that inhibit NO production via activated microglia is crucial in treating neurodegenerative diseases.

In our continuing search for neuroinflammatory components from Korean medicinal plants,[4–8] the $CHCl_3$-soluble fraction of *Firmiana simplex* was found to strongly inhibit nitric oxide production in lipopolysaccharide-activated BV-2 cells.

F. simplex (Sterculiaceae) is a deciduous tree that is distributed in Korea and China.[9] It is called a Chinese parasol tree, which is characterized by large stems and leaves, and is popular as an ornamental plant.[10] *F. simplex* seeds have been used to treat diarrhea and stomach disorders.[11] Recently, Our earlier phytochemical investigation on *F. simplex* resulted in the isolation of cytotoxic triterpenes.[12] Using bioactivity-guided isolation techniques, nineteen lignan derivatives including two new lignans (**1**–**2**) were further isolated from the most active $CHCl_3$-soluble fraction (Fig. 1). In the present study, we report the isolation and structural elucidation of compounds **1**–**19** and their NO production activity.

Compound (**1**) was obtained as a colorless gum. The molecular formula of **1** was determined to be $C_{19}H_{18}O_6$ by the negative mode HR-FABMS data at *m*/*z* 341.1020 $[M-H]^-$ (calcd for $C_{19}H_{17}O_6$, 341.1020). The ^1H NMR spectrum (Table 1) of **1** showed signals of 1,3,4-trisubstitued-aromatic ring protons at δ_H 6.99 (1H, d, *J* = 1.5 Hz, H-2), 6.92 (1H, d, *J* = 7.5 Hz, H-6), and 6.81 (1H, dd, *J* = 7.5, 1.5 Hz, H-5), of 1,4-disubstitued-aromatic protons at δ_H 7.27 (2H, d, *J* = 9.0 Hz, H-2′, 6′) and 6.80 (2H, d, *J* = 9.0 Hz, H-3′, 5′) of dioxymethylene at 5.95 (2H, s, —OCH_2O—), of two oxygenated methines at 4.86 (1H, d, *J* = 5.5 Hz, H-7) and 4.69 (1H, s, H-7′), of four oxygenated methylenes at δ_H 4.46 (1H, t, *J* = 9.0 Hz, H-9a), 4.03 (1H, d, *J* = 9.0 Hz, H-9′a), 3.87 (1H, d, *J* = 9.0 Hz, H-9′b), and 3.78 (1H, dd, *J* = 8.5, 5.5 Hz, H-9b), of one methine proton at 3.00 (1H, dt, *J* = 7.5, 3.5 Hz, H-8).

The ^{13}C NMR spectrum (Table 2) revealed resonances for 19 carbons attributable to twelve aromatic carbons, one dioxymethylene carbon (δ_C 102.4), five oxygenated carbons (δ_C 92.7, 89.2, 87.5, 76.3, and 72.1), and one methine carbons (δ_C 62.6). The ^1H and ^{13}C NMR spectra of **1** were very close to those of (+)-beechenol, which was isolated from *Zanthoxylum beecheyanum*,[13] except that the proton and carbon signal of methine (H-8′) in (+)-beechenol were absent, and instead, the resonances of oxygenated carbon at δ_C 92.7 was present in **1**. The gross planar structure of **1** was confirmed by analysis of 2D NMR experiments (^1H–^1H COSY, HMQC, and HMBC) (Fig. 2).

