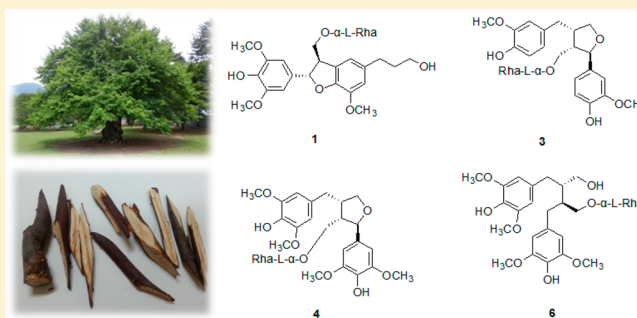


Lignan Glycosides from the Twigs of *Chaenomeles sinensis* and Their Biological ActivitiesChung Sub Kim,[†] Lalita Subedi,[‡] Sun Yeou Kim,^{‡,§} Sang Un Choi,[‡] Ki Hyun Kim,[†] and Kang Ro Lee^{*,†}[†]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea[‡]Gachon Institute of Pharmaceutical Science and [§]College of Pharmacy, Gachon University, Incheon 406-799, Republic of Korea[‡]Korea Research Institute of Chemical Technology, Daejeon 305-343, Republic of Korea

Supporting Information

ABSTRACT: Phytochemical investigation of the twigs of *Chaenomeles sinensis* led to the isolation and identification of six new lignan glycosides, chaenomisine A–F (1–6), along with five known ones (7–11). Their chemical structures were determined by spectroscopic methods, including NMR, MS, ECD, and GC/MS analyses. All the isolated compounds (1–11) were tested for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide-activated murine microglial cells and the secretion of nerve growth factor (NGF) in a C6 rat glioma cell line. Compound 6 significantly reduced NO levels in the murine microglia BV2 cells with an IC₅₀ value of 21.3 μ M, and compounds 1, 3, and 6 were potent stimulants of NGF release with stimulation levels of 151.74 \pm 6.77%, 144.31 \pm 7.49%, and 167.61 \pm 18.5%, respectively.



Chaenomeles sinensis Koehne (Rosaceae) is a woody plant widely distributed in eastern Asian countries, including Korea, Japan, and China. The fruits of this plant have been used in Korean traditional medicine to treat throat diseases, diarrhea, inflammatory diseases, and dry beriberi, which is caused by damage to the nervous system.^{1–3} Pharmacological activities have been identified in the components and/or extract of the fruit of *C. sinensis*.^{4–7} Antiviral activity against influenza A virus and antidiabetic, antiacetylcholinesterase, antihyperglycemic, antihyperlipidemic, and antioxidant effects in streptozotocin-induced diabetic rats have been reported from the extract of *C. sinensis* fruit.^{4–7} Also, tissue factor inhibitory flavonoids and triterpenoids and antipruritic flavonoids were isolated from the same sources.^{1–3} However, research to isolate anti-inflammatory and/or neuroprotective agents from the twigs of *C. sinensis* has not been conducted.

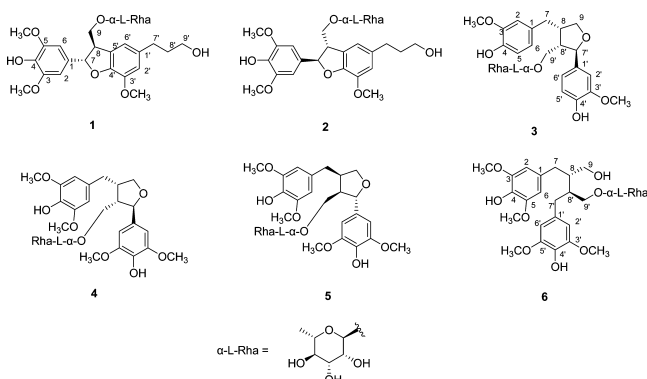
In the continuing search for new anti-inflammatory substances from Korean medicinal plants, we isolated and identified a potential neuroprotective agent from the twigs of *C. sinensis*. The EtOAc layer of the methanolic extract exhibited significant NGF induction activity in C6 cells (226.7 \pm 11.9%; positive control *Zingiberis Rhizoma*, 148.4 \pm 6.1%). The column chromatographic purification of the most active EtOAc layer led to the isolation of six new lignan glycosides, chaenomisine A–F (1–6), along with five known ones (7–11). The chemical structures of isolated compounds were determined by their NMR spectroscopic data (¹H and ¹³C NMR, ¹H–¹H COSY, DEPT, HMQC, and HMBC), MS and ECD spectra, and hydrolysis. All isolates (1–11) were evaluated for their anti-inflammatory and neuroprotective

activities. In this study, we report the isolation and structure elucidation of bioactive components from the twigs of *C. sinensis* as well as their antineuroinflammatory and neuroprotective activities.

