Bioorganic & Medicinal Chemistry Letters 26 (2016) 1877–1880

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Bioactive lignan constituents from the twigs of *Sambucus williamsii*

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A R T I C L E I N F O

Article history:
Received 25 January 2016
Revised 4 March 2016
Accepted 8 March 2016
Available online 8 March 2016

Keywords:
Sambucus williamsii
Lignan
Anti-inflammation
NGF regulation
Cytotoxicity

A B S T R A C T

As part of our ongoing search for bioactive constituents of natural Korean medicinal plants, three new lignan derivatives, sambucasinol A–C (**1**–**3**), together with 7 known compounds (**4**–**10**) were isolated from the twigs of *Sambucus williamsii*. The structures of these new compounds were determined by a combination of 1D and 2D NMR spectroscopic data analysis, as well as circular dichroism (CD) spectroscopy studies. Here, we evaluated the anti-inflammatory effects of **1**–**10** in lipopolysaccharide (LPS)-stimulated murine microglia BV-2 cells. Compounds **1**–**3** exhibited significant inhibitory effects on nitric oxide production in LPS-activated BV-2 cells, with IC_{50} values of 6.82, 7.04, and 14.70 μM, respectively. Additionally, we evaluated the effects of compounds **1**–**10** on NGF induction in a C6 rat glioma cell line. Compounds **1**–**3** upregulated NGF secretion to 183.95 ± 2.63%, 153.99 ± 5.15%, and 155.96 ± 5.15%, respectively, without any significant cell toxicity. Moreover, all isolates were evaluated for their cytotoxicity against A549, SK-OV-3, SK-MEL-2, and XF498 cell lines. Compounds **1**–**3** showed consistent cytotoxicity against the four human cell lines, with IC_{50} values in the range of 11.07–19.62 μM.
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Sambucus williamsii var. *coreana* Nakai (Caprifoliaceae) is a deciduous shrub, which is widely distributed throughout Korea, Japan, and China.[1] *S. williamsii* has long been used as a traditional Korean medicine for the treatment of bone fractures and osteoporosis.[2] Previous phytochemical investigation regarding *S. williamsii* has reported the presence of terpenes, fatty acids, phenolic compounds, and lignans.[3–5] Particularly, some lignans showed anti-proliferative activity on osteoblast-like UMR106 cells.[3] However, only a few constituents associated with the anti-tumor activity of *S. williamsii* have been reported. In our continuing search for bioactive constituents of Korean medicinal plants,[6,7] we attempted to investigate the active constituents of the twigs of *S. williamsii*. The MeOH extract from the twigs of *S. williamsii* was suspended in distilled water and then successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. In order to identify the bioactive constituents responsible for the anti-cancer activity, each fraction was evaluated for cytotoxicity against human cancer cell lines using a sulforhodamine B (SRB) assay.[8] The active fraction was shown to be the $CHCl_3$ extract, which also inhibited NO production in LPS-stimulated BV-2 microglial cells. The $CHCl_3$-soluble fraction was separated using repeated silica gel and C-18 column chromatography followed by preparative HPLC, to afford ten lignan derivatives (**1**–**10**) including three new lignans, named sambucasinol A–C (**1**–**3**). Herein, we report the isolation and structural determination of the isolates (**1**–**10**) and their biological activities.

