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Biological evaluation of phenolic constituents from the trunk of *Berberis koreana*

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ABSTRACT

A bioassay-guided fractionation and chemical investigation of the trunk of *Berberis koreana* resulted in the isolation and identification of a new sesquilignan, named berbikonol (**1**), along with fourteen known lignan derivatives (**2–15**) and a new phenolic compound, named berfussinol (**16**), together with five known ones (**17–21**). The structures of these new compounds were elucidated on the basis of 1D and 2D NMR spectroscopic data analysis as well as circular dichroism (CD) spectroscopy studies. Compounds 1–**5**, **7–8**, **11**, and **14** showed significant cytotoxicity against the XF498 cell line with IC_{50} values of 7.14–19.32 μM. In addition, compounds **3–8** and **15** strongly reduced nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells, a microglial cell line.

Berberis koreana Palibin (Berberidaceae), well-known as 'Korean barberry', is a dense growing deciduous shrub that is native to Korea. Koreans have used an extract of this plant for the treatment of various disorders such as fever, gastroenteritis, sore throats, and conjunctivitis.[1] As part of a continuing search for bioactive constituents from Korean medicinal plant sources, a MeOH extract of the trunk of *B. koreana* exhibited significant cytotoxicity against some human tumor cell lines. Previously, our chemical investigation of the bioactive extract led to the isolation of biphenyls and triterpenoids with cytotoxicity.[2,3]

Our interest in further research on bioactive constituents from this plant led us to investigate this source in the current study. The MeOH extract of the trunk of *B. koreana* was suspended in distilled water and then partitioned successively with *n*-hexane, $CHCl_3$, and *n*-BuOH. To identify the active ingredients responsible for the cytotoxic activity, each fraction was evaluated for cytotoxicity against human cancer cell lines using a sulforhodamine B (SRB) assay.[4] The active fraction, the $CHCl_3$-soluble fraction, was separated using silica gel and C-18 open-column chromatography, followed by preparative HPLC to afford a new sesquilignan, named berbikonol (**1**), along with 14 known lignan derivatives (**2–15**) and a new phenolic compound, named berfussinol (**16**), together with five known ones (**17–21**) (Fig. 1). With the aim of evaluating the potential of *B. koreana* as an herbal medicine with anti-cancer and anti-neuroinflammatory effects, herein we report the isolation, structural determination of new compounds (**1** and **16**) and biological activity of isolates (**1–21**).

Berbikonol (**1**) was obtained as a yellowish gum with positive optical rotation ($[\alpha]_D^{25}$ +84.0). The molecular formula of **1** was determined to be $C_{32}H_{40}O_{11}$ by positive mode HR-FABMS data at *m/z* 601.2635 $[M+H]^+$ (calcd for $C_{32}H_{41}O_{11}$, 601.2649). The IR spectrum of **1** showed the presence of hydroxyl (3417 cm^{-1}) and aromatic functions (1594 and 1502 cm^{-1}). In the UV spectrum of **1**, absorption maxima were observed at 232 and 281 nm. The ^{13}C NMR spectrum (Table 1) of **1** showed 27 carbon signals except for 5 methoxy signals, indicating **1** to be a sesquilignan which contained a glycerol moiety and a 3,4-dimethoxyphenyl unit from the ^1H and ^{13}C NMR spectra.[5] Overall, the proton and carbon signals in the ^1H and ^{13}C NMR data of **1** were very similar to those of dihydrobuddlenol B, except for addition of a methoxy group in **1**.[6] Careful analysis of fragments of the molecular ion in the EIMS of **1** showed peaks at *m/z* 378, 228, 212, and 152, as shown in Figure 2. The significant relative abundance of a fragment peak at *m/z* 228 indicated the presence of 1-(3,4-dimethoxyphenyl)propane-1,2,3-triol ($C_{11}H_{16}O_5$). The connection of functional groups was confirmed by the HMBC correlations (Fig. 3). The planar structure of **1** was established on the basis of the consideration and analysis of 2D NMR experiments (^1H–^1H COSY, HMQC, and HMBC). The absolute configuration of **1** was clarified by the CD spectroscopic study. The chemical shifts at C-2 and C-3 (δ_H 5.56/δ_C 87.4 and δ_H 3.64/δ_C 54.5) and their relatively small coupling constant (J = 6.0 Hz) led us to clarify the relative configuration of these protons, the *anti* configuration.[7–9] The similar CD data [$\Delta\varepsilon$ +1.6 (210 nm) and $\Delta\varepsilon$