The 80% aqueous methanolic extract from the twigs of *C. sinensis* was sequentially partitioned with *n*-hexane, CHCl₃, EtOAc, and *n*-BuOH. Repeated chromatographic purification of the EtOAc fraction yielded six new lignan glycosides, chaenomisine A–F (1–6), and five known ones, (7R,8S)-dihydrodehydrodiconiferyl alcohol 9-*O*- β -D-glucopyranoside (7),⁸ (7S,8R)-dihydrodehydrodiconiferyl alcohol 9-*O*- α -L-rhamnoside (8),⁹ (+)-9'-*O*-(β -D-glucopyranosyl)lyoniresinol (9),¹⁰ (–)-9'-*O*-(α -L-rhamnopyranosyl)lyoniresinol (10),¹¹ and avicularin (11),¹² which were identified by comparing their spectroscopic data and specific rotations with reported data.

Compound 1 was obtained as a colorless gum. The molecular formula was determined to be C₂₇H₃₆O₁₁ from the ¹³C NMR data and the [M – H][–] ion in the negative ion HRFABMS. The ¹H NMR spectrum displayed the presence of two 1,2,3,5-tetrasubstituted aromatic rings [δ _H 6.77 (1H, s, H-6'), 6.73 (1H, s, H-2'), and 6.69 (2H, s, H-2 and H-6)], one oxygenated methine [δ _H 5.50 (1H, d, *J* = 6.6 Hz, H-7)], two oxygenated methylenes [δ _H 3.92 (1H, dd, *J* = 9.8, 7.9 Hz, H-9a), 3.77 (1H, dd, *J* = 9.8, 5.1 Hz, H-9b), and 3.59 (2H, t, *J* = 6.6 Hz, H-9')], one methine [δ _H 3.67 (1H, m, H-8)], two methylenes [δ _H 2.66 (2H, t, *J* = 7.5 Hz, H-7') and 1.85 (2H, m, H-8')], three methoxy groups [δ _H 3.89 (3H, s, 3'-OCH₃), 3.85

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(6H, s, 3-OCH₃ and 5-OCH₃), and one α -rhamnopyranosyl unit [δ_{H} 4.77 (1H, d, $J = 1.5$ Hz, H-1''), 3.85 (1H, overlap, H-2''), 3.63 (1H, dd, $J = 9.4$, 3.4 Hz, H-3''), 3.56 (1H, dq, $J = 9.4$, 6.1 Hz, H-5''), 3.40 (1H, t, $J = 9.4$ Hz, H-4''), and 1.28 (1H, d, $J = 6.1$ Hz, H-6'')]. The ¹³C NMR spectrum revealed 24 peaks for 27 carbons, including 12 aromatic carbons for two aromatic rings [δ_{C} 149.5 (C-3 and C-5), 136.6 (C-4), 134.0 (C-1), and 104.3 (C-2 and C-6), and 147.6 (C-4'), 145.4 (C-3'), 137.2 (C-1'), 129.6 (C-5'), 117.8 (C-6'), and 114.4 (C-2')] and one anomeric carbon [δ_{C} 101.8 (C-1')]. These NMR data of **1** (Table 1) were similar to those of **8** with a major difference being the one aromatic ring of **8**, indicating the presence of a methoxy group at C-5 in **1**. The planar structure of **1** was determined through 2D NMR analysis, including ¹H–¹H COSY, HMQC, and HMBC spectra (Figure 1). The HMBC correlation of H-1'' to C-9 indicated that the rhamnose unit was linked to the oxygen at C-9, and the J value of the anomeric proton ($J = 1.5$ Hz) confirmed it as α -rhamnose.¹³ Enzymatic hydrolysis of **1** afforded the aglycone (7*S*,8*R*)-3,3',5'-trimethoxy-4',7'-epoxy-8,5'-neolignan-4,9,9'-triol (**1a**) and L-rhamnose ($[\alpha]_{\text{D}}^{25} +9.0$), which was identified by co-TLC confirmation and GC/MS analysis.^{14,15} The identification of the aglycone was done by comparing its ¹H NMR, MS, and optical rotation data.¹⁶ The C-7/C-8 relative configuration was assigned as *trans* from the small coupling constant (6.6 Hz, **1**; 7.4 Hz, *trans*-form; 8.4 Hz, *cis*-form).¹⁷ The absolute configuration of **1** was confirmed as 7*S* and 8*R* from the negative Cotton effect at 222 nm and positive ones at 246 and 289 nm as seen in the electronic circular dichroism (ECD) spectrum (Figure S31).¹⁸ Thus, the structure of **1** was established as (7*S*,8*R*)-3,5,3'-trimethoxy-4',7'-epoxy-8,5'-neolignan-4,9,9'-triol 9-*O*- α -L-rhamnopyranoside, and this compound was named chaenomside A.