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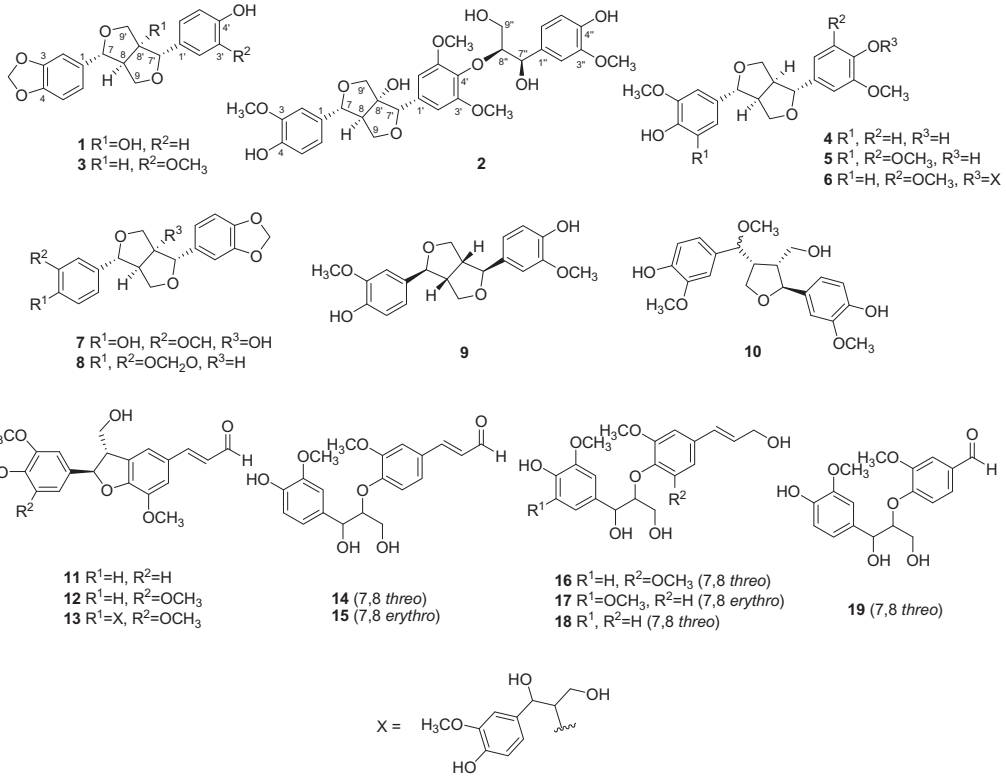

Figure 1. Chemical structures of compounds **1**–**19** from *F. simplex*.

Table 1
[1]H NMR data of compounds **1** and **2** (CD_3OD, 700 MHz, δ in ppm, J in Hz)[a]

Position	1		2	
	δ_H	HMBC	δ_H	HMBC
1	—		—	
2	6.99 (d, 2.0)	4, 6, 7	7.08 (d, 2.0)	4, 6, 7
3	—		—	
4	—		—	
5	6.81 (d, 9.0)	1, 3	6.80 (d, 8.0)	1, 3
6	6.92 (dd, 8.5, 1.5)	2, 3, 7	6.90 (dd, 8.0, 2.0)	2, 3, 7
7	4.86 (d, 5.5)	2, 6, 8, 9, 8′, 9′	4.8 (overlap)	2, 6, 8, 9, 8′, 9′
8	3.00 dt (7.5, 5.5)	1, 8′	3.07 m	1, 8′
9a	4.46 (t, 9.0)	7, 8, 7′, 8′	4.51 (t, 9.0)	7, 8, 7′, 8′
9b	3.78 (dd, 8.5, 5.5)		3.81 (dd, 9.0, 6.0)	
1′	—		—	
2′	7.27 (d, 9.0)	2′, 3′, 4′, 7′	6.80 s	4′, 6′, 7′
3′	6.80 (d, 9.0)	1′, 3′, 4′	—	
4′	—		—	
5′	6.80 (d, 9.0)	1′, 3′, 4′	—	
6′	7.27 (d, 9.0)	2′, 3′, 4′, 7′	6.80 s	2′, 4′, 7′
7′	4.69 s	8, 9, 2′, 8′, 9′	4.75 s	9, 1′, 2′, 8′, 9′
8′	—		—	
9′a	4.03 (d, 9.0)	7, 8, 7′, 8′	4.14 (d, 9.0)	7, 8, 7′, 8′
9′b	3.87 (d, 9.0)		3.91 (d, 9.0)	
1″			—	
2″			7.03 br s	4″, 6″, 7″
3″			—	
4″			—	
5″			6.77 (d, 8.0)	1″, 3″
6″			6.82 (overlap)	2″, 4″, 7″
7″			4.97 (d, 5.0)	1″, 2″, 6″, 8″, 9″
8″			4.24 m	4′
9″a			3.90 m	7″
9″b			3.58 (dd, 12.0, 3.5)	
3-OCH_3			3.88 s	3
3′-OCH_3			3.87 s	3′
3″-OCH_3			3.86 s	3″
-OCH_2O-	5.95 s	3	—	

[a] Assignments were based on HMQC, and HMBC experiments.

The configuration of **1** was determined on the basis of the NOESY correlations [H-7/H-9b, H-8/H-9a, H-9b/H-7′, H-7′/H-9′a] (Fig. 3) and positive optical rotation value ($[\alpha]_D^{25}$ +18.0, CH_3OH) in comparison to (+)-syringaresinol.[14] Thus, the structure of **1** was established to be (+)-8′-hydroxybeechenol, and was named firmianol A.