Compound **1** was obtained as a yellowish gum with a negative optical rotation ($[\alpha]_D^{25}$ −6.4). The molecular formula of **1** was determined to be $C_{41}H_{46}O_{14}$ by negative mode HR-FABMS data at *m*/*z* 761.2804 $[M-H]^-$ (calcd for $C_{41}H_{45}O_{14}$, 761.2804). The IR spectrum of **1** showed the presence of hydroxyl (3414 cm^{-1}) and aromatic functions (1645 and 1470 cm^{-1}). The ^1H NMR spectrum (Table 1) of **1** indicated characteristic signals attributable to a pair of olefinic protons [δ_H 7.57 (1H, d, *J* = 16.0 Hz, H-7‴)/6.36 (1H, d, *J* = 16.0 Hz, H-8‴)], two 1,3,4-trisubstituted aromatic ring moieties [δ_H 6.99 (1H, d, *J* = 2.0 Hz, H-2″), 6.76 (1H, d, *J* = 8.0 Hz, H-5″), and 6.79 (1H, dd, *J* = 8.0, 2.0 Hz, H-6″)/7.19 (1H, d, *J* = 2.0 Hz, H-2‴), 6.83 (1H, d, *J* = 8.0 Hz, H-5‴), and 7.08 (1H, dd, *J* = 8.0, 2.0 Hz, H-6‴)], two 1,3,4,5-tetrasubstituted aromatic ring systems [δ_H 6.72 (2H, s, H-2,5), 6.78 (1H, br s, H-2′), and 6.75 (1H, br s, H-5′)], one *trans* olefinic moiety [δ_H 7.57 (1H, d, *J* = 16.0 Hz, H-7‴), and 6.36 (1H, d, *J* = 16.0 Hz, H-8‴)] and five methoxyl groups [δ_H 3.90 (3H, s, 3‴-OCH_3), 3.88 (3H, s, 3′-OCH_3), 3.83 (3H, s, 3″-OCH_3), 3.79 (6H, s, 3,5-OCH_3)]. The ^{13}C NMR spectrum revealed resonances for 36 carbon signals, with the exception of five methoxy signals, indicating **1** to be a dihydrobenzofuranlignan, with one guaiacyl glycerol moiety and one feruloyl moiety. Overall, the proton and carbon signals in the ^1H and ^{13}C NMR data of **1** were very similar to those of

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http://dx.doi.org/10.1016/j.bmcl.2016.03.023
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buddlenol G, which was isolated from the same plant,[9] except for the addition of a methoxy group at C-3′ in **1**. The planar structure of **1** was established on the basis of the consideration and analysis of 2D NMR experiments (^{1}H–^{1}H COSY, HMQC, and HMBC). The HMBC correlation indicated that the ester group linked a *trans*-feruloyl moiety to C-9′ (Fig. 2). The absolute configuration at C-7 and C-8 were identified to be 7*R* and 8*S*, respectively, based on the coupling constants (J = 6.0 Hz) between H-7 and H-8 in the ^{1}H NMR spectrum of **1**, and the CD spectrum showing a negative Cotton effect at 289 nm.[10–12] Moreover, the coupling constant of 4.7 Hz between H-7′ and H-8′ suggested the *erythro* relative configuration.[13] The CD spectrum of **1** showed a positive Cotton effect at 230 nm, indicating that the absolute configurations at C-7″ and C-8″ of **1** were 7″*R* and 8″*S*.[13,14] Thus, the structure of **1** was elucidated as shown in Figure 1, and named sambucasinol A (**1**).

Compound **2** was obtained as a yellowish gum and assigned the molecular formula $C_{41}H_{46}O_{14}$ by positive mode HR-FABMS data at *m*/*z* 785.2779 [M+Na]$^+$ (calcd for $C_{41}H_{46}Na_1O_{14}$, 785.2780). The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, with the exception of the large coupling constant (J = 6.8 Hz) between H-7″ and H-8″, and the chemical shifts for the glycerol moiety [**2**: δ_H 5.00 (1H, d, J = 6.8 Hz, H-7″), 4.09 (1H, m, H-8″); **1**: δ_H 5.00 (1H, d, J = 6.8 Hz, H-7″), 4.09 (1H, m, H-8″)], suggesting the 7″,8″-*threo*-configuration for **2**.[13] The structure of **2** was further confirmed by ^{1}H–^{1}H COSY, HMQC, and HMBC. The absolute configuration at C-7, 8, 7″, and 8″ were determined to be *R*, *S*, *R*, and *R*, respectively, as the CD spectrum of **2** showed the negative Cotton effect at 232 nm and 290 nm.[12,14] Thus, the structure of **2** was elucidated as shown in Figure 1, and named sambucasinol B (**2**).

Compound **3** was obtained as a yellowish gum. The molecular formula of **3** was established as $C_{41}H_{46}O_{14}$ using HR-FABMS, which showed a positive ion [M + Na]$^+$ at *m*/*z* 785.2780 (Calcd for $C_{41}H_{46}Na_1O_{14}$ 785.2780). The ^{1}H and ^{13}C NMR data of **3** were similar to those of **2**, except that a 9‴-*O*-(*Z*)-feruloyl moiety replaced the 9‴-*O*-(*E*)-feruloyl moiety of **2**. This is supported by the *cis*-coupling constant (J = 13.0 Hz) for H-7‴ and H-8‴ of **3**.[15] The structure of **3** was further confirmed by ^{1}H–^{1}H COSY, HMQC, and HMBC (Fig. 2). A comparison of the CD spectrum of **3** with that of **2** suggested that compound **3** also possessed the same 7″*R*, and 8″*R* configuration. Thus, the structure of **3** was elucidated as shown in Figure 1, and named sambucasinol C (**3**).

