***Regular Article***

# Secoiridoid Glucosides from the Twigs of *Syringa oblata* var. *dilatata* and Their Neuroprotective and Cytotoxic Activities

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**Phytochemical investigation of the twigs of *Syringa oblata* var. *diatata* led to the isolation of two new secoiridoid glucosides, dilatioside A–B (1–2), along with thirteen known ones (3–15). The structures were determined by spectroscopic methods including one and two dimensional (1- and 2D-) NMR techniques, high resolution (HR)-FAB-MS, and chemical methods. The isolated compounds (1–15) were tested for the induction of nerve growth factor (NGF) secretion in a C6 rat glioma cell line and their cytotoxicity against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, HCT15) *in vitro* using a sulforhodamine B bioassay. Compounds 5, 7, 8, 10, and 14 were found to induce upregulation of NGF secretion without causing significant cell toxicity.**

**Key words** *Syringa oblata* var. *dilatata*; Oleaceae; secoiridoid glucoside; nerve growth factor; cytotoxicity

Current research is focused on finding neurotrophin signaling-mediated neuroprotection against neurodegenerative diseases.[1)] Phytochemicals that trigger the production of neurotrophins such as nerve growth factor (NGF) will protect neurons against neuronal degeneration. NGF mainly acts in the growth, development, and survival of neurons, and has been suggested to play an important role in neurodegenerative diseases. NGF not only helps the survival and growth but also the differentiation of sensory and sympathetic neurons in the central and peripheral nervous systems. NGF signaling serves neuroprotective and repair functions.[2)] Higher NGF production ensures the protection of axons and myelin sheathes against inflammation *via* modulation of the immune system and reduction of endotoxin- or inflammation-induced toxicity in the brain.[3)] Moreover, deficiency in NGF results in neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, and diabetic polyneuropathy.[4,5)] Thus, regulation of NGF secretion or treatment with NGF mimetics is one method for the prevention and repair of neurodegenerative disorders. The immune modulatory and strong neuroprotective efficacy of NGF has resulted in it becoming a potential target for the screening of phytochemicals for neurodegenerative diseases.

*Syringa* genus has long been used for the treatment of asthma, inflammation, and liver and intestinal disorders.[6–9)] Previous phytochemical investigations regarding this genus have reported the isolation of secoiridoid glucosides, lignans, and phenolic compounds with anti-radical scavenging and cytotoxic activity.[10)] However, only a few phytochemical studies on *S. oblata* var. *dilatata* have been reported. We found that the MeOH extract of the twig of *S. oblata* var. *dilatata* induces increases in the levels of endogenous NGF in C6 glioma cells. Thus, we investigated the bioactive constituents of the aerial parts of *S. oblata* var. *dilatata*. The column chromatographic purification of the EtOAc-soluble fraction led to the isolation of two new secoiridoid glucosides, dilatioside A–B (**1**–**2**), along with thirteen known ones (**3**–**15**). The chemical structures of the isolated compounds were determined by their NMR spectroscopic data, MS, and methanolysis. The isolated compounds were tested for their ability to induce NGF secretion and for their cytotoxic activities. This paper describes the isolation and structural elucidation of the two new compounds (**1**–**2**) as well as their neuroprotective and antiproliferative activities.

## Results and Discussion

The methanol extract of the twigs of *S. oblata* var. *dilatata* was partitioned successively with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. Repeated column chromatographic purification of the EtOAc-soluble fraction afforded two new secoiridoid glucosides (**1**–**2**), together with thirteen known secoiridoid glucoside derivatives (**3**–**15**) (Fig. 1).

