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Bioactive Lignans from the Trunk of *Abies holophylla*

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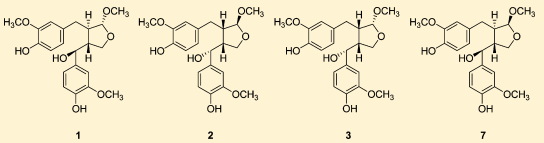
Supporting Information

ABSTRACT: Six new lignans (**1**−**6**) were isolated from the trunk of *Abies holophylla* MAXIM, together with 11 known lignans (**7**−**17**). The structures of **1**−**7** were elucidated by spectroscopic methods, acid hydrolysis, and use of the modified Mosher's method. The effects of the isolates on nerve growth factor induction in a C6 rat glioma cell line were evaluated. Compounds **6**, **7**, and **13** showed significant induction of nerve growth factor secretion at concentrations of 10 μM. Compounds **1**, **5**, **6**, and **16** showed moderate inhibitory effects on nitric oxide production in lipopolysaccharide-activated BV-2 cells (IC_{50} 28.5−36.4 μM).



Abies holophylla MAXIM (Pinaceae), also known as Manchurian Fir or Needle Fir, is an evergreen and coniferous tree that is widely distributed in Korea, China, and Russia.[1,2] Several *Abies* species have been used in Korean folk medicine for the treatment of colds, stomach aches, indigestion, rheumatic diseases, and vascular and pulmonary diseases.[2] Previous phytochemical investigations on *A. holophylla* reported lignans, terpenoids, steroids, and phenolic compounds, and their cytotoxic activities against several tumor cell lines or inhibitory effect against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages.[1,3]

As part of our efforts to discover constituents of Korean medicinal plants with antineuroinflammatory activity, we found that an EtOAc phase obtained from a MeOH extract (see Experimental Section) of the trunk of *A. holophylla* exhibited inhibitory effects on NO production in murine microglia BV-2 cells. The EtOAc solubles were successively chromatographed through silica gel, Sephadex LH-20, and prep high-performance liquid chromatography (HPLC). The result was the isolation of six new lignans (**1**−**6**) and 11 known lignans (**7**−**17**). Their structures were determined by spectroscopic methods, including one-dimensional (1D) and two-dimensional (2D) NMR, HRMS, CD experiments, acid hydrolysis, and the use of the modified Mosher's method. Herein, we report the isolation and structural elucidation of compounds **1**−**7**, along with NGF secretion and inhibitory effects on NO production of isolates (**1**−**17**). (see Figure S1 of the Supporting Information for structures of the known compounds).