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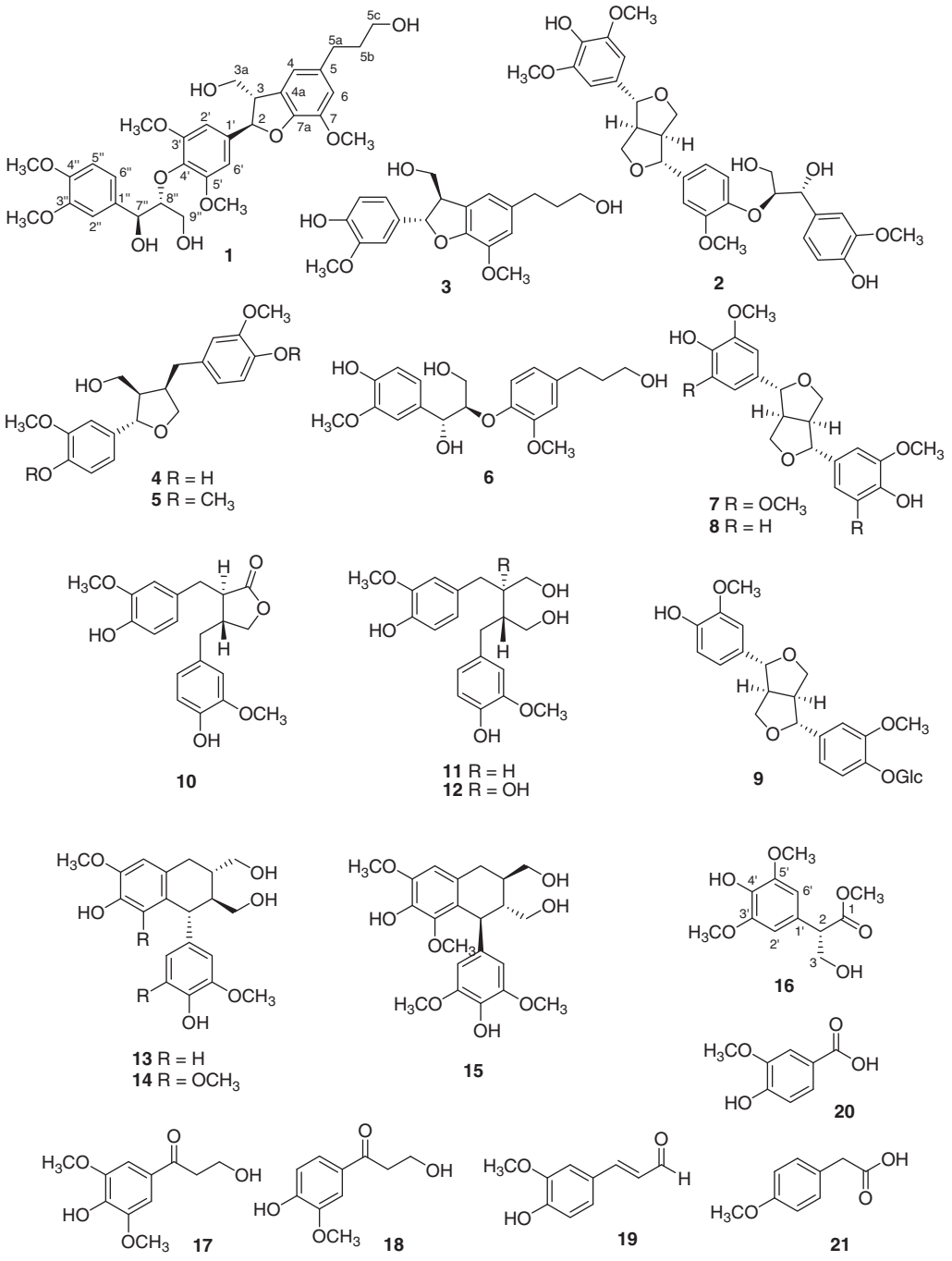

Figure 1. The structures of compounds **1**–**21**.