Compound **1** was obtained as an amorphous gum. The molecular formula of **1** was determined to be $C_{37}H_{48}O_{16}$ on the basis of a $[M+Na]^+$ peak at *m/z* 771.2844 (Calcd for $C_{37}H_{48}NaO_{16}$, 771.2840) in positive high-resolution (HR)-FAB-MS. The $^1$H-NMR spectrum displayed the typical oleoside methyl ester moiety[11)]; two olefinic protons [$\delta_H$ 7.56 (1H, s, H-3), 6.13 (1H, m, H-8)], one hemiacetalic proton [$\delta_H$ 5.99 (1H, br s, H-1)], one methine [$\delta_H$ 4.03 (1H, dd, $J$=9.0, 4.6 Hz, H-5)], one methylene [$\delta_H$ 2.53 (1H, dd, $J$=14.0, 9.0 Hz, H-6a), 2.75 (1H, dd, $J$=14.0, 4.6 Hz, H-6b), one methyl [$\delta_H$ 1.70 (3H, dd, $J$=7.1, 1.4 Hz, H-10)], one methyl ester group [$\delta_H$ 3.71 (3H, s, 11-$OCH_3$)], and one glucopyranosyl unit [$\delta_H$ 4.83 (1H, d, $J$=7.8 Hz, H-1′), 3.32 (1H, m, H-2′), 3.42 (1H, t, $J$=9,1 Hz, H-3′), 3.27 (1H, m, H-4′), 3.33 (1H, overlap, H-5′), 3.62 (1H, dd, $J$=12.0, 6.4 Hz, H-6′a), 3.88 (1H, dd, $J$=11.9, 2.1 Hz, H-6′b) ]. Furthermore, the $^1$H-NMR data exhibited the presence of a (−)-secoisolariciresinol[12)]; six aromatic protons [$\delta_H$ 6.69 (2H, d, $J$=8.0 Hz, H-5‴, 5⁗), 6.61 (1H, d, $J$=1.8 Hz, H-2⁗), 6.58 (1H, dd, $J$=8.0, 1.8 Hz, H-6‴), 6.55 (1H, dd, $J$=8.0, 1.8 Hz, H-6⁗), and 6.53 (1H, d, $J$=1.8 Hz, H-2‴)], two oxygenated methylenes [$\delta_H$ 4.29 (1H, dd, $J$=11.1, 5.8 Hz,

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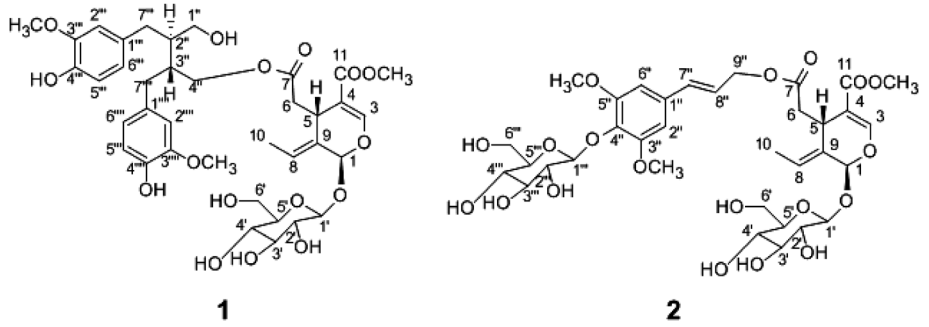