Compound **1** had a molecular formula of $C_{21}H_{26}O_7$, as determined from the ion peak $[M + H]^+$ at m/z 391.1757 in positive ion high-resolution fast-atom bombardment mass spectrometry (HRFABMS). The ^{1}H NMR spectrum showed the presence of two 1,3,4-trisubstituted aromatic rings [δ_H 6.77 (1H, d, J = 2.0 Hz, H-2), 6.71 (1H, d, J = 7.5 Hz, H-5), and 6.62 (1H, dd, J = 7.5, 2.0 Hz, H-6) and 6.87 (1H, d, J = 1.5 Hz, H-2′), 6.75 (1H, d, J = 8.0 Hz, H-5′), and 6.74 (1H, dd, J = 8.0, 1.5 Hz, H-6′)], one acetal methine [δ_H 4.67 (1H, d, J = 1.0 Hz, H-9)], one oxymethine [δ_H 4.52 (1H, d, J = 9.0 Hz, H-7′)], three OCH_3 groups [δ_H 3.84 (3H, s, 3-OCH_3), 3.83 (3H, s, 3′-OCH_3), and 3.19 (3H, s, 9-OCH_3)], one oxymethylene [δ_H 3.71 (1H, t, J = 8.5 Hz, H-9′a) and 3.51 (1H, t, J = 8.5 Hz, H-9′b)], one methylene [δ_H 2.75 (1H, dd, J = 13.0, 4.5 Hz, H-7a) and 2.48 (1H, m, H-7b)], and two methine protons [δ_H 2.56 (1H, m, H-8) and 2.35 (1H, m, H-8′)]. The ^{13}C NMR spectrum contained 21 signals, including 12 aromatic carbons for two aromatic rings, one acetal methine [δ_C 109.5 (C-9)], one oxymethine [δ_C 75.8 (C-7′)], one oxymethylene [δ_C 68.5 (C-9′)], three OCH_3 groups [δ_C 55.2 (3-OCH_3), 55.1 (3′-OCH_3), and 53.5 (9-OCH_3)], two methine [δ_C 51.6 (C-8′) and 50.3 (C-8)], and one methylene [δ_C 39.2 (C-7)] carbons. These spectroscopic data suggested that **1** was a 9-O-9′ subtype tetrahydrofuran lignan,[4,5] and these data were similar to those of *iso*-α-intermedianol except for the absence of signals of an OCH_3 at C-7′ [δ_H 3.33 (3H, s); δ_C 56.3].[5] ^{1}H−^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum correlation spectroscopy (HMQC), and heteronuclear multiple bond correlation spectroscopy (HMBC) spectra (Figure S2 of the Supporting Information) confirmed the planar structure of **1**. The relative configuration of **1** was elucidated from the coupling constants and NOESY correlations. The small J value between H-8/H-9 (1.0 Hz) showed that H-8 and H-9 were in the *trans* form[5−7] and the NOESY correlations of H-7/H-9 and H-8′ and H-8/H-7′ corroborated the relative configurations at C-8, C-9, C-7′, and C-8′ (Figure S3 of the Supporting Information). In the CD spectrum, negative Cotton effects at 224 and 284 nm indicated that **1** had 8*R* and 8′*R* absolute configuration.[8,9] Through Mosher's method with (*R*)- and (*S*)-

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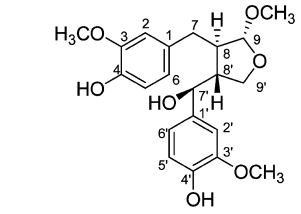
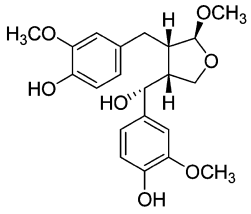
MPA, the absolute configuration at C-7′ was confirmed as *S* (Figure S4 of the Supporting Information).[10,11] (−)-Koreanol[12] was reported without determination of the configuration at C-7′, and its ^{1}H and ^{13}C NMR spectra were quite similar to those of **1**. The *J* value of H-7′ in (−)-koreanol (d, *J* = 5.2 Hz) was reported to be different from that of **1** (d, *J* = 9.0 Hz), indicating that (−)-koreanol could be the C-7′ epimer of **1**. Thus, compound **1** was determined to be (8*R*,9*R*,7′*S*,8′*R*)-4,4′,7′-trihydroxy-3,3′,9-trimethoxy-9,9′-epoxylignan, and it was named holophyllol A.



1



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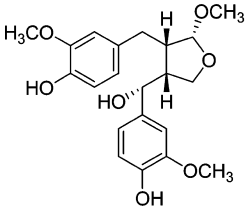

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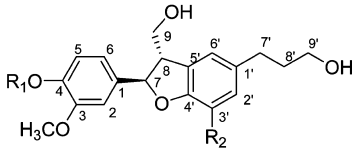
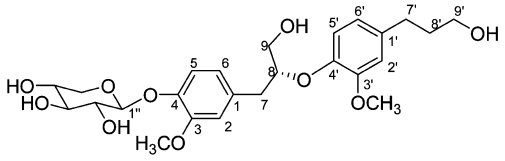


	R_1	R_2
4	α-L-Rha	H
5	3-*O*-Methyl-α-L-rha	OH



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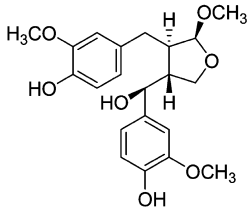