Compound **2** was obtained as a colorless gum with the same molecular formula of C₂₇H₃₆O₁₁ as **1**. The NMR data of **2** were similar to those of **1** except for the slightly shifted peaks of H-9 of **2** [δ_{H} 4.02 (1H, dd, $J = 9.2$, 5.2 Hz, H-9a) and 3.63 (1H, overlap, H-9b)], compared to those of **1** [δ_{H} 3.92 (1H, dd, $J = 9.8$, 7.9 Hz, H-9a) and 3.77 (1H, dd, $J = 9.8$, 5.1 Hz, H-9b)], suggesting that **2** was a stereoisomer of **1**. A full NMR analysis demonstrated that the planar structure of **2** was the same as **1**. The ECD spectra of **1** and **2** were opposite from 220 to 300 nm (positive Cotton effects at 231 nm and negative ones at 258 and 293 nm), indicating that the aglycone moieties of **1** and **2** were enantiomeric. Thus, the structure of **2** was determined as (7*R*,8*S*)-3,5,3'-trimethoxy-4',7'-epoxy-8,5'-neolignan-4,9,9'-triol 9-*O*- α -L-rhamnopyranoside and named chaenomside B.

The molecular formula of compound **3** was determined to be C₂₆H₃₄O₁₀ from positive ion HRFABMS and ¹³C NMR data.

Table 1. ¹³C (175 MHz) and ¹H (700 MHz) NMR Assignments of Compounds **1**–**3** in Methanol-*d*₄

pos.	1		2		3	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
1	134.0		133.8		133.5	
2	104.3	6.69, s	104.4	6.69, s	113.5	6.79, overlap
3	149.5		149.5		149.2	
4	136.6		136.6		146.0	
5	149.5		149.5		116.4	6.74, d (8.0)
6	104.3	6.69, s	104.4	6.69, s	122.2	6.67, dd (8.0, 1.7)
7a	89.8	5.50, d (6.6)	89.7	5.49, d (6.5)	34.2	2.91, dd (13.7, 5.1)
7b						2.56, dd (13.7, 10.9)
8	53.0	3.67, m	53.3	3.65, overlap	44.2	2.78, m
9a	70.6	3.92, dd (9.8, 7.9)	70.8	4.02, dd (9.2, 5.2)	74.0	4.03, dd (8.4, 6.5)
9b		3.77, dd (9.8, 5.1)		3.63, overlap		3.73, dd (8.4, 6.7)
1'	137.2		137.3		135.7	
2'	114.4	6.73, s	114.3	6.77, s	110.8	6.93, d (1.3)
3'	145.4		145.4		149.2	
4'	147.6		147.6		147.3	
5'	129.6		129.7		116.2	6.80, overlap
6'	117.8	6.77, s	118.0	6.76, s	120.1	6.81, overlap
7'	33.0	2.66, t (7.5)	33.0	2.66, t (7.6)	84.9	4.80, d (6.8)
8'	35.9	1.85, m	36.0	1.85, m	51.6	2.49, quin (6.8)
9'a	62.3	3.59, t (6.6)	62.3	3.59, t (6.6)	67.0	3.82, overlap
9'b						3.66, overlap
3-OCH ₃	57.0	3.85, s	57.0	3.85, s	56.6	3.86, s
5-OCH ₃	57.0	3.85, s	57.0	3.85, s		
3'-OCH ₃	56.9	3.89, s	56.9	3.88, s	56.6	3.87, s
1''	101.8	4.77, d (1.5)	102.4	4.77, d (1.5)	102.3	4.69, d (1.7)
2''	72.3	3.85, overlap	72.3	3.85, overlap	72.4	3.82, overlap
3''	72.6	3.63, dd (9.4, 3.4)	72.6	3.67, overlap	72.7	3.66, overlap
4''	74.0	3.40, t (9.4)	74.0	3.41, overlap	74.0	3.39, t (9.5)
5''	70.4	3.56, dq (9.4, 6.1)	70.3	3.62, overlap	70.4	3.59, dq (9.5, 6.2)
6''	18.2	1.28, d (6.1)	18.2	1.27, d (6.2)	18.2	1.27, d (6.2)