1 2

Fig. 1. Chemical Structures of Compounds **1** and **2**

H-4″b), 3.93 (1H, dd, *J*=11.1, 6.3 Hz, H-4″a), 3.70 (1H, dd, *J*=10.9, 6.7 Hz, H-1″b), 3.53 (1H, m, H-1″a)], two methylenes [$\delta_H$ 2.71 (1H, dd, *J*=13.9, 6.5 Hz, H-7‴b), 2.57 (1H, dd, *J*=13.9, 8.7 Hz, H-7‴a), 2.62 (2H, m, H-7⁗)], two methines [$\delta_H$ 2.13 (1H, m, H-3″), 1.94 (1H, m, H-2″) and two aromatic methoxy groups [$\delta_H$ 3.76, 3.75 (3H each, s, 3‴,3⁗-$OCH_3$)] (Table 1). The $^{13}$C-NMR spectrum showed signals for an oleoside methyl ester moiety and (−)-secoisolariciresinol[12] (Table 1). The connectivity between C-7 and C-4″ (oleoside dimethyl ester (**1a**) and (−)-secoisolariciresinol (**1b**)) was confirmed through heteronuclear multiple bond connectivity (HMBC) correlation (Fig. 2). The HMBC correlation of H-1′ to C-1 indicated that the glucopyranose unit was linked to the oxygen at C-1, and the *J* value of the anomeric proton (*J*=7.8 Hz) confirmed it as the *β*-configuration.[13] This gross structure was confirmed by analysis of the $^1$H–$^1$H correlation spectroscopy (COSY), $^1$H-detected heteronuclear multiple quantum coherence (HMQC), and HMBC spectra (Fig. 2). The configuration of the oleoside dimethyl ester moiety (**1a**) was identified by comparison of the NMR and physical data with oleoside dimethyl ester (**3**) previously isolated from *Fraxinus excelsior*.[11] (−)-Secoisolariciresinol moiety (**1b**) was confirmed by comparison the optical rotation and $^1$H-NMR data with previously reported values.[12] The stereochemistry in **1** was reconfirmed through nuclear Overhauser effect spectroscopy (NOESY) correlations and biosynthetic aspect[14] (Fig. 3). Alkaline methanolysis of **1** afforded oleoside dimethyl ester (**1a**) and (−)-secoisolariciresinol (**1b**).[12] **1a** was identified by comparison of their $^1$H-NMR data and **1b** was identified as (2″*R*,3″*R*)-secoisolarisiresinol by comparison with their $^1$H-NMR data and negative specific rotation {$[\alpha]_D^{25}$ −20.0 (*c*=0.05, MeOH)} with the reported values, respectively.[12] And the configurations at C-2″ and C-3″ in **1b** were confirmed by comparison of negative Cotton effects at 228 and 287 nm in the circular dichroism (CD) spectrum.[12] Acid hydrolysis of **3**(**1a**) afforded D-glucopyranose, which was identified by co-TLC with authentic samlple ($CHCl_3$–MeOH–$H_2O$=2 : 1 : 0.1, *Rf*=0.3) and specific optical rotation {$[\alpha]_D^{25}$ +105.0 (*c*=0.04, MeOH)}.[15]

Thus, the structure of **1** was established as shown in Fig. 1, and this compound was named dilatioside A.

Compound **2** was isolated as an amorphous gum with the molecular formula $C_{34}H_{46}O_{19}$ based on the positive HR-FAB-MS data (*m/z* 781.2534 $[M+Na]^+$, Calcd for $C_{34}H_{46}NaO_{19}$, 781.2531). The $^1$H- and $^{13}$C-NMR spectra displayed the typical oleoside methyl ester[11] moiety. Moreover, the $^1$H-NMR data exhibited the presence of a syringin moiety[16]; aromatic protons [$\delta_H$ 6.80 (2H, s, H-2″, 6″)], one *trans*-substituted double bond [$\delta_H$ 6.64 (1H, d, *J*=15.7 Hz, H-7″), 6.30 (1H, dt, *J*=15.9, 6.3 Hz, H-8″)], one oxygenated methylene [$\delta_H$ 4.78 (1H, m, H-9″a, 4.68 (1H, m, H-9″b)], two methoxy groups [$\delta_H$ 3.90 (6H, s, 3′,5′-$OCH_3$)]; $\delta_C$ 154.57 (C-3″,5″), 136.48 (C-4″), 135.08 (C-7″), 134.64 (C-1″), 124.51 (C-8″), 105.88 (C-2″,6″) and 66.39 (C-9″), and a glucopyranose unit [$\delta_H$ 4.92 (1H, d, *J*=6.5 Hz, H-1‴), 3.50 (1H, m, H-2‴), 3.45 (1H, m, H-3‴), 3.44 (1H, m, H-4‴), 3.25 (1H, m, H-5‴), 3.69 (1H, m, H-6‴a), 3.81 (1H, m, H-6‴b)], together with the $^{13}$C-NMR data [$\delta_C$ 105.4 (C-1‴), 75.9 (C-2‴), 78.0 (C-3‴), 71.5 (C-4‴), 78.6 (C-5‴), 62.8 (C-6‴)] (Table 1). The connectivity between C-7 and C-9″ (oleoside dimethyl ester (**1a**) and syringin (**2a**)) was confirmed through HMBC correlation (Fig. 2). The HMBC correlation from H-1‴ to C-4″ showed that the D-glucopyranose unit was located at C-4″, and the *J* value of the anomeric proton (*J*=6.5 Hz) confirmed it as *β*-D-glucopyranose.[13] This gross structure was confirmed by analysis of the $^1$H–$^1$H COSY, HMQC, and HMBC spectra (Fig. 2). The configuration of the oleoside dimethyl ester moiety (**1a**) was assumed by comparison of the NMR and physical data with previously isolated oleoside dimethyl ester (**3**).[11] The stereochemistry in **2** was reconfirmed through NOESY correlations and biosynthetic aspect[14] (Fig. 3). Alkaline methanolysis of **2** afforded oleoside dimethyl ester (**1a**) and syringin (**2a**). **2a** was identified by comparison of the co-TLC; ($CHCl_3$–MeOH–$H_2O$=3 : 1 : 0.1, *Rf*=0.38) and $^1$H-NMR data with the reported values, respectively.[16] Thus, the structure of **2** was determined as shown in Fig. 1, and named dilatioside B. The thirteen known secoiridoid glucoside derivatives were identified as oleoside dimethyl ester (**3**),[11] ligustroside (**4**),[11] oleuropein (**5**),[17] (2″*R*)-2″-methoxyoleuropein (**6**),[18] fraxamoside (**7**),[19] hydroxyframoside A (**8**),[20] syringalactone A (**9**),[21] syringalactone B (**10**),[21] (8*E*)-nüzhenide (**11**),[22] (8*Z*)-nuezhenide A (**12**),[23] jaspolyanoside (**13**),[13] jaspolyoside (**14**),[24] oleonuezhenide (**15**)[25] by comparison of their spectroscopic data with the data reported in the literatures.