7

Compound **2** was assigned the molecular formula of $C_{21}H_{26}O_7$ by high-resolution electrospray ionization mass spectrometry (HRESIMS). The ^{1}H and ^{13}C NMR spectra of **2** closely resembled those of **1** but with an upfield shift of H-9′ (δ_H 4.18 and 3.99) and downfield shifts of H-7, 8, 7′, and 8′ (δ_H 2.40 and 2.04, 1.95, 4.42, and 2.22, respectively), indicating that **2** was a stereoisomer of **1**. The small *J* value between H-8/H-9 (1.0 Hz) and NOESY correlations of H-7/H-9 and H-7′ and H-8/H-8′ and H-9′-OCH$_3$ confirmed the relative configuration of **2**.[5−7] The CD spectrum of **2**, which showed negative Cotton effects at 228 and 286 nm, was quite similar to that of (8*S*,8′*R*,9*S*)-cubebin,[9] thus, the absolute configurations at C-8 and C-8′ were determined as *S* and *R*, respectively. Mosher's esterification with (*R*)- and (*S*)-MPA revealed that the absolute configuration at C-7′ of **2** was *R*. Therefore, the structure of **2**, named holophyllol B, was unequivocally defined as (8*S*,9*S*,7′*R*,8′*R*)-4,4′,7′-trihydroxy-3,3′,9-trimethoxy-9,9′-epoxylignan.

Compound **3** also had a molecular formula of $C_{21}H_{26}O_7$. Comparison of the NMR spectra of **3** with those of **2** showed they were very similar. However, upfield shifts of C-7 (δ_C 32.9) and C-9 (δ_C 105.4) were observed in ^{13}C NMR spectrum of **3**, suggesting that the relative configuration of **3** was identical with that of **2**, except at C-9. The large *J* value H-8/H-9 (4.5 Hz) and NOESY correlations of H-7/H-7′ and H-8/H-8′ and H-9 confirmed the relative configuration of **3**.[5,6,13] The absolute configurations at C-8 and C-8′ were determined as *S* and *R*, respectively, from the CD spectrum which was very similar to that of **2**, and the 7′*R* configuration was determined by the modified Mosher's method. Thus, the structure of **3** was established as (8*S*,9*R*,7′*R*,8′*R*)-4,4′,7′-trihydroxy-3,3′,9-trimethoxy-9,9′-epoxylignan, and it was named holophyllol C.

Compound **4** was obtained as a colorless gum. The molecular formula was determined to be $C_{25}H_{32}O_9$ from the $[M + Na]^+$ ion in the positive ion HRESIMS. The ^{1}H and ^{13}C NMR spectra of **4** suggested that **4** was a dihydrobenzofuran neolignan glycoside and were similar to those of **11**,[14] except for the absence of signals of an OH group at C-5′ (δ_C 145.1) of **11**. ^{1}H−^{1}H COSY, HMQC, and HMBC correlations confirmed the planar structure of **4**. The HMBC correlation of H-1″ to C-4 indicated that the rhamnose unit was linked to the oxygen at C-4, and the *J* value of anomeric proton (*J* = 1.5 Hz) confirmed it as α-rhamnose.[15] Acid hydrolysis of **4** afforded the aglycone, cedrusinin, and L-rhamnose ($[\alpha]_D^{25}$ +9.0), which was identified by co-TLC confirmation and gas chromatography (GC) analysis.[16,17] The identification of the aglycone was by comparison of its ^{1}H NMR and MS data.[18,19] The trans-configuration between H-7 and H-8 was confirmed through the *J* value (6.0 Hz),[20] and its CD spectrum showed the negative Cotton effect at 236 nm confirming the absolute configurations as 7*R* and 8*S*.[21,22] Cedrusinin-4-*O*-α-L-rhamnopyranoside was isolated without determination of the absolute configuration at C-7 and C-8,[15] and its ^{1}H and ^{13}C NMR spectra were quite similar to those of **4**. From the opposite sign of optical rotation values between cedrusinin-4-*O*-α-L-rhamnopyranoside ($[\alpha]_D^{23}$ −50.0) and **4** ($[\alpha]_D^{25}$ +16.0), cedrusinin-4-*O*-α-L-rhamnopyranoside should be 7*S* and 8*R* forms. Thus, the structure of **4** was determined to be (7*R*,8*S*)-cedrusinin 4-*O*-α-L-rhamnoside.