The known compounds were identified as lariciresinol (**4**),[16] (7α*H*,8′α*H*)-4,4′,8α,9-tetrahydroxy-3,3′-dimethoxy-7,9′-epoxylignan (**5**),[17] berchemol (**6**),[18] 7-hydroxylariciresinol (**7**),[19] (−)-medioresinol (**8**),[20] (−)-pinoresinol (**9**),[16] and 7*R*,8*S*-dihydrodehydrodiconiferyl alcohol (**10**),[21] by comparison of their spectroscopic and physical data with previously reported values. The cytotoxic activity of the isolates (**1**–**10**) was evaluated by

Table 1
^{1}H and ^{13}C NMR data of **1**–**3** in CD_3OD (δ in ppm, 700 MHz for ^{1}H and 175 MHz for ^{13}C)[a]

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		139.6		136.9		136.7
2	6.72 s	104.1	6.74 s	104.0	6.75 s	103.8
3		154.7		154.4		154.3
4		136.4		139.8		139.8
5	6.72 s	154.7	6.74 s	154.4	6.75 s	154.3
6		104.1		104.0		103.8
7	5.53 d (6.0)	88.8	5.53 d (6.0)	88.8	5.58 d (6.0)	88.6
8	3.50 m	55.7	3.48 m	55.8	3.47 m	55.8
9	3.88 m, 3.76 m	65.2	3.86 m, 3.75 m	65.1	3.89 m, 3.77 m	65.1
1′		136.6		136.6		136.3
2′	6.78 br s	114.3	6.79 br s	114.3	6.76 br s	114.1
3′		145.4		145.5		145.4
4′		147.7		147.7		147.5
5′	6.75 br s	129.8	6.76 br s	139.8	6.72 br s	139.7
6′		118.1		118.2		117.9
7′	2.70 t (7.3)	33.3	2.73 t (7.5)	33.3	2.65 t (7.5)	33.1
8′	2.02 m	31.9	2.04 m	31.9	1.98 m	31.8
9′	4.19 t (6.5)	65.0	4.20 t (6.5)	65.0	4.16 m	64.7
1″		133.8		133.6		133.4
2″	6.99 d (2.0)	111.5	7.05 d (2.0)	111.7	7.02 br s	111.6
3″		148.8		148.8		148.7
4″		146.9		147.3		147.1
5″	6.76 d (8.0)	115.8	6.76 d (8.0)	115.9	6.77 d (8.0)	115.9
6″	6.79 dd (8.0, 2.0)	120.8	6.89 dd (8.0, 2.0)	120.9	6.88 dd (8.0, 2.0)	120.8
7″	4.92 d (4.7)	74.1	5.00 d (6.8)	74.6	5.01 d (6.5)	74.6
8″	4.26 m	87.5	4.09 m	88.9	4.10 m	88.9
9″	3.60 m, 3.91 m	61.7	3.77 m, 3.35 m	61.8	3.78 m, 3.35 m	61.8
1‴		127.8		127.7		127.8
2‴	7.19 br s	111.8	7.20 br s	111.8	7.72 br s	114.9
3‴		149.5		149.6		148.3
4‴		150.7		150.9		149.6
5‴	6.83 d (8.0)	116.6	6.83 d (8.0)	116.6	6.78 d (8.0)	115.8
6‴	7.08 dd (8.0, 2.0)	124.2	7.10 dd (8.0, 2.0)	124.2	7.11 dd (8.0, 2.0)	126.6
7‴	7.57 d (16.0)	146.8	7.57 d (16.0)	146.8	6.87 d (13.0)	145.3
8‴	6.36 d (16.0)	115.7	6.36 d (16.0)	115.4	5.80 d (13.0)	116.8
9‴		169.4		169.5		168.7
3,5-OCH_3	3.79 s	56.7	3.85 s	56.8	3.86 s	56.8
3′-OCH_3	3.88 s	56.6	3.91 s	56.6	3.88 s	56.6
3″-OCH_3	3.83 s	56.3	3.86 s	56.5	3.84 s	56.6
3‴-OCH_3	3.90 s	56.9	3.93 s	56.9	3.87 s	56.9