The neuroprotective activities of the isolates (**1**–**15**) were evaluated by determining their effects on NGF secretion in C6 cells (Table 2). Of the tested compounds at 50 $\mu$M, compounds **5**, **7**, **8**, **10**, and **14** were potent stimulants of NGF release, with stimulation levels of 201.58±4.41, 207.48±15.41, 205.64±4.84, 196.85±4.71, and 171.64±1.61%, respectively (the positive control 6-shogaol was 168.58±7.16%), while compounds **6**, **12**, and **13** exhibited moderate activities (Table 2). Interestingly, structural differences in the tested compounds displayed different NGF secretion stimulatory levels; that is, although the structures of **4**, **9**, and **13** are quite similar to those of **5**, **10**, and **14**, with the exception of the presence of the hydroxy group at C-3 in the aromatic ring, their effects on NGF

Table 1. $^{1}H$- (700 MHz) and $^{13}C$- (175 MHz) NMR Spectral Data of **1** and **2** in $CD_3OD$ ($\delta$ in ppm)[a)]

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 5.99 brs | 95.1 | 5.97 s | 95.6 |
| 3 | 7.56 s | 155.3 | 7.56 s | 155.3 |
| 4 | | 109.5 | | 109.5 |
| 5 | 4.03 dd (9.0, 4.6) | 32.2 | 4.05 m | 32.1 |
| 6 | 2.53 dd (14.0, 9.0) | 41.5 | 2.58 m | 41.3 |
| | 2.75 dd (14.0, 4.6) | | 2.79 d (13.6) | |
| 7 | | 173.4 | | 173.1 |
| 8 | 6.13 m | 124.9 | 6.13 m | 125.1 |
| 9 | | 130.8 | | 130.7 |
| 10 | 1.70 dd (7.1, 1.4) | 13.8 | 1.75 brs | 13.8 |
| 11 | | 168.8 | | 168.8 |
| 11-$OCH_3$ | 3.71 s | 52.1 | 3.74 s | 52.1 |
| 1′ | 4.83 d (7.8) | 100.8 | 4.78 brs | 101.2 |
| 2′ | 3.32 m | 74.9 | 3.31 m | 74.9 |
| 3′ | 3.42 t (9.1) | 78.6 | 3.43 m | 78.1 |
| 4′ | 3.27 m | 71.7 | 3.31 m | 71.6 |
| 5′ | 3.33 overlap | 78.1 | 3.32 m | 78.5 |
| 6′ | 3.62 dd (12.0, 6.4) | 62.9 | 3.66 m | 62.9 |
| | 3.88 dd (11.9, 2.1) | | 3.89 m | |
| 1″ | 3.53 m | 63.0 | | 134.64 |
| | 3.70 dd (10.9, 6.7) | | | |