Compound **5** displayed an HRESIMS ion peak, $[M + Na]^+$ at *m*/*z* 529.2052, consistent with the molecular formula $C_{26}H_{34}O_{10}$. The ^{1}H and ^{13}C NMR spectra were very similar to those of **13**,[23] except for absence of signals of an OCH$_3$ [δ_H 3.86 (3H, s); δ_C 55.6]. The location of the two OCH$_3$ groups in **5** was established by the HMBC correlations from 3-OCH$_3$ (δ_H 3.85) to C-3 (δ_C 152.2) and from 3″-OMe (δ_H 3.53) to C-3″ (δ_C 82.0). Additionally, the HMBC correlation from H-1′ to C-4 showed that the sugar unit was located at C-4. Acid hydrolysis of **5** gave the aglycone, **8**, which was identified as cedrusin by comparison of its ^{1}H NMR and MS data,[24] and 3-*O*-methyl-L-rhamnose, which was identified by co-TLC confirmation, optical rotation ($[\alpha]_D^{25}$ +10.3), ^{1}H NMR and MS data,[25,26] and GC analysis. The relative configuration between H-7 and H-8 of **5** was determined by the *J* value (6.0 Hz) as *trans*,[20] and the negative Cotton effect at 245 nm in the CD spectrum confirmed the 7*R* and 8*S* configuration.[21,22] Thus, **5** was elucidated as (7*R*,8*S*)-cedrusin 4-*O*-(3-*O*-methyl-α-L-rhamnoside).

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Compound **6** had a molecular formula of $C_{25}H_{34}O_{10}$, as determined by the ion peak $[M + Na]^+$ at m/z 517.2048. The ^1H and ^{13}C NMR spectra were similar to those of ligraminol E,[27] except for the presence of signals of a xylose unit [δ_H 4.80 (1H, d, J = 7.0, H-1″), 3.89 and 3.29 (each 1H, m, H-5″), 3.55 (1H, m, H-4″), 3.46 (1H, m, H-2″), and 3.42 (1H, m, H-3″); δ_C 102.2 (C-1″), 75.9 (C-3″), 73.2 (C-2″), 69.6 (C-4″), and 65.4 (C-5″)]. This was supported by $^1H-^1H$ COSY, HMQC, and HMBC spectra. The xylose unit was placed at C-4 by the observation of an HMBC correlation from H-1″ to C-4. Acid hydrolysis of **6** afforded the aglycone, ligraminol E, and D-xylose ($[\alpha]_D^{25}$ +21.1), which was identified by co-TLC and GC analysis.[16,17] The aglycone was identified as ligraminol E by comparison of its ^1H NMR and MS data.[27] The absolute configuration of **6** at C-8 was confirmed to be 8*R* by the negative Cotton effect at 232 nm in the CD spectrum of ligraminol E.[27] On the basis of these results, the structure of **6** was established as ligraminol E 4-*O*-*β*-D-xyloside.

Compound **7** had the molecular formula of $C_{21}H_{26}O_7$. Compounds **7** and **1** had very similar NMR spectra, but marked differences included an upfield shift of C-7 (δ_C 34.2) and C-9 (δ_C 106.2) in the ^{13}C NMR spectrum and a small change of chemical shift of methine and methylene protons at H-7, 8, 9, 7′, 8′, and 9′ in the ^1H NMR spectrum. Extensive 1D and 2D NMR analyses confirmed that the planar structure of **7** was the same as that of **1** but that the configuration at C-9 was different. The large J value between H-8/H-9 (4.5 Hz) confirmed that H-8 and H-9 were in the *cis* form[5,6,13] and NOESY correlations of H-8/H-9 and H-7′ and H-7/H-8′ confirmed the relative configurations at C-8, C-9, C-7′, and C-8′. Pseudolarkaemin A was isolated from *Pseudolarix kaempferi* without determination of the absolute configuration.[13] ^1H and ^{13}C NMR, HRMS, specific optical rotation, UV, and IR data of pseudolarkaemin A were almost identical with those of **7**. The absolute configurations at C-8 and C-8′ were determined to be *R* by CD spectrum, which was quite similar to that of **1**. The absolute configuration at C-7′ was elucidated to be *S* using the same method as for **1**. The irregular $\Delta\delta$ value of H-8′ (+0.023) was due to the anisotropic effect of the α-aromatic group.[28] Thus, **7** was established as (8*R*,9*S*,7′*S*,8′*R*)-4,4′,7′-trihydroxy-3,3′,9-trimethoxy-9,9′-epoxylignan. Although compound **7** was a known, pseudolarkaemin A, the absolute configuration of it had not been reported earlier.