Compound (**2**) was obtained as a colorless gum. The molecular formula of **2** was determined to be $C_{31}H_{35}O_{12}$ by the positive mode HR-FABMS data at m/z 623.2100 $[M+Na]^+$ (calcd for $C_{31}H_{35}O_{12}Na$, 623.2099). Its NMR spectra were analogous to those of (+)-1-hydroxypinoresinol, which was isolated from *Saussurea pulchella*,[15]

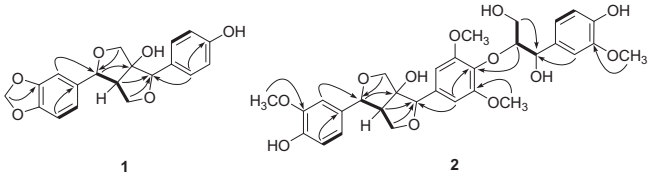

Figure 2. Key COSY (bold line) and HMBC (arrow) correlations of **1** and **2**.

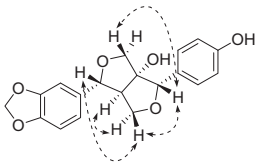

Figure 3. Key NOESY correlations of **1**.

Table 2
^{13}C NMR data of compounds **1** and **2** (CD_3OD, 175 MHz, δ in ppm, J in Hz)[a]

δ_C	1	2
1	136.5	133.7
2	107.8	111.2
3	149.5	149.1
4	148.8	147.4
5	109.0	116.0
6	121.0	120.4
7	87.5	87.8
8	62.6	62.6
9	72.1	72.3
1′	128.5	134.2
2′	130.9	106.2
3′	115.8	154.3
4′	158.5	136.3
5′	115.8	154.3
6′	130.9	106.2
7′	89.2	89.2
8′	92.7	93.2
9′	76.3	76.2
1″		133.9
2″		111.2
3″		148.7
4″		146.8
5″		115.7
6″		120.4
7″		74.1
8″		87.7
9″		61.6
3-OCH_3		56.8
3′-OCH_3		56.8
3″-OCH_3		56.3
—OCH_2O—	102.4	—

[a] Assignments were based on HMQC, and HMBC experiments.

except for the additional guiacylglycerol moiety [δ_H 7.03 (1H, s, H-2″), 6.82 (1H, overlap, H-6″), 6.77 (1H, d, J = 8.0 Hz, H-5″), 4.97 (1H, d, J = 5.0 Hz, H-7″), 4.24 (1H, m, H-8″), 3.90 (1H, m, H-9″a), 3.58 (1H, dd, J = 12.0, 3.5 Hz, H-9″b), and 3.86 (3H, s, 3″-OCH_3) in the ^{1}H NMR; δ_C 148.7 (C-3″), 146.8 (C-4″), 133.9 (C-1″), 120.4 (C-6″), 115.7 (C-5″), 111.2 (C-2″), 87.7 (C-8″), 74.1 (C-7″), 61.6 (C-9″), 56.3 (3″-OCH_3) in the ^{13}C NMR]. The location of the guiacylglycerol group was deduced to be at C-4′ by analysis of the HMBC data showing correlation from H-8″ to C-4′ (Fig. 2). The configuration of two aryl substituents in **2** was identified to be the same as **1** by NOESY correlation and positive optical rotation value ($[\alpha]_D^{25}$ +8.8, CH_3OH). The small coupling constant for $J_{7''/8''}$ (5.0 Hz) in guiacylglycerol moiety indicated an *erythro* configuration. The CD spectrum exhibited the positive cotton effect at 240 nm confirming the absolute configurations as 7″*R* and 8″*S*.[16] Thus, the structure of **2** was determined as shown in Figure 1, and was named firmianol B.