| 2″ | 1.94 m | 44.4 | 6.80 s | 105.88 |
| 3″ | 2.13 m | 40.5 | | 154.57 |
| 4″ | 3.93 dd (11.1, 6.3) | 66.4 | | 136.48 |
| | 4.29 dd (11.1, 5.8) | | | |
| 5″ | | | | 154.57 |
| 6″ | | | 6.80 s | 105.88 |
| 7″ | | | 6.64 d (15.7) | 135.08 |
| 8″ | | | 6.30 dt (15.9, 6.3) | 124.51 |
| 9″ | | | 4.78 m | 66.39 |
| | | | 4.68 m | |
| 1‴ | | 133.8 | 4.92 d (6.5) | 105.4 |
| 2‴ | 6.53 d (1.8) | 113.4 | 3.50 m | 75.9 |
| 3‴ | | 148.9 | 3.45 m | 78.0 |
| 4‴ | | 145.7 | 3.44 m | 71.5 |
| 5‴ | 6.69 d (8.0) | 116.0 | 3.25 m | 78.6 |
| 6‴ | 6.58 dd (8.0, 1.8) | 122.9 | 3.69 m | 62.8 |
| | | | 3.81 m | |
| 7‴ | 2.57 dd (13.9, 8.7) | 35.9 | | |
| | 2.71 dd (13.9, 6.5) | | | |
| 1⁗ | | 133.3 | | |
| 2⁗ | 6.61 d (1.8) | 113.6 | | |
| 3⁗ | | 149.0 | | |
| 4⁗ | | 145.8 | | |
| 5⁗ | 6.69 d (8.0) | 116.0 | | |
| 6⁗ | 6.55 dd (8.0, 1.8) | 123.0 | | |
| 7⁗ | 2.62 m | 36.1 | | |
| 3′-$OCH_3$ | | | 3.90 s | 57.3 |
| 5′-$OCH_3$ | | | 3.90 s | 57.3 |
| 3‴-$OCH_3$ | 3.76 s | 56.4 | | |
| 3⁗-$OCH_3$ | 3.75 s | 56.4 | | |

*a*) Assignments were based on 2D-NMR including HMQC and HMBC. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hz in parentheses.

synthesis induction is so different (Fig. S1, Table 2). These data suggest that the presence of a 2-(3,4-dihydroxylphenyl)-ethoxycarbonyl moiety may be important for NGF induction.

The antiproliferative activities of compounds **1**–**15** were evaluated by the determination of their inhibitory effects on four tumor cell lines including A549 (lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells) using the SRB bioassay.[26)]

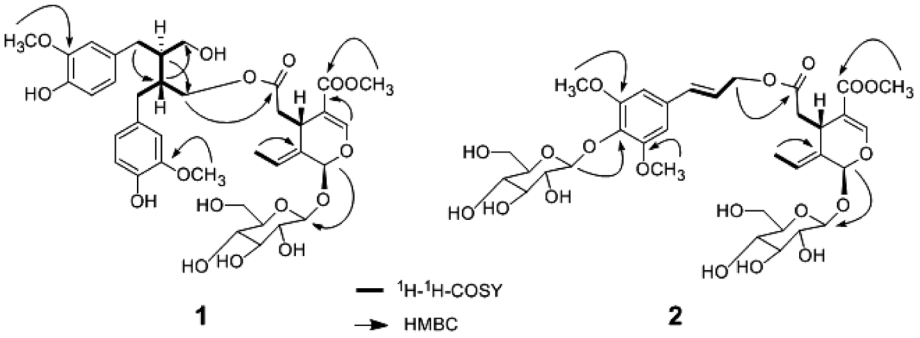


Fig. 2. Key HMBC and $^{1}H$–$^{1}H$ COSY Correlations of **1** and **2**

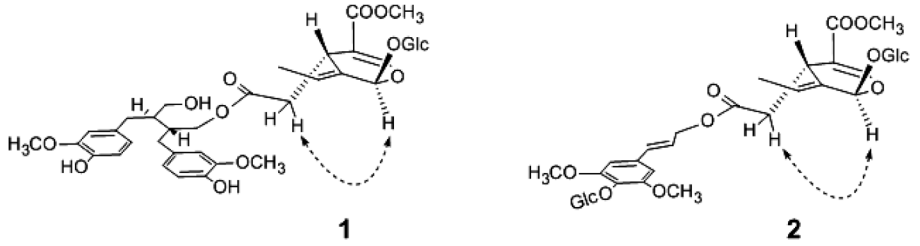


Fig. 3. Key NOESY Correlations of **1** and **2**

Table 2. Effects of Compounds **1**–**15** on NGF Secretion and Cell Viability in C6 Cells[a)]