The 11 known compounds were identified as (8*R*,9*S*,7′*S*,8′*R*)-4,4′,7′-trihydroxy-3,3′,9-trimethoxy-9,9′-epoxylignan (**7**), cedrusin (**8**),[24] (2*R*,3*S*)-2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4′-*O*-*β*-D-glucopyranoside (**9**),[29] 7*R*,8*S*-dihydrodehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside (**10**),[22] isomassonianoside B (**11**),[14] icariside E_4 (**12**),[30] juniperigiside (**13**),[23] (−)-secoisolariciresinol (**14**),[31] (2*R*,3*R*)-2*β*-(4″-hydroxy-3″-methoxybenzyl)-3*α*-(4′-hydroxy-3′-methoxybenzyl)-*γ*-butyrolactone (**15**),[32] conidendrin (**16**),[33] and tanegool (**17**)[34] by comparison of their spectroscopic data and specific optical rotation with the reported data (see the Supporting Information for structures of the known compounds).

Compounds **1**−**17** were evaluated for their effects on NGF secretion using an enzyme-linked immunosorbant assay (ELISA) development kit from C6 glioma cells to measure NGF release into the medium and in a cell viability by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. As shown in Table S1 of the Supporting Information, compounds **6**, **7**, and **13** exhibited significant induction of NGF secretion (169 ± 9%, 165 ± 9%, and 159 ± 11%, respectively), which displayed more activitiy than 6-shogaol (129 ± 11%), a positive control, at a concentration of 10 μM. The other compounds were considered moderately active or inactive.

Antineuroinflammatory activities of the isolates (**1**−**17**) were also tested via measurement of NO levels using bacterial endotoxin, LPS, in the murine microglia BV-2 cell line. Compounds **1**, **5**, **6**, and **16** moderately inhibited NO production with IC_{50} values of 36.4, 32.9, 31.0, and 28.5 μM, respectively, in LPS-stimulated BV-2 cells without cell toxicity (Table S2 of the Supporting Information).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. Infrared spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. CD spectra were measured on a JASCO J-810 spectropolarimeter. UV spectra were recorded using an Agilent 8453 UV−visible spectrophotometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C), respectively. HRFABMS spectra were obtained on a JEOL JMS700 mass spectrometer and HRESIMS spectra were recorded on a Micromass QTOF2-MS. Preparative 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 (CC). TLC was performed using Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates. The packing material for molecular sieve CC was Sephadex LH-20 (Pharmacia Company). Low-pressure liquid chromatography was performed over Merck Lichroprep Lobar-A (240 × 10 mm) column with an FMI QSY-0 pump (ISCO).