The known structures **3**–**19** were confirmed as (+)-piperitol (**3**),[17] (+)-pinoresinol (**4**),[17] (+)-syringaresinol (**5**),[18] buddlenol E (**6**),[19] (1*S**,2*R**,5*R**,6*S**)-6-(4-hydroxy-3-methoxyphenyl)-2-(3,4-methoxylenedioxyphenyl)-3,7-dioxybicyclo[3.3.0]-oactan-1-ol (**7**),[20] (+)-sesamin (**8**),[17] (−)-pinoresinol (**9**),[21] (+)-7′-methoxylariciresinol (**10**),[22] balanophonin (**11**),[23] (−)-5-methoxybalanophonin (**12**),[24] buddlenol A (**13**),[25] *threo*-(7*R*,8*R*)-guaiacylglycerol-β-coniferyl aldehyde ether (**14**),[26] *erythro*-(7*S*,8*R*)-guaiacylglycerol-β-coniferyl aldehyde ether (**15**),[26] *threo*-guaiacylglycerol-8-*O*-4′-sinapyl alcohol ether (**16**),[27] *erythro*-syringylglycerol-8-*O*-4′-coniferyl alcohol ether (**17**),[27] *threo*-guaiacylglycerol-8-*O*-4′-coniferyl alcohol ether (**18**),[27] and *threo*-guaiacylglycerol 8′-vanillin ether (**19**)[28] by comparing their spectroscopic data with the reported data (Supplementary data).

Microglia cells are considered to be the major immune cells that play a crucial role in inflammatory, immune and degenerative processes in the brain.[3] In order to determine the potential antineuroinflammatory properties of isolated lignans, compounds (**1**–**19**) were tested for their inhibitory effect on lipopolysaccharide (LPS)-stimulated nitric oxide (NO) production in microglia cells. In this study, compounds **14** and **15** strongly inhibited NO levels in LPS-stimulated BV-2 without cell toxicity (IC_{50} value of 1.05 and 0.929 μM, respectively) (Table 3). Similarly, compounds **11**–**13** and **17** also exhibited IC_{50} values ranging from 7.07 to

Table 3
Inhibitory effect on NO production of compounds **1**–**19** in LPS-activated BV-2 cells

Compounds	IC_{50}[a] (μM)	Cell viability[b] (%)
Firmianol A (**1**)	35.39	109 ± 3.6
Firmianol B (**2**)	>500	108.5 ± 3.9
(+)-Piperitol (**3**)	32.65	113. ± 9.4
(+)-Pinoresinol (**4**)	25.1	114.8 ± 5.8
(+)-Syringaresinol (**5**)	27.53	110.1 ± 7.5
Buddlenol E (**6**)	19.33	121 ± 8.5
(1*S**,2*R**,5*R**,6*S**)-6-(4-Hydroxy-3-methoxy-phenyl)-2-(3,4-methoxylenedioxy phenyl)-3,7-dioxybicyclo[3.3.0]-oactan-1-ol (**7**)	>500	108 ± 6.7
(+)-Sesamin (**8**)	26.26	126 ± 5.3
(−)-Pinoresinol (**9**)	31.1	111.1 ± 6.1
(+)-7′-Methoxylariciresinol (**10**)	32.99	115 ± 5.5
Balanophonin (**11**)	7.07	115. ± 5.9
(−)-5-Methoxybalanophonin (**12**)	10.0	119.5 ± 5.5
Buddlenol A (**13**)	15.28	129 ± 10.0
threo-(7*R*,8*R*)-Guaiacylglycerol-β-coniferyl aldehyde ether (**14**)	1.05	122.6 ± 4.8
erythro-(7*S*,8*R*)-Guaiacylglycerol-β-coniferyl aldehyde ether (**15**)	0.929	128.9 ± 6.3
threo-Guaiacylglycerol-8-*O*-4′-sinapyl alcohol ether (**16**)	23.53	120.2 ± 3.3
erythro-Syringylglycerol-8-*O*-4′-coniferyl alcohol ether (**17**)	9.14	123.4 ± 4.2
threo-Guaiacylglycerol-8-*O*-4′-coniferyl alcohol ether (**18**)	32.56	116.8 ± 4.8
threo-Guaiacylglycerol 8′-vanillin ether (**19**)	47.59	116.4 ± 6.9
NMMA[c]	16.27	98.2 ± 2.6

[a] IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells.
[b] Cell viability after treatment with 20 μM of each extract was expressed as a percentage (%) of the LPS only treatment group. The results are averages of three independent experiments, and the data are expressed as mean ± SD.
[c] NMMA as a positive control.

15.28 μM which were less than the value of the positive control N^G-Mono-methyl-L-arginine (L-NMMA). The presence of α,β-unsaturated aldehyde group in these lignans played an especially important role in the compounds, anti-inflammatory properties.

In conclusion, our current study indicates that these isolated lignan compounds from *F. simplex* may be candidates as anti-neuroinflammatory agents with the potential to be therapeutic agents in the treatment of neurodegenerative diseases.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.01.008.

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