| Compounds | NGF secretion (%) | Cell viability[b)] (%) |
|---|---|---|
| **1** | 97.36±0.77 | 103.24±2.70 |
| **2** | 93.20±15.02 | 112.47±7.27 |
| **3** | 69.32±16.90 | 97.28±0.81 |
| **4** | 94.79±10.36 | 90.20±4.78 |
| **5** | 201.58±4.41*** | 95.28±0.81 |
| **6** | 138.16±4.80* | 102.44±4.40 |
| **7** | 207.48±15.41*** | 101.56±1.38 |
| **8** | 205.64±4.84*** | 103.43±1.52 |
| **9** | 92.72±10.72 | 108.62±3.31 |
| **10** | 196.85±4.71*** | 97.11±0.04 |
| **11** | 78.47±10.27 | 99.08±1.31 |
| **12** | 139.33±10.58* | 108.59±2.10 |
| **13** | 114.40±2.62 | 111.70±1.42 |
| **14** | 171.64±1.61*** | 107.22±0.56 |
| **15** | 72.39±9.48 | 106.27±1.15 |
| 6-Shogaol[c)] | 168.58±7.16*** | 125.80±0.93 |

*a*) C6 cells were treated with 50 $\mu$g/mL compounds **1**–**15**. After 24 h, NGF secretion in C6-conditioned media was measured by ELISA and is expressed as a percentage of the untreated control. The data shown represent the mean±standard deviation (S.D.) of three independent experiments performed in triplicate. *b*) Cell viability after treatment with 50 $\mu$g/mL each extract was determined by an MTT assay and is expressed as a percentage of the untreated control (%). The results are the average of three independent experiments, and the data are expressed as the mean±S.D. *c*) 6-Shogaol as a positive control. *$p<0.05$, and ***$p<0.001$ indicate statistically significant differences in comparison to untreated control group.

Compounds **5** and **6** exhibited antiproliferative activity against the SK-MEL-2 cells, with $IC_{50}$ values of 10.86 and 14.64 $\mu$M, respectively, and compound **10** showed activity against the SK-OV-3 and SK-MEL-2 cells, with $IC_{50}$ values of 16.83 and 10.45 $\mu$M, respectively, but most of the compounds were inactive ($IC_{50}>30.0$ $\mu$M) against the human tumor cell lines tested.

## Experimental

**General Experimental Procedures** Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were recorded on a JASCO FT/IR-4600 spectrometer, and NMR spectra were recorded on a Bruker AVANCEIII 700 NMR spectrometer operating at 700 MHz ($^{1}H$) and 175 MHz ($^{13}C$) with chemical shifts given in ppm ($\delta$). FAB and HR-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer, and preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and a Phenomenex Luna 10 $\mu$m column (250×10 mm). Silica gel 60 (Merck, Darmstadt, 70–230, and 230–400 mesh) and RP-$C_{18}$ silica gel (Merck, 230–400 mesh) were used for column chromatography. Ion exchange resin (Dowex® 50WX8 hydrogen form, Sigma-Aldrich, U.S.A.) was used for alkali elimination. TLC was performed using Merck pre-coated silica gel $F_{254}$ plates and RP-18 $F_{254s}$ plates. Spots were detected under UV light or by heating after spraying with 10% $H_2SO_4$ in EtOH (v/v). Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar-A Si gel 60 (240×10 mm) with an FMI QSY-0 pump (ISCO).

**Plant Material** The twigs of *S. oblata* var. *dilatata* were collected at Suwon, Korea in June 2014. The plant was identified by one of the authors (K. R. Lee). A voucher specimen (SKKU-NPL 1404) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Republic of Korea.