−0.6 (290 nm)] of **1** with that of an analogous compound, (+)-7*R*,8*S*-5-methoxydihydrodehydroconiferyl alcohol revealed that the absolute configuration at C-2 and C-3 was 2*R*,3*S*.[9] Moreover, the small coupling constant (J = 4.0 Hz) observed between H-7″ and H-8″ and the chemical shift of C-7″ (δ_C 72.8) indicated that the glycerol moiety of **1** possessed an *erythro*-configuration.[10,11] The CD spectrum of **1** also showed a negative Cotton effect at 240 nm, indicating that the absolute configurations at C-7″ and C-8″ of **1** were 7″*S* and 8″*R* form.[11,12] Thus, the structure of **1** was elucidated as shown in Figure 1, and named berbikonol.

The known compounds were identified as ficusesquilignan A (**2**),[13] (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol (**3**),[14] (+)-lariciresinol (**4**),[15] (+)-lariciresinol dimethyl ether (**5**),[16] (1*R*,2*R*)-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (**6**),[17] (−)-syringaresinol (**7**),[18] (−)-pinoresinol (**8**),[19] (−)-pinoresinol 4-*O*-β-D-glucopyranoside (**9**),[20] (−)-matairesinol (**10**),[21] (−)-secoisolariciresinol (**11**),[15] (−)-carinol (**12**),[22] *ent*-isolariciresinol (**13**),[23] (−)-lyoniresinol (**14**),[24] and (+)-lyoniresinol (**15**),[24] by comparison of their spectroscopic data with previously reported values. The absolute configurations of the above known compounds (**2–15**) were established on the basis of their ^{1}H NMR coupling constant values, optical rotation values, and CD spectroscopic data. To the best of our knowledge, this is the first time that the above known lignan derivatives (**2–15**) have been isolated from *B. koreana*.

Berfussinol (**16**) was obtained as an amorphous gum. The molecular formula of **16** was determined to be $C_{12}H_{16}O_6$ by positive mode HR-FABMS data at *m/z* 257.1034 $[M+H]^+$ (calcd for

Table 1
^{1}H and ^{13}C NMR data of **1** in CD_3OD (δ in ppm, 500 MHz for ^{1}H and 125 MHz for ^{13}C)[a]

Position	1	
	δ_H	δ_C
2	5.56 d (6.0)	87.4
3	3.64 m	54.5
3a	3.88 m, 3.76 m	63.8
4	6.69 br s	116.7
4a		128.3
5		136.0
5a	2.63 t (7.5)	31.7
5b	1.82 m	34.6
5c	3.57 t (6.5)	61.0
6	6.72 br s	112.9
7		144.0
7a		146.2
1′		138.5
2′	6.72 br s	102.7
3′		153.4
4′		135.0
5′		153.4
6′	6.72 br s	102.7
1″		132.6
2″	6.98 d (1.5)	110.1
3″		147.4
4″		147.7
5″	6.73 d (8.0)	114.5
6″	6.77 dd (8.0, 1.5)	119.5
7″	4.95 d (4.0)	72.8
8″	4.24 m	86.2
9″	3.91 m, 3.87 m	60.4
7-OCH_3	3.87 s	55.5
3′,5′-OCH_3	3.79 s	55.6
3″-OCH_3	3.83 s	55.4
4″-OCH_3	3.81 s	55.1

[a] J values are in parentheses and reported in Hz; the assignments were based on ^{1}H–^{1}H COSY, HMQC, and HMBC experiments.

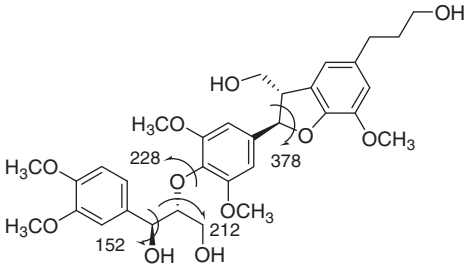

Figure 2. Proposed EIMS fragmentation of **1**.