The ¹H and ¹³C NMR spectra were similar to those of lariciresinol,^{19,20} except for the presence of α -rhamnose signals [δ_{H} 4.69 (1H, d, $J = 1.7$ Hz, H-1''), 3.82 (1H, overlap, H-2''), 3.66 (1H, overlap, H-3''), 3.59 (1H, dq, $J = 9.5$, 6.2 Hz, H-5''), 3.39 (1H, t, $J = 9.5$ Hz, H-4''), and 1.27 (1H, d, $J = 6.2$ Hz, H-6''); δ_{C} 102.3 (C-1''), 74.0 (C-4''), 72.7 (C-3''), 72.4 (C-2''), 70.4 (C-5''), and 18.2 (C-6'')] and a deshielded signal of C-9' (3, δ_{C} 67.0; lariciresinol, δ_{C} 60.5). The HMBC correlation from H-1'' to C-9' showed that the sugar unit was located at C-9'. Enzymatic hydrolysis of **3** afforded (–)-lariciresinol, identified by the ¹H NMR, FABMS, and negative specific rotation $\{[\alpha]_{\text{D}}^{25} -18.0$ (c 0.05, MeOH)},^{19,20} and L-rhamnose, which was confirmed by GC/MS analysis.^{14,15} The relative configuration of **3** was confirmed by NOESY correlations of H-2'/H-8 and H-8', and the 8*S*,7'*R*,8'*S* configuration of **3** was deduced from

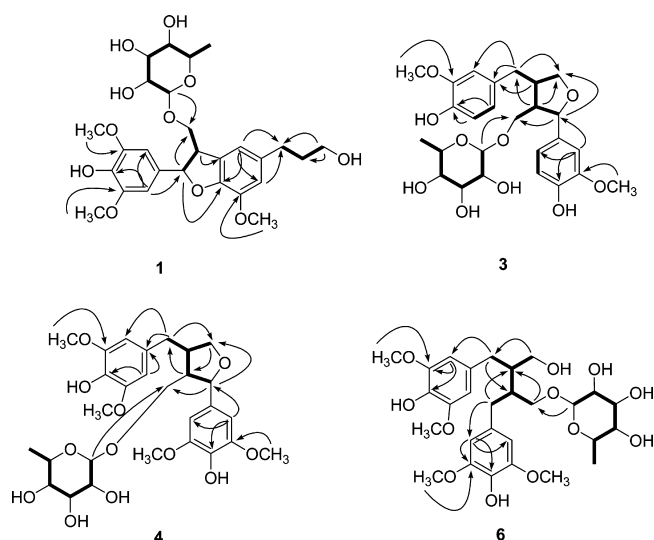


Figure 1. Key correlations of ^1H - ^1H COSY (bold lines) and HMBC (arrows) of 1, 3, 4, and 6.

the positive Cotton effects at 231 and 277 nm and negative one at 246 nm as seen in the ECD spectrum (Figure S32).²¹ An extensive NMR analysis including 2D NMR data confirmed the

structure of 3 (chaenomide C) as (–)-lariciresinol 9'-O- α -L-rhamnopyranoside.

Inspection of the ^1H and ^{13}C NMR data of 4 (Table 2) revealed that the two aromatic rings [δ_{H} 6.52 (2H, s, H-2 and H-6) and 3.86 (6H, s, 3-OCH₃ and 5-OCH₃), and 6.65 (2H, s, H-2' and H-6') and 3.87 (6H, s, 3'-OCH₃ and 5'-OCH₃); δ_{C} 149.5 (C-3 and C-5), 135.0 (C-4), 132.7 (C-1), and 107.0 (C-2 and C-6), and 149.4 (C-3' and C-5'), 136.2 (C-4'), 134.9 (C-1'), and 104.5 (C-2' and C-6')] are symmetrical 1,2,3,5-tetrasubstituted moieties. This was supported by ^1H - ^1H COSY, HMQC, and HMBC spectra. The sugar analysis and determination of stereochemistry of 4 were performed by the same method as for 3. The ECD spectrum of 4 showed a similar pattern of Cotton effects (positive at 224 and 282 nm and negative at 246 nm) to those of 3, which confirmed the same absolute configuration as 3. Thus, the structure of 4 was determined as (8*S*,7'*R*,8'*S*)-5,5'-dimethoxylariciresinol 9'-O- α -L-rhamnopyranoside and named chaenomide D.