Plant Material. *A. holophylla* trunk material was collected in Seoul, Korea, in January 2012, and the plant was identified by one of the authors (K.R.L.). A voucher specimen (SKKU-NPL 1205) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The trunk material of *A. holophylla* (5.0 kg) was extracted with 80% aq MeOH under reflux and was filtered. The filtrate was evaporated under reduced pressure to obtain a MeOH extract (280 g), which was suspended in distilled H_2O and successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-butanol, yielding 23, 43, 17, and 35 g of residues, respectively. The EtOAc-soluble fraction (17 g) was separated over a RP-C_{18} silica gel column with 50% aq MeOH to yield eight subfractions (E1-E8). Fraction E3 (3.4 g) was separated on a silica gel column ($CHCl_3$−MeOH−H_2O, 4:1:0.1) to give ten subfractions (E31−E310). Fraction E34 (130 mg) was separated on a Lichroprep Lobar-A RP-C_{18} column (25% aq CH_3CN) and then by preparative HPLC (25% aq CH_3CN), to yield compound **17** (6 mg). Fraction E35 (50 mg) was separated by preparative HPLC (25% aq CH_3CN), to give compound **5** (4 mg). Fraction E38 (610 mg) was separated on a RP-C_{18} silica gel column (20% aq CH_3CN) and further by preparative HPLC (25% aq CH_3CN), to yield compounds **10** (7 mg) and **11** (20 mg). Compound **9** (9 mg) was obtained from E310 (90 mg) by preparative HPLC (20% aq CH_3CN). Fraction E5 (2.1 g) was separated over a Sephadex LH-20 column (60% aq MeOH) to yield eight subfractions (E51−E58). Fraction E52 (460 mg) was separated on a silica gel column ($CHCl_3$−MeOH, 8:1) and further by preparative HPLC (30% aq CH_3CN), to give compounds **6** (4 mg), **12**, (20 mg), **13** (7 mg), and **15** (2 mg). Fraction E55 (300 mg) was separated on a silica gel Waters Sep-Pak Vac 6 cm^3 ($CHCl_3$−MeOH, 30:1) and then by preparative HPLC (40% aq CH_3CN) to yield compounds **1** (15 mg), **7** (8 mg), **8** (2 mg), and **14** (2 mg). Fraction E6 (1.2 g) was separated by silica gel CC (CH_3Cl−MeOH, 7:1) to give eight subfractions (E61−E68). Fraction E61 (79 mg) was purified by preparative HPLC (30% aq CH_3CN) to yield compounds **2** (5 mg), **3** (3 mg), and **16** (4 mg). Fraction E68 (100 mg) was separated on a Sephadex LH-20 column (CH_2Cl_2-

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MeOH, 1:1) and further by preparative HPLC (30% aq CH_3CN) to give compound **4** (4 mg).

Holophyllol A (1). Colorless needles; mp 175−178 °C; $[\alpha]_D^{25}$ −50.6 (*c* 0.75, MeOH); IR (KBr) ν_{max} 3379, 2941, 2834, 1517, 1453, 1274, 1032 cm^{-1}; UV (MeOH) λ_{max} (log ε) 281 (1.5), 230 (3.0) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 284 (−1.2), 224 (−5.2) nm; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table S3; positive HRFABMS *m*/*z* 391.1757 $[M + H]^+$ (calcd for $C_{21}H_{27}O_7$, 391.1757).

Holophyllol B (2). Colorless needles; mp 159−161 °C; $[\alpha]_D^{25}$ −25.0 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3382, 2947, 2833, 1453, 1032 cm^{-1}; UV (MeOH) λ_{max} (log ε) 281 (1.6), 229 (3.0) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 286 (−1.5), 228 (−3.9) nm; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table S3; positive HRESIMS *m*/*z* 413.1570 $[M + Na]^+$ (calcd for $C_{21}H_{26}NaO_7$, 413.1576).

Holophyllol C (3). Colorless needles; mp 164−166 °C; $[\alpha]_D^{25}$ +12.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3358, 2945, 2832, 1452, 1033 cm^{-1}; UV (MeOH) λ_{max} (log ε) 281 (1.6), 229 (3.4) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 284 (−1.0), 228 (−2.4) nm; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table S3; positive HRESIMS *m*/*z* 413.1570 $[M + Na]^+$ (calcd for $C_{21}H_{26}NaO_7$, 413.1576).

Compound 4. Colorless gum; $[\alpha]_D^{25}$ +16.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3358, 2945, 2832, 1453, 1033 cm^{-1}; UV (MeOH) λ_{max} (log ε) 282 (1.6), 229 (4.0) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 292 (+1.7), 236 (−9.8) nm; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table S4; positive HRESIMS *m*/*z* 499.1938 $[M + Na]^+$ (calcd for $C_{25}H_{32}NaO_9$, 499.1944).

