Anti-inflammatory terpenoid derivatives from the twigs of Syringa oblata var. dilatata

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ABSTRACT

As a part of our ongoing search for bioactive constituents from Korean medicinal sources, chemical investigations of Syringa oblata var. dilatata twigs were carried out, leading to the isolation and characterization of two new terpenoid derivatives (1-2) and five known compounds (3-7). The chemical structures of the compounds 1 and 2 were determined by extensive NMR data (1H and 13C NMR, 1H-1H COSY, HSQC, HMBC, and NOESY), HRMS, and chemical methods. These compounds (1-7) were evaluated for their nitric oxide (NO) inhibitory effects in lipopolysaccharide (LPS)-activated microglial cells (BV-2). Compounds 2 and 4 exhibited significant anti-inflammatory activities, with IC50 values of 23.3 and 13.9 μM, respectively, which displayed similar activity to the positive control, NOS-monomethyl-L-arginine (L-NMMA, IC50 20.7 μM), without any cell toxicity.

1. Introduction

Syringa oblata var. dilatata (Nakai) Redher (Oleacea), commonly called “Lilac”, is a deciduous shrub that grows in calcareous zones of Korea and China. It has been used for the treatment of vomiting, diarrhea, and rheumatalgia (Zhao et al., 2016). Several species in the Syringa genus have been used for the treatment of asthma, inflammation, liver, and intestinal disorders (Wang et al., 2012; Xu et al., 2010; Yin et al., 2008; Park et al., 1999). According to previous investigations on S. oblata, several secoiridoid glucosides, iridoids, and flavonoids with antioxidant activity have been reported (Zhao et al., 2016; Oh et al., 2003; Sun and Guo, 2013).

In a continuing search for bioactive constituents from Korean medicinal plants, we recently reported secoiridoid glucosides correlated with neuroprotective and antiapoptotic activities from S. oblata var. dilatata (Park et al., 2017). In the process of finding new compounds from this plant, we further isolated two terpenoid derivatives, including a new megastigmane (1) and a new homomonoterpenoid (2), together with five known ones (3-7) from the n-hexane and CHCl3 soluble fractions (Fig. 1). The structures of the compounds 1 and 2 were elucidated by NMR (1H and 13C NMR, 1H-1H COSY, HSQC, HMBC, and NOESY), HRMS, and chemical methods. All isolated compounds (1-7) were assessed for their effects on nitric oxide (NO) production from lipopolysaccharide (LPS)-activated BV-2 cells. Herein, we report the isolation and structural elucidation of compounds 1-7 and their inhibition activity of NO generation.

2. Results and discussion

The MeOH extract of S. oblata var. dilatata twigs was subjected to liquid–liquid solvent-partitioning to yield n-hexane, CHCl3, EtOAc, and n-BuOH soluble fractions. Each fraction was assessed for its inhibitory effect on nitric oxide (NO) production in lipopolysaccharide-activated murine microglial cells. It was found that the n-hexane and CHCl3 soluble fractions showed anti-inflammatory activities, with IC50 values of 42.4 and 29.4 μM, respectively, while the EtOAc and n-BuOH soluble fractions exhibited weak activity (72.9 and 500.1 μM, respectively). Therefore, the active n-hexane and CHCl3 soluble fractions were investigated and resulted in the isolation of a new megastigmane (1) and a new homomonoterpenoid (2), together with five known compounds (3-7) (Fig. 1).

Compound 1 was purified as a colorless gum and its molecular formula was confirmed as C13H20O4 based on its molecular ion peak [M + Na]+ at m/z 263.1264 (calcd for C13H20O4Na, 263.1259) in the positive-ion HRESIMS. The UV and IR spectra showed absorption bands associated with hydroxy and α, β-unsaturated carbonyl groups (Fig. S2, S3). The 1H NMR spectrum of 1 displayed signals for an olefinic proton [δH 5.90 (1H, s, H-1)], an oxymethine proton [δH 4.28 (1H, m, H-4)], and four methyl groups [δH 2.26 (3H, s, H-4), 1.45 (6H, s, H-7′ and 9′), 1.22 (3H, s, H-8)]. The 13C NMR spectrum exhibited 13...
resonances characteristic for two carbonyl carbons ($\delta^1 C = 211.6$ and 200.9), two olefinic carbons ($\delta^1 C = 120.1$ and 101.2), an oxygenated tertiary carbon ($\delta^1 C = 72.5$), an oxymethine carbon ($\delta^1 C = 64.5$), two methine carbons ($\delta^1 C = 50.1$ and 49.9), a quaternary carbon ($\delta^1 C = 37.1$), and four methyl carbons ($\delta^1 C = 32.4, 30.9, 29.4,$ and 26.6). These spectroscopic data (Table 1) were similar to those of cannabiside D (Wu et al., 2006), except for the absence of the glucopyranose signals (see above). The planar structure of 1 was established through 2D NMR analysis, including $^1H$–$^1H$ COSY, HSQC, and HMBC spectra (Fig. 2). The HMBC cross-peak from H-1 to C-2 and from H-4 to C-3 confirmed the positions of the two carbonyl groups at C-2 and C-3, respectively (Fig. 2). The relative configuration of 1 was deduced by analyzing the NOESY data. In the NOESY spectrum (Fig. 2), strong and weak correlations of H-4′/H-5′a, H-4′/H-7′, H-5′/H-9′ were observed, which indicated H-4′ and CH$_3$-7′ are cofacial. The absolute configuration of C-4′ was determined by the modified Mosher’s method (Kim et al., 2011). Treatment of 1 with (R)- and (S)-MTPA-Cl gave the (S)- and (R)-MTPA esters 1s and 1r, respectively. The assigned $^1H$ NMR signals of the two MTPA esters were calculated as the $\Delta \delta$ values ($\delta^S - \delta^R$) (Fig. 3). The results indicated that the absolute configuration is 4′R. Therefore, compound 1 was elucidated as shown in Fig. 1, and named dilatanone (1).