The ^1H and ^{13}C NMR data of 5 resembled those of 4, but there were slight shifts in the signals of H-9' [δ_{H} 3.89 (1H, overlap, H-9'a) and 3.69 (1H, overlap, H-9'b); 4, δ_{H} 4.00 (1H, dd, J = 9.9, 6.7 Hz, H-9'a) and 3.54 (1H, dd, J = 9.9, 6.5 Hz, H-9'b)], indicating that 5 could be a stereoisomer of 4. The planar structure of 5 was determined by 2D NMR (^1H - ^1H COSY, HMQC, and HMBC) spectroscopic analysis. The ECD spectra of 4 and 5 were opposite from 220 to 285 nm (negative CEs at

Table 2. ^{13}C (175 MHz) and ^1H (700 MHz) NMR Assignments of Compounds 4–6 in Methanol-*d*₄

pos.	4		5		6	
	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)
1	132.7		132.8		133.2	
2	107.0	6.52, s	107.0	6.52, s	107.2	6.32, s
3	149.5		149.5		149.2	
4	135.0		135.0		134.6 ^a	
5	149.5		149.5		149.2	
6	107.0	6.52, s	107.0	6.52, s	107.2	6.32, s
7a	34.6	2.90, dd (13.6, 5.3)	34.7	2.92, dd (13.6, 5.4)	36.7	2.75, dd (13.8, 6.1)
7b		2.58, dd (13.6, 11.2)		2.57, dd (13.6, 10.8)		2.54, dd (13.8, 9.2)
8	44.2	2.80, m	44.2	2.80, m	44.3	1.95, m
9a	73.9	4.06, dd (8.4, 6.6)	74.0	4.04, dd (8.4, 6.7)	63.1	3.78, overlap
9b		3.76, dd (8.4, 6.5)		3.76, dd (8.4, 6.9)		3.52, dd (11.0, 7.3)
1'	134.9		135.0		133.0	
2'	104.5	6.65, s	104.4	6.65, s	107.2	6.30, s
3'	149.4		149.4		149.2	
4'	136.2		136.1		134.5 ^a	
5'	149.4		149.4		149.2	
6'	104.5	6.65, s	104.4	6.65, s	107.2	6.30, s
7'a	85.0	4.84, d (6.9)	85.1	4.81, d (6.5)	37.0	2.67, dd (13.8, 6.7)
7'b						2.60, dd (13.8, 8.8)
8'	51.8	2.48, m	51.7	2.51, m	41.0	2.11, m
9'a	66.9	4.00, dd (9.9, 6.7)	67.1	3.89, overlap	69.6	3.85, dd (9.9, 5.6)
9'b		3.54, dd (9.9, 6.5)		3.69, overlap		3.39, overlap
3,5-OCH ₃	56.9	3.86, s	56.9	3.86, s	56.7 ^a	3.77 ^a , s
3',5'-OCH ₃	57.0	3.87, s	57.0	3.87, s	56.8 ^a	3.76 ^a , s
1''	102.3	4.74, d (1.4)	102.3	4.71, d (1.5)	102.5	4.65, d (1.5)
2''	72.4	3.86, overlap	72.4	3.83, dd (3.3, 1.5)	72.6	3.86, dd (3.3, 1.5)
3''	72.7	3.69, dd (9.5, 6.3)	72.7	3.67, overlap	72.8	3.72, dd (9.4, 3.3)
4''	74.0	3.41, t (9.5)	74.0	3.40, t (9.3)	74.0	3.42, t (9.4)
5''	70.5	3.64, dq (9.5, 6.2)	70.4	3.60, dq (9.3, 6.1)	70.3	3.67, dq (9.4, 6.2)
6''	18.2	1.27, d (6.2)	18.2	1.27, d (6.1)	18.2	1.28, d (6.2)

^aExchangeable peaks.

223 and 271 nm and a positive CE at 244 nm), suggesting that the aglycone moieties of **4** and **5** were enantiomeric. Thus, the structure of **5** was determined as (8*R*,7'*S*,8'*R*)-5,5'-dimethoxyariciresinol 9'-*O*- α -L-rhamnopyranoside and named chaenomisode E.