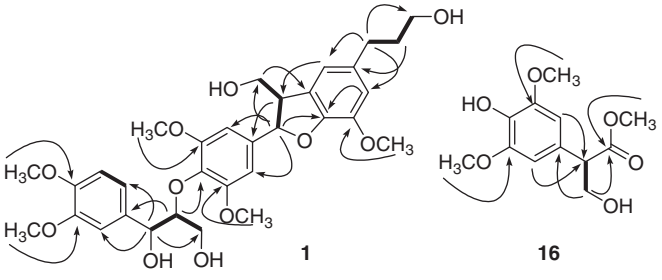

Figure 3. Key ^{1}H–^{1}H COSY (—) and HMBC (→) correlations of **1** and **16**.

$C_{12}H_{17}O_6$, 257.1025). The IR spectrum of **16** showed the presence of hydroxyl (3380 cm^{-1}) and carbonyl groups (1730 cm^{-1}). A survey of the literature revealed that the ^{1}H and ^{13}C NMR data of **16** are very similar to those of goldfussinol, except for the chemical shift and splitting pattern of H-2 and H-3 [δ_H 4.07 (1H, dd, J = 12.0, 11.0 Hz, H-3a), 3.72 (1H, m, H-2), and 3.70 (1H, m, H-3b) in **16**; δ_H 4.23 (1H, dd, J = 10.8, 7.2 Hz, H-3a), 4.17 (1H, dd, J = 10.8, 7.1 Hz, H-3b), and 4.11 (1H, dd, J = 7.2, 7.1 Hz, H-2) in goldfussinol],[25] which suggested that they have a different stereochemistry at C-2. Analysis of the ^{1}H–^{1}H COSY, HMQC, and HMBC correlations led to the establishment of the planar structure for **16** (Fig. 3). Compound **16** is structurally related with a methyl tropate derivative.[26] The negative specific rotation ($[\alpha]_D^{25}$ −81.3 in $CHCl_3$) of **16** revealed that the absolute configuration at C-2 was 2*S* on the basis of Watson's confirmation of the *S*-configuration of (−)-methyl tropate.[26] In conclusion, the structure of **16** was assigned as shown in Figure 1, and named berfussinol. In addition, goldfussinol isolated from *Goldfussia psilostachys*, may have the *R*-form because of its positive specific rotation ($[\alpha]_D^{25}$ +5.0 in MeOH).[25]

Other known compounds were identified as 3-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)propan-1-one (**17**),[27] 3-hydroxy-1-(3-methoxy-4-hydroxyphenyl)propan-1-one (**18**),[27] *trans*-coniferyl aldehyde (**19**),[28] vanillic acid (**20**),[29] and 4-methoxyphenylacetic acid (**21**),[30] by comparison of their spectroscopic data with literature values. To the best of our knowledge, above known compounds (**17–21**) were isolated from *B. koreana* for the first time.

The cytotoxic activities of the isolates (**1–21**) were evaluated by determining their inhibitory effects on four human tumor cell lines including A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and XF498 (human CNS cancer) using a SRB assay.[4] The results (Table 2) showed that all the tested lignan derivatives (**1–15**) had consistent cytotoxicity against the XF498 cell line with IC_{50} values ranging from 7.14 to 27.62 μM. Of the phenolic compounds (**16–21**), compound **16** showed cytotoxicity against the SK-MEL-2 cell line (IC_{50}: 27.17 μM), compounds **17–18** and **21** exhibited cytotoxicity against the XF498 cell line (IC_{50}: 24.30–28.41 μM), and compound **19** showed cytotoxicity against the A549 cell line with an IC_{50} value of 21.18 μM, but compound **20** was inactive. Compound **5** showed cytotoxicity against all of the cell lines tested with IC_{50} values of 20.13, 21.50, 25.12, and 15.40 μM, respectively, for the A549, SK-OV-3, SK-MEL-2, and XF498 cell lines. We also evaluated for inhibitory effects of lignan derivatives (**1–15**) on NO production