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

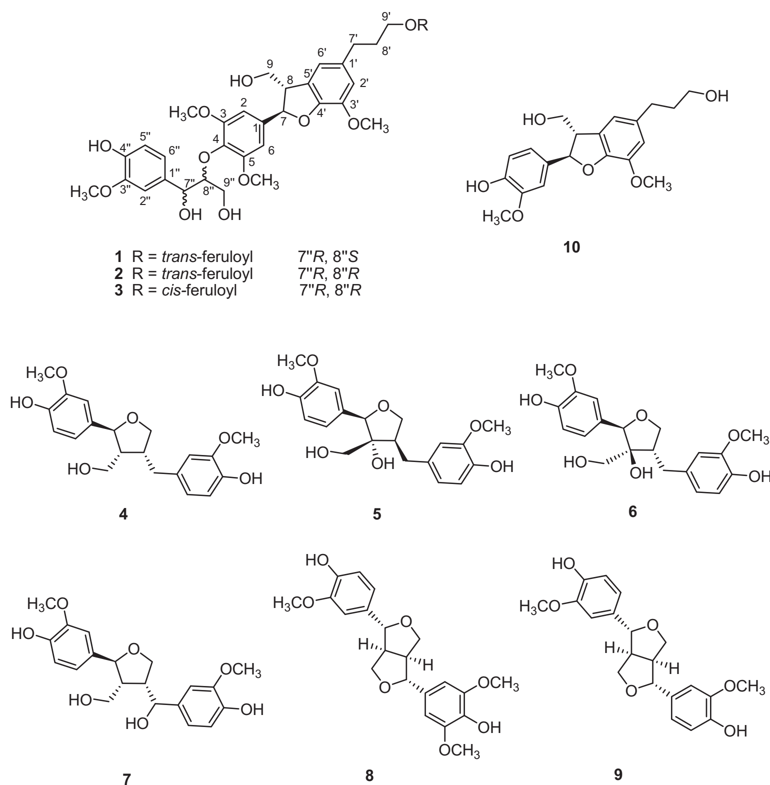

Figure 1. Chemical structures of compounds **1**–**10**.

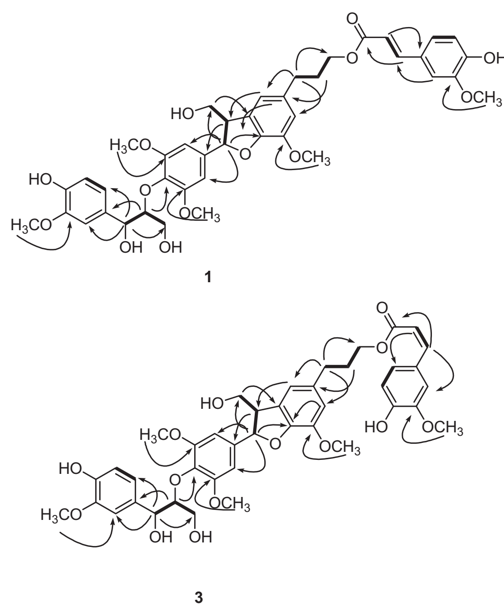

Figure 2. Key ^{1}H–^{1}H COSY (—) and HMBC (→) correlations of **1** and **3**.

determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and XF498) using the SRB bioassay.[8] The results (Table 2) showed that compounds **1**–**3** had consistent cytotoxicity against all the cell lines tested, with IC_{50} values in the range of 11.07–19.62 μM. The other compounds were shown to be inactive (IC_{50} > 30 μM). Etoposide was used as a positive control (IC_{50}: 2.06, 0.84, 1.84, and 1.94 μM, for the A549, SK-OV-3, SK-MEL-2, and XF498 cell lines, respectively).