**Extraction and Isolation** The air-dried and pulverized twigs of *S. oblata* var. *dilatata* (6.9 kg) were extracted with 80% MeOH three times at room temperature. The resultant MeOH extract (450 g) was suspended in distilled water and successively partitioned with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH, yielding 15, 25, 48 and 213 g, respectively. The EtOAc soluble fraction (48.0 g) was separated over a silica gel column (230–400 mesh, 350 g) eluted with $CHCl_3$–MeOH [20 : 1 (1.5 L), 15 : 1 (1.5 L), 10 : 1 (1.5 L), 6 : 1 (1.0 L), 3 : 1 (1.0 L) and 1 : 1 (1.5 L)] to afford ten fractions [Fr. A, 20 : 1, 1.0 L; Fr. B, 20 : 1, 0.5 L; Fr. C, 15 : 1, 1.0 L; Fr. D, 15 : 1, 0.5 L; Fr. E,

10 : 1, 0.5 L; Fr. F, 10 : 1, 0.5 L; Fr. G, 10 : 1, 0.5 L; Fr. H, 6 : 1, 1.0 L; Fr. I, 3 : 1, 1.0 L; Fr. J, 1 : 1, 1.5 L]. Fraction F (1.5 g) was separated over an RP-$C_{18}$ silica gel column (230–400 mesh, 80 g, 50% MeOH) to give seven subfractions [Fr. F1–F7 (each 1.0 L)]. Fraction F2 (274 mg) was purified by semi-preparative reversed-phase HPLC (flow rate; 2 mL/min, 40% MeCN) to yield **3** (7 mg, $t_R$=21.2 min). Fraction F4 (567 mg) was separated by semi-preparative reversed-phase HPLC (flow rate; 2 mL/min, 30% MeCN), as described above, to yield **4**, (130 mg, $t_R$=22.0 min). Fraction G (7.6 g) was chromatographed over an RP-$C_{18}$ silica gel column (230–400 mesh, 400 g, 50% MeOH) to give nine subfractions [Fr. G1–G9 (each 1.0 L)]. Fraction G3 (3.9 g) was separated over a silica gel column (230–400 mesh, 20 g, $CHCl_3$–MeOH–$H_2O$=8.5 : 1 : 0.1) to give six subfractions [Fr. G31–G36]. Fraction G34 (2.5 g) was separated by preparative reversed-phase HPLC (flow rate; 2 mL/min, 25% MeCN) to yield **5** (8 mg, $t_R$=27.8 min). Fraction G32 (2.5 g) was separated by preparative reversed-phase HPLC (flow rate; 2 mL/min, 25% MeCN) to yield **7** (4 mg, $t_R$=25.2 min) and **9** (4 mg, $t_R$=18.6 min). Fraction G6 (70 mg) was purified by preparative reversed-phase HPLC (flow rate; 2 mL/min, 30% MeCN) to yield **1** (11 mg, $t_R$=22.3 min). Fraction H (1.8 g) was separated over an RP-$C_{18}$ silica gel column (230–400 mesh, 80 g, 45% MeOH) to give twelve subfractions [Fr. H1–H12 (each 1.0 L)]. Fraction H3 (72 mg) was further separated using a Lobar-A Si gel 60 (240×10 mm) column ($CHCl_3$–MeOH–$H_2O$=5 : 1 : 0.1) to yield **10** (6 mg) and **11** (3 mg). Fraction H5 (94 mg) was separated by preparative reversed-phase HPLC (flow rate; 2 mL/min, 27% MeCN) to yield **6** (3 mg, $t_R$=21.5 min) and **12** (10 mg, $t_R$=18.9 min). Fraction H6 (166 mg) was separated using a Lobar-A Si gel 60 (240×10 mm) column ($CHCl_3$–MeOH–$H_2O$=6 : 1 : 0.1) to yield **2** (4 mg). Fraction H9 (91 mg) was separated by preparative reversed-phase HPLC (flow rate; 2 mL/min, 30% MeCN) to yield **14** (6 mg, $t_R$=18.2 min) and **15** (6 mg, $t_R$=15.7 min). Fraction H10 (102 mg) was separated by semi-preparative reversed-phase HPLC (flow rate; 2 mL/min, 30% MeCN) to yield **8** (3 mg, $t_R$=24.3 min) and **13** (5 mg, $t_R$=26.1 min).