The molecular formula of **6** was determined to be C₂₈H₄₀O₁₂ from the [M – H][–] ion in the negative ion HRFABMS and ¹³C NMR data. The ¹H and ¹³C NMR spectra were similar to those of (8*S*,8'*S*)-bisdihydrosiringenin,²² except for the presence of α -rhamnose signals [δ_{H} 4.65 (1H, d, *J* = 1.5 Hz, H-1''), 3.86 (1H, dd, *J* = 3.3, 1.5, H-2''), 3.72 (1H, dd, *J* = 9.4, 3.3, H-3''), 3.67 (1H, dq, *J* = 9.4, 6.2 Hz, H-5''), 3.42 (1H, t, *J* = 9.4 Hz, H-4''), and 1.28 (1H, d, *J* = 6.2 Hz, H-6''); δ_{C} 102.5 (C-1''), 74.0 (C-4''), 72.8 (C-3''), 72.6 (C-2''), 70.3 (C-5''), and 18.2 (C-6'')] and a deshielded signal of C-9' [**6**, δ_{C} 69.6; (8*S*,8'*S*)-bisdihydrosiringenin, δ_{C} 61.7], indicating the location of the sugar moiety at C-9'. This was corroborated by the HMBC correlation of H-1'' to C-9'. Enzymatic hydrolysis of **6** yielded the aglycone (8*S*,8'*S*)-bisdihydrosiringenin (**6a**), whose ¹H NMR, FABMS, and negative specific rotation {[α]_D²⁵ +30.2 (c 0.05, MeOH)} were in good agreement with the reported value,²² and L-rhamnose was identified by the same method as **1**. Thus, the structure of **6** was established as (8*S*,8'*S*)-bisdihydrosiringenin 9'-*O*- α -L-rhamnopyranoside and named chaenomisode F.

To investigate the effect of compounds (**1**–**11**) on neuroinflammation, we measured nitric oxide (NO) levels in murine microglia BV2 cells stimulated by lipopolysaccharide (LPS) (Table S1). Among the isolates, compound **6** significantly reduced NO levels in the medium with an IC₅₀ value of 21.3 μ M, which displayed more activity than the positive control, L-NMMA (IC₅₀ 24.8 μ M). Compounds **5** and **9**–**11** exhibited moderate activities with IC₅₀ values ranging from 29.8 to 40.2 μ M. None of the compounds showed any significant cellular toxicity up to 20 μ M. Interestingly, although the chemical structures of compounds **4** and **5** were quite similar, they differed substantially with respect to their inhibitory effect on NO production (**4**, IC₅₀ 199.3 μ M; **5**, IC₅₀ 29.8 μ M). The data suggest that the 8*R*,7'*S*,8'*R*-form of the aglycone moiety in tetrahydrofuran-type lignans is important for the NO inhibitory activity, which was supported by the weak activity of **3** (IC₅₀ 179.7 μ M), possessing an (8*S*,7'*R*,8'*S*)-tetrahydrofuran moiety.

We also evaluated the neuroprotective activities of the isolated compounds (**1**–**11**) by determining their effects on NGF secretion in C6 cells (Table S2). Of the tested compounds at 20 μ M, **1**, **3**, and **6** were potent stimulants of NGF release with stimulation levels of 151.74 \pm 6.77%, 144.31 \pm 7.49%, and 167.61 \pm 18.5%, respectively (positive control 6-shogaol was 141.75 \pm 9.43%), while compounds **5**, **7**, and **11** displayed moderate activities, with NGF secretion levels of 124.67 \pm 7.80%, 140.04 \pm 16.06%, and 123.06 \pm 1.36%, respectively. Among the dihydrobenzofuran-type neolignans possessing a rhamnosyl unit at C-9 (**1**, **2**, and **8**), **1** was the most potent stimulant of NGF release (**1**, 151.74 \pm 6.77%; **2**, 106.85 \pm 4.25%; **8**, 102.88 \pm 6.40%), which implies that both the 7*S*,8*R*-configuration and a C-5 methoxy group were essential for an effect on NGF release.

The antiproliferative activities of compounds **1**–**11** were evaluated by determining their inhibitory effects on four human tumor cell lines, namely, A549 (non-small-cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melano-

ma), and HCT-15 (colon adenocarcinoma), using the SRB bioassay,²³ but they were inactive (IC₅₀ > 10.0 μ M).