Table 2
Cytotoxic activities of compounds **1**–**3** and **10** isolated from *S. williamsii*

Compound	IC_{50}[a] (μM)			
	A549	SK-OV-3	SK-MEL-2	XF498
1	13.22	15.72	11.07	18.14
2	16.37	17.46	14.08	18.39
3	15.82	17.58	11.36	19.62
10	>30.0	>30.0	>30.0	>30.0
Etoposide	2.06	0.84	1.83	1.94

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

Subsequently, the anti-neuroinflammatory effect of the compounds (**1**–**10**) isolated from *S. williamsii* was evaluated by measuring the NO production levels using bacterial endotoxin lipopolysaccharide in the murine microglia BV-2 cell line. The inhibitory activity of the isolated compounds on NO production is expressed as the concentration that elicits 50% inhibition (IC_{50}). As shown in Table 3, compounds **1**–**3** significantly decreased the production of NO, with IC_{50} values of 6.82, 7.04, and 14.70 μM, respectively, in LPS-induced BV-2 cells, with no cell toxicity. Their activity was more potent than that of the positive control, NG-monomethyl-L-arginine (L-NMMA), which inhibited NO production with an IC_{50} value of 20.78 μM. Compounds **8**, **9**, and **10** showed moderate activity with IC_{50} values of 45.59, 34.25, and 39.97 μM, respectively.

We also evaluated the neuroprotective activity of the isolated compounds (**1**–**10**) by determining their effects on nerve growth factor (NGF) secretion in C6 cells (Table 4). Among the isolates, Compounds **1**–**3** were significantly potent stimulants of NGF release with stimulation levels of 183.95 ± 2.63%, 153.99 ± 5.15%, and 155.96 ± 5.15%, respectively (positive control 6-shogaol was 151.75 ± 8.43%), without any cell toxicity at a concentration of

Table 3
Inhibitory effects on NO production of compounds **1–10** in LPS-activated BV-2 cells

Compounds	IC_{50}[a] (μM)	Cell viability[b] (%)
1	6.82	157.08 ± 10.22
2	7.04	146.18 ± 9.77
3	14.70	137.59 ± 9.91
4	72.58	147.08 ± 2.62
5	>500	128.15 ± 7.52
6	215.41	150.73 ± 3.97
7	128.97	114.13 ± 9.97
8	45.59	127.23 ± 5.30
9	34.25	147.82 ± 0.68
10	39.97	129.33 ± 2.84
[c]L-NMMA	20.78	101.56 ± 5.76

[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 compound was determined by MTT assay and is expressed in percentage (%). The results are averages of three independent experiments, and the data are expressed as mean ± SD.
[c] L-NMMA as positive control.

Table 4
Effects of compounds **1–10** on NGF secretion and cell viability in C6 cells[a]

Compounds	NGF secretion	Cell viability
1	183.95 ± 2.63	92.26 ± 1.56
2	153.99 ± 5.15	82.70 ± 2.06
3	155.96 ± 5.15	96.99 ± 2.5
4	77.31 ± 7.57	101.45 ± 0.02
5	107.97 ± 5.38	106.34 ± 0.76
6	107.88 ± 5.23	102.57 ± 3.93
7	101.55 ± 0.85	104.46 ± 0.57
8	96.39 ± 4.67	103.19 ± 1.56
9	106.93 ± 8.99	104.87 ± 2.35
10	97.14 ± 5.67	105.30 ± 1.27
[b]6-Shogaol	151.75 ± 8.43	104.731 ± 10.38

[a] C6 cells were treated with 20 μM of compounds. After 24 h, the content of NGF secretion in C6-conditioned media was measured by ELISA. The level of secreted NGF cells is expressed as percentage of the untreated control. The data shown represent the means ± SD of three independent experiments performed in triplicate.
[b] 6-Shogaol as positive control.

20 μM. Interestingly, compounds **1** and **2** have the same planar structures but different stereochemistry. These data suggest that the stereochemistry in the guaiacyl glycerol moiety at C-8″ may be very important for the secretion of NGF from C6 cells.

In conclusion, this study indicates that lignan derivatives are the main components of the twigs of *S. williamsii*. Additionally, three new lignan derivatives, sambucasinol A–C (**1–3**), were isolated from this plant. Moreover, we investigated the cytotoxic activity, anti-inflammatory, and neuroprotective activities of isolates from *S. williamsii*. As a result, new compounds **1–3** showed better results than other lignan from all the activity tests. In particular, compounds **1–3** exhibited a potent inhibitory effect on NO production in LPS-stimulated BV-2 cells and NGF secretion in C6 cells, and could be useful for the development of novel anti-inflammatory and neuroprotective agents.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2A10005315). We are thankful to the Korea Basic Science Institute (KBSI) for acquiring the MS data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.03.023.

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