Compound 5. Colorless gum; $[\alpha]_D^{25}$ −16.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3358, 2945, 2832, 1452, 1033 cm^{-1}; UV (MeOH) λ_{max} (log ε) 280 (1.3), 231 (4.0) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 286 (−7.8), 250 (−4.8), 218 (+2.5) nm; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table S4; positive HRESIMS *m*/*z* 529.2052 $[M + Na]^+$ (calcd for $C_{26}H_{34}NaO_{10}$, 529.2050).

Compound 6. Colorless gum; $[\alpha]_D^{25}$ + 20.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3358, 2945, 2832, 1452, 1031 cm^{-1}; UV (MeOH) λ_{max} (log ε) 279 (1.5), 231 (4.5) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 232 (−1.9) nm; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table S4; positive HRESIMS *m*/*z* 517.2048 $[M + Na]^+$ (calcd for $C_{25}H_{34}NaO_{10}$, 517.2050).

Compound 7. Colorless needles; mp 168−170 °C; $[\alpha]_D^{25}$ +20.0 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3359, 2944, 2832, 1515, 1452, 1272, 1032 cm^{-1}; UV (MeOH) λ_{max} (log ε) 281 (1.6), 228 (2.9) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 286 (−1.5), 225 (−5.5) nm; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table S3; positive HRFABMS *m*/*z* 391.1757 $[M + H]^+$ (calcd for $C_{21}H_{27}O_7$, 391.1757).

Acid Hydrolysis of 4, 5, and 6. Compounds **4**, **5**, and **6** (each 2.0 mg) were individually hydrolyzed by 1 N HCl (1.0 mL) under reflux conditions for 2 h. The reaction mixtures were extracted with $CHCl_3$, and the organic layers evaporated under reduced pressure to yield cedrusinin,[18,19] cedrusin,[24] and ligraminol E[27] (each 1.0 mg). L-Rhamose, 3-*O*-methyl-L-rhamnose, and D-xylose were obtained from the aqueous layers of **4**, **5**, and **6**, respectively, by neutralization through an Amberlite IRA-67 column. L-Rhamose and D-xylose were identified by co-TLC confirmation with the authentic sample[16] and by optical rotation ($[\alpha]_D^{25}$ +9.0 for L-rha and $[\alpha]_D^{25}$ +21.1 for D-xyl). Identification of 3-*O*-methyl-L-rhamnose was performed by comparing the ^1H NMR, HRESIMS, and the optical rotation ($[\alpha]_D^{25}$ +10.3) in the literature[25,26] and co-TLC confirmation with that of **13** [solvent system ($CHCl_3$−MeOH, 4:1), R_f 0.25], which was hydrolyzed as above. The absolute configuration of the sugars were determined by a GC experiment according to reported procedures.[16,17]

(7R,8S)-Cedrusinin. Colorless gum; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 292 (+1.8), 236 (−10.0) nm; UV (MeOH) λ_{max} (log ε) 282 (2.0), 229 (4.1) nm; ^1H NMR (CD_3OD, 500 MHz) δ 7.10 (1H, br s, H-2′), 7.01 (1H, d, *J* = 2.0 Hz, H-2), 7.00 (1H, d, *J* = 8.0 Hz, H-6′), 6.90 (1H, d, H-6), 6.89 (1H, d, *J* = 8.0 Hz, H-5), 6.80 (1H, d, *J* = 8.0 Hz, H-5′), 5.48 (1H, d, *J* = 6.5 Hz, H-7), 3.85 (1H, m, H-9a), 3.82 (3H, s, 3-OCH_3), 3.76 (1H, m, H-9b), 3.56 (2H, t, *J* = 6.5 Hz, H-9′), 3.47 (1H, m, H-8), 2.66 (2H, t, *J* = 8.0 Hz, H-7′), 1.87 (2H, m, H-8′); positive ESIMS *m*/*z* 353 $[M + Na]^+$.