This work shows that the twigs of *C. sinensis* are rich in lignan glycosides, the main bioactive constituents with anti-inflammatory and neuroprotective activities. In particular, chaenomisode F (**6**), which exhibited a potent inhibitory effect on NO production in LPS-stimulated BV-2 cells and NGF secretion activity in C6 cells, could be useful for the development of novel anti-inflammatory and neuroprotective agents.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded with a Jasco P-1020 polarimeter. UV spectra were acquired on an Agilent 8453 UV–visible spectrophotometer. IR spectra were measured with a Bruker IFS-66/S FT-IR spectrometer. ECD spectra were recorded with a JASCO J-810 spectropolarimeter. NMR spectra were recorded in methanol-*d*₄ solutions on a Bruker AVANCE III 700 NMR spectrometer operating at 700 MHz (¹H) and 175 MHz (¹³C). HRFABMS were measured with a Waters SYNAPT G2 and a JEOL JMS700 mass spectrometer. Semipreparative HPLC was performed on a Gilson 306 pump equipped with a Shodex refractive index detector at a flow rate of 2 mL/min. Silica gel 60 (Merck, 230–400 mesh) and RP-C₁₈ silica gel (Merck, 230–400 mesh) were used for column chromatography. LPLC was performed over a LiChroprep Lobar-A Si 60 column (Merck, 240 mm \times 10 mm i.d.) equipped with an FMI QSY-0 pump. Sephadex LH-20 (Pharmacia Co. Ltd.) was used as a packing material for molecular sieve column chromatography.

Plant Material. Twigs of *C. sinensis* were collected in Seoul, Korea, in January 2012. A voucher specimen of the plants (SKKU-NPL 1206) was identified by one of the authors (K.R.L.) and deposited at the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. Twigs of *C. sinensis* (7.0 kg) were extracted three times with 80% aqueous MeOH (each 10 L \times 1 day) under reflux and filtered. The filtrate was evaporated under vacuum to obtain a MeOH extract (320 g), which was suspended in distilled H₂O and successively partitioned with *n*-hexane, CHCl₃, EtOAc, and *n*-BuOH, to yield **3**, **15**, **6**, and **30** g fractions, respectively. The EtOAc-soluble layer (6 g) was applied to a Sephadex LH-20 column with a solvent system of 90% aqueous MeOH to yield five subfractions (E1–E5). Fraction E1 (3.8 g) was subjected to silica gel column chromatography (CHCl₃–MeOH, 10:1) to give 10 subfractions (E1A–E1J). Fraction E1E (400 mg) was chromatographed over a Lobar-A RP-18 column (60% aqueous MeOH) to give five subfractions (E1E1–E1E5), and fraction E1E1 (170 mg) was further separated on a Lobar-A RP-18 (50% MeOH(aq)) to give five subfractions (E1E1A–E1E1E). Compounds **4** (5 mg) and **5** (5 mg), and **2** (5 mg) and **3** (4 mg) were acquired by purification of fractions E1E1D (30 mg) and E1E1E (21 mg) using semipreparative HPLC (35% MeOH(aq)), respectively. Fraction E1F (280 mg) was separated over a Lobar-A RP-18 with a solvent system of 50% aqueous MeOH and further purified by semipreparative HPLC (35–50% MeOH(aq)) to yield compounds **1** (6 mg), **8** (8 mg), and **10** (20 mg). Fraction E1G (350 mg) was subjected to a Lobar-A RP-18 with a solvent system of 40% MeOH(aq) and purified by semipreparative HPLC (40–50% MeOH(aq)) to yield compounds **6** (2 mg) and **11** (20 mg). Fraction E1I (120 mg) was chromatographed on a Lobar-A RP-18 with a solvent system of 50% MeOH(aq) and purified by semipreparative HPLC (40–50% MeOH(aq)) to yield compounds **7** (10 mg) and **9** (6 mg).

Chaenomisode A (1): colorless gum; [α]_D²⁵ –6.4 (c 0.2, MeOH); IR (KBr) ν_{max} 3358, 2945, 2832, 1452, 1033 cm^{–1}; UV (MeOH) λ_{max} (log ϵ) 280 (1.31), 231 (4.03) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 289 (+5.12), 246 (+7.92), 222 (–2.91) nm; ¹H and ¹³C NMR data, see Table 1; negative HRFABMS *m/z* 535.2174 [M – H][–] (calcd for C₂₇H₃₅O₁₁, 535.2179).