Dilatioside A (**1**)

Amorphous gum; $[\alpha]_D^{25}$ −181.8 ($c$=0.56, MeOH); IR (KBr): $\nu_{max}$ 3419, 3185, 2965, 2845, 1710, 1633, 1516, 1270, 1033, 1009 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) nm (log$\varepsilon$): 204 (1.7), 231 (0.7), 282 (0.3) nm; $^1$H- (700 MHz) and $^{13}$C- (175 MHz) NMR data, see Table 1; HR-FAB-MS: $m/z$=771.2844 $[M+Na]^+$ (Calcd for $C_{37}H_{48}NaO_{16}$, 771.2840).

Dilatioside B (**2**)

Amorphous gum; $[\alpha]_D^{25}$ −47.7 ($c$=1.11, MeOH); IR (KBr): $\nu_{max}$ 3417, 3192, 2968, 2360, 2336, 1054, 1032, 1012 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) nm (log$\varepsilon$): 224 (0.11), 243 (0.05), 270 (0.04) nm; $^1$H- (700 MHz) and $^{13}$C- (175 MHz) NMR data, see Table 1; HR-FAB-MS: $m/z$=781.2534 $[M+Na]^+$ (Calcd for $C_{34}H_{46}NaO_{19}$, 781.2531).

**Alkaline Methanolysis of Compounds 1 and 2** Compounds **1** and **2** (each 1.0 mg) were hydrolyzed with 0.5 mol/L KOH in MeOH (1 mL) at room temperature for 1 h. The mixture was subsequently eluted using an ion exchange column (Dowex® 50WX8 hydrogen form, Sigma-Aldrich) in 100% MeOH to remove KOH. The reaction mixtures of **1** were separated through semi-prep. HPLC (30% MeCN) to give **1a** (=**3**) (0.3 mg) and **1b** (0.4 mg), which were identified as oleoside dimethyl ester (**1a**) and (−)-secoisolariciresinol (**1b**) by comparison of $^1$H-NMR. The reaction mixtures of **2** were evaporated under reduced pressure to yield a mixture of oleoside dimethyl ester and syringin (0.6 mg), identified by $^1$H-NMR and co-TLC as dimethyl ester (**1a**) and syringin (**2a**) ($CHCl_3$–MeOH–$H_2O$=3 : 1 : 0.1, $Rf$=0.38).[13]

**Acid Hydrolysis of Compound 3 (=1a) and Sugar Analysis** Compound **3** (=**1a**) (1.1 mg) was refluxed with 1 mL of 1 N HCl (aq.) at 90°C for 3 h. The hydrolysate was extracted with EtOAc, and the aqueous layer was neutralized using an Amberlite IRA-67 column to yield the sugar. The $H_2O$ layer yielded D-glucose which was identified with authentic sample (Sigma-Aldrich) using silica gel co-TLC ($CHCl_3$–MeOH–$H_2O$=2 : 1 : 0.1, $Rf$=0.3).[27]

**NGF and Cell Viability Assays** In this study, C6 glioma cells were used to measure the induction of NGF release into the culture medium. C6 cells were seeded onto 24-well plates at a density of $1\times10^5$ cells/well, and after 24 h, the cells were treated with serum-free Dulbecco's modified Eagle's medium (DMEM) containing different concentrations of compound for an additional 24 h. The medium was collected from the cultured plates and the NGF level was measured using an enzyme-linked immunosorbent assay (ELISA) kit. Cell viability was measured using an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described previously.[26] The results are expressed as a percentage of the control group (untreated cells). 6-Shogaol was used as the positive control.[28]

**Cytotoxicity Assay** A sulforhodamine B bioassay (SRB) was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines.[29] The cell lines (National Cancer Institute, Bethesda, MD, U.S.A.) used were A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). Cisplatin (Sigma Chemical Co., ≥98%) was used as a positive control. The cytotoxic activities of cisplatin against the A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines showed $IC_{50}$ values of 1.96, 2.11, 1.17, and 3.04 $\mu$M, respectively. Tested compounds were demonstrated to be pure as evidenced by NMR and HPLC analysis (purity ≥95%).

**Statistical Analysis** All results are presented as the mean±standard error of the mean (S.E.M.). Significant differences between experimental groups were determined using one-way ANOVA followed by a Newman–Keuls *post hoc* test using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, U.S.A.). $p$<0.05 was considered statistically significant.

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**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials. NMR spectra and HR-MS of **1** and **2** are available as supplementary materials.

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