(7R,8S)-Cedrusin. Colorless gum; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 286 (−8.0), 250 (−5.8), 218 (+3.0) nm; UV (MeOH) λ_{max} (log ε) 280 (1.5), 231 (4.5) nm; ^1H NMR (CD_3OD, 500 MHz) δ 6.98 (1H, br s, H-2), 6.84 (1H, br d, *J* = 8.5 Hz, H-6), 6.76 (1H, d, *J* = 8.5 Hz, H-5), 6.60 (1H, s, H-6′), 6.57 (1H, s, H-2′), 5.48 (1H, d, *J* = 6.5 Hz, H-7), 3.85 (1H, m, H-9a), 3.82 (3H, s, 3-OCH_3), 3.74 (1H, dd, *J* = 11.0, 7.5 Hz, H-9b), 3.55 (2H, t, *J* = 6.5 Hz, H-9′), 3.45 (1H, dd, *J* = 11.0, 6.5 Hz, H-8), 2.56 (2H, t, *J* = 8.0 Hz, H-7′), 1.79 (2H, m, H-8′); positive ESIMS *m*/*z* 369 $[M + Na]^+$.

Ligraminol E. Colorless gum; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 232 (−2.0) nm; UV (MeOH) λ_{max} (log ε) 279 (2.0), 231 (4.8) nm; ^1H NMR (CD_3OD, 500 MHz) δ 6.82 (1H, d, *J* = 8.5 Hz, H-5′), 6.81 (2H, br s, H-2 and 2′), 6.70 (1H, br d, *J* = 8.5 Hz, H-6′), 6.68 (1H, br d, *J* = 8.5 Hz, H-6), 6.67 (1H, d, *J* = 8.5 Hz, H-5), 4.35 (1H, m, H-8), 3.81 (3H, s, 3′-OCH_3), 3.79 (3H, s, 3-OCH_3), 3.63 (2H, m, H-9), 3.55 (2H, t, *J* = 6.5 Hz, H-9′), 2.89 (2H, dd, *J* = 6.0, 2.0 Hz, H-7), 2.61 (2H, t, *J* = 8.0 Hz, H-7′), 1.79 (2H, m, H-8′); positive ESIMS *m*/*z* 385 $[M + Na]^+$.

NGF and Cell Viability Assay. C6 Glioma cells were used to measure NGF release into the medium.[35] C6 cells were purchased from the Korean Cell Line Bank (Seoul, Korea) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) in a humidified incubator with 5% CO_2. To measure NGF content in medium and cell viability, C6 cells were seeded into 24-well plates (1×10^5 cells/well). After 24 h, the cells were treated with DMEM containing 2% FBS and 1% PS with 20 μM of each sample for one day. Media supernatant was used for the NGF assay using an ELISA development kit (R&D System, Minneapolis, MN). Cell viability was assessed by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay.

Measurement of NO Production and Cell Viability. BV-2 cells were maintained in DMEM supplemented with 5% FBS and 1% penicillin-streptomycin. To measure NO production, BV-2 cells were plated onto a 96-well plate (3×10^4 cells/well). After 24 h, the cells were pretreated with compounds for 30 min and stimulated with 100 ng/mL LPS for 24 h. Nitrite, a soluble oxidation product of NO, was measured in the culture media using the Griess reaction. The supernatant was harvested and mixed with an equal volume of Griess reagent (1% sulphanilamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid). After 10 min, the absorbance at 540 nm was measured using an Emax microplate reader (Molecular Devices, Sunnyvale, CA). Sodium nitrite was used as a standard to calculate the nitrite concentration. Cell viability was measured using the MTT assay. N^G-Monomethyl-L-arginine (L-NMMA; Sigma-Aldrich, St. Louis, MO), a well-known NO synthase inhibitor, was tested as a positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Bioactivities of **1**−**17**, ^1H and ^{13}C NMR data of **1**−**7**, structures of known compounds **8**−**17**, HRMS and 1D and 2D NMR data of **1**−**7**, CD spectra and $\Delta\delta_{R-S}$ values for the (*R*)- and (*S*)-MPA esters of **1**−**3** and **7**, ^1H NMR data of **1r**−**3r** and **7r**, **1s**−**3s** and **7s**, **4a**−**6a**, and 3-*O*-methyl-L-rhamnose. This 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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