Chaenomisode B (2): colorless gum; [α]_D²⁵ +10 (c 0.1, MeOH); IR (KBr) ν_{max} 3359, 2946, 2831, 1451, 1032 cm^{–1}; UV (MeOH) λ_{max} (log

ϵ) 282 (1.36), 231 (4.11) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 293 (−3.79), 258 (−1.82), 231 (+5.21) nm; ^1H and ^{13}C NMR data, see Table 1; negative HRFABMS m/z 535.2173 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{27}\text{H}_{35}\text{O}_{11}$, 535.2179).

Chaenomisode C (3): colorless gum; $[\alpha]_{\text{D}}^{25}$ −20 (c 0.1, MeOH); IR (KBr) ν_{\max} 3405, 2942, 2834, 1614, 1450 cm^{-1} ; UV (MeOH) λ_{\max} ($\log \epsilon$) 281 (5.21), 225 (6.02), 212 (4.33) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 277 (+10.41), 246 (−4.97), 231 (+2.62) nm; ^1H and ^{13}C NMR data, see Table 1; positive HRFABMS m/z 507.2230 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{26}\text{H}_{35}\text{O}_{10}$, 507.2230).

Chaenomisode D (4): colorless gum; $[\alpha]_{\text{D}}^{25}$ −12 (c 0.1, MeOH); IR (KBr) ν_{\max} 3406, 2942, 2833, 1614, 1451 cm^{-1} ; UV (MeOH) λ_{\max} ($\log \epsilon$) 281 (5.01), 224 (5.91), 210 (4.01) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 282 (+0.91), 246 (−2.11), 224 (+3.12) nm; ^1H and ^{13}C NMR data, see Table 2; negative HRFABMS m/z 565.2281 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{28}\text{H}_{37}\text{O}_{12}$, 565.2285).

Chaenomisode E (5): colorless gum; $[\alpha]_{\text{D}}^{25}$ +14.2 (c 0.04, MeOH); IR (KBr) ν_{\max} 3401, 2941, 2832, 1613, 1449 cm^{-1} ; UV (MeOH) λ_{\max} ($\log \epsilon$) 283 (5.29), 225 (6.06), 212 (4.31) nm; CD (MeOH) λ_{\max} ($\Delta\epsilon$) 271 (−2.23), 244 (+2.12), 223 (−4.12) nm; ^1H and ^{13}C NMR data, see Table 2; negative HRFABMS m/z 565.2279 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{28}\text{H}_{37}\text{O}_{12}$, 565.2285).

Chaenomisode F (6): colorless gum; $[\alpha]_{\text{D}}^{25}$ +30 (c 0.1, MeOH); IR (KBr) ν_{\max} 3401, 2941, 2832, 1613, 1449 cm^{-1} ; UV (MeOH) λ_{\max} ($\log \epsilon$) 283 (1.31), 230 (1.72) nm; ^1H and ^{13}C NMR data, see Table 2; negative HRFABMS m/z 567.2436 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{28}\text{H}_{39}\text{O}_{12}$, 567.2442).

Enzymatic Hydrolysis of 1–6. A solution of each sample (1.0–2.0 mg) in H_2O (2 mL) was individually hydrolyzed with naringinase (30 mg, from *Penicillium* sp.; ICN Biomedicals Inc.) at 40 °C for 24 h. Each reaction mixture was extracted with CHCl_3 to yield 0.5–1.0 mg of **1a–6a** (Supporting Information).

Acid Hydrolysis of 1–6 and Sugar Analysis. Compounds **1–6** (each 1.0–2.0 mg) were refluxed with 1 mL of 1 N HCl for 1 h at 90 °C. The hydrolysate was extracted with EtOAc, and the aqueous layer was neutralized using an Amberlite IRA-67 column to yield the sugar. The sugar acquired from the hydrolysis was dissolved in anhydrous pyridine (0.5 mL), and 2.0 mg of L-cysteine methyl ester hydrochloride (Sigma) was added. The mixture was stirred at 60 °C for 1.5 h and trimethylsilylated through adding 0.1 mL of 1-trimethylsilylimidazole (Sigma) for 2 h. The mixture was partitioned with *n*-hexane and H_2O (1.0 mL each), and the *n*-hexane layer (1.0 μL) was analyzed through GC/MS. Identification of L-rhamnose ($[\alpha]_{\text{D}}^{25}$ +9.0) was performed by co-injection of the hydrolysate with authentic samples, giving a single peak at 9.712 min. An authentic sample (Sigma) treated in the same way displayed a single peak at 9.730 min.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR data of **1–6**; enzymatic hydrolysis and bioassay procedures. The material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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