Table 2
Cytotoxic activities of compounds (**1–21**) isolated from *B. koreana*

Compound	IC_{50}[a] (μM)			
	A549	SK-OV-3	SK-MEL-2	XF498
1	>30.0	>30.0	27.31	19.32
2	>30.0	>30.0	11.52	9.47
3	22.40	>30.0	23.34	13.42
4	24.51	>30.0	>30.0	11.65
5	20.13	21.50	25.12	15.40
6	>30.0	>30.0	>30.0	26.56
7	>30.0	>30.0	14.47	8.82
8	>30.0	>30.0	26.60	13.32
9	>30.0	>30.0	>30.0	25.37
10	23.80	>30.0	>30.0	23.65
11	>30.0	>30.0	21.47	18.75
12	>30.0	>30.0	>30.0	27.62
13	20.19	>30.0	24.88	22.81
14	21.22	>30.0	>30.0	26.53
15	>30.0	>30.0	>30.0	7.14
16	>30.0	>30.0	27.17	>30.0
17	>30.0	>30.0	>30.0	24.30
18	>30.0	>30.0	>30.0	28.41
19	21.18	>30.0	>30.0	>30.0
20	>30.0	>30.0	>30.0	>30.0
21	>30.0	>30.0	>30.0	28.05
Etoposide	1.85	1.81	1.17	1.72

[a] IC_{50} value of compounds against each cancer cell line. The IC_{50} value was defined as the concentration (μM) that caused 50% inhibition of cell growth in vitro.

Table 3
Inhibitory effect on NO production of compounds (**1**–**15**) isolated from *B. koreana*

Compound	IC_{50}[a] (μM)
1	90.86
2	>500
3	28.80
4	51.80
5	44.42
6	58.49
7	13.48
8	26.19
9	144.33
10	94.53
11	87.62
12	200.90
13	151.87
14	113.34
15	69.14
NMMA	17.08

[a] The IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production by LPS-activated BV-2 cells.

in lipopolysaccharide (LPS)-activated BV-2 cells, a microglial cell line.[31] NO is a gaseous signaling molecule which has pivotal roles in immune and inflammatory responses and neuronal transmission in the brain.[32] In the normal condition, NO has neuroprotective and antioxidative effects. However, overproduced NO from activated microglia causes various neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease through mitochondrial dysfunction and neuronal cell death.[33] An investigation of the ability of various lignin derivatives to inhibit neuroinflammation has already been reported.[34–37] Therefore, we sought to determine whether it is intended that lignin derivatives from *B. koreana* influence the suppression of NO production in LPS-activated microglia. As shown in Table 3, compounds **3–8** and **15** significantly inhibited NO production. Compound **7** most strongly reduced NO levels with an IC_{50} value of 13.48 μM. This is significant because NO produced by activated microglia is one of the main proinflammatory mediators in the central nervous system. Therefore, these results suggest that lignan derivatives from the trunk of *B. koreana*, including (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol (**3**), (+)-lariciresinol (**4**), (+)-lariciresinol dimethyl ether (**5**), (1*R*,2*R*)-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (**6**), (−)-syringaresinol (**7**), (−)-pinoresinol (**8**), and (+)-lyoniresinol (**15**), suppress neuroinflammation via reduction of NO production by overactivated microglia.

In conclusion, this study indicates that lignan derivatives are the main active constituents of the trunk of *B. koreana*. Moreover, this study led to the isolation and identification of a new sesquilignan named berbikonol (**1**) and a new phenolic compound named berfussinol (**16**). Anti-cancer and anti-neuroinflammatory effects of lignin derivatives and phenolic compounds isolated from this plant source were confirmed. In particular, seven lignan derivatives **3**–**8** and **15** showed both cytotoxicity in various cancer cell lines and an inhibitory effect on NO production by LPS-activated BV-2 cells. The present study thus indicates that these compounds will be good bioactive molecules for the treatment of cancers and neurodegenerative diseases.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.104.

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