Compound 2 was isolated as a colorless gum. The molecular formula was established as C$_{10}$H$_{18}$O$_4$ from the [M + Na]$^+$ ion peak at m/z 225.1109 (calcd for C$_{10}$H$_{18}$O$_4$Na, 225.1103) in the positive-ion

Table 1

<table>
<thead>
<tr>
<th>Position</th>
<th>$^1H$ [ppm, mult., ($J$ in Hz)]</th>
<th>$^13C$ NMR spectroscopic data of compounds 1 and 2 in Methanol-d$_4$.</th>
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<tr>
<td></td>
<td>$\delta^1 C$</td>
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<tr>
<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>7</td>
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</tr>
<tr>
<td>8</td>
<td>125.1</td>
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<tr>
<td>9</td>
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<tr>
<td>OCH$_3$</td>
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</table>

*a* Recorded at $700 (\delta^1 H)$ and $175 (\delta^1 C)$ MHz.

*b* Recorded at $500 (\delta^1 H)$ and $125 (\delta^1 C)$ MHz.

Fig. 2. Key $^1H$–$^1H$ COSY, HMBC, and NOESY correlations of 1 and 2.
HRESIMS. The $^1$H NMR spectrum of 2 exhibited the presence of an olefinic proton ($\delta_H 5.64$ (1H, q, $J = 6.9$ Hz, H-8)), two oxyethylene ($\delta_H 4.00$ (2H, brd, $J = 2.3$ Hz, H-1) and 3.51 (2H, m, H-3)), a methine proton ($\delta_H 3.27$ (1H, m, H-5)), two methylene protons ($\delta_H 2.50$ (2H, dd, $J = 7.8$ 3.1 Hz, H-6), 1.73 (2H, m, H-4)), a methyl group ($\delta_H 1.67$ (3H, dt, $J = 6.9$, 1.1 Hz, H-10)), and a methoxy group ($\delta_H 3.62$ (3H, s, COOCH$_3$)). The $^{13}$C NMR spectrum of 2 displayed 10 carbon signals including an ester carbonyl carbon ($\delta_C 175.1$), two olefinic carbons ($\delta_C 140.8$ and 125.1), two oxyethylene carbons ($\delta_C 64.7$ and 61.2), two methylene carbons ($\delta_C 39.9$ and 37.1), a methine carbon ($\delta_C 33.6$), a methyl carbon ($\delta_C 13.4$), and a methoxy carbon ($\delta_C 52.1$). The gross structure of 2 was established by analysis of the 2D NMR data (1H COSY, HSQC, and HMBC) (Fig. 2). The location of the methoxy group was determined to be C-7 through the HMBC cross-peak between $\delta_H 3.62$ (3H, s, COOCH$_3$) and $\delta_C 175.1$. The $^{13}$C NMR chemical shifts at C-5 between compound 2 and 13C NMR chemical shifts at C-5 between compound 2 and 1 were determined using the MTT assay and is expressed as a percentage (%). Data are presented as the mean ± SD of three independent experiments.

The known compounds were identified as megaritalexanolon (3) (Mousouri et al., 2014), 4-epi-cryptomeriadiol (4) (Ando et al., 1994), cryptomeriadiol (5) (Ando et al., 1994), maslinic acid (6) (Rudiansyah and Garson, 2006), and arjunolic acid (7) (Rudiansyah and Garson, 2006) by comparison of their spectroscopic data with the reported data in the references. The anti-inflammatory effects of the isolates (1-7) were evaluated through the measurement of NO production levels in the LPS-stimulated murine microglia BV-2 cells (Table 2). Compound 4 exhibited significant inhibitory effect on NO production, with an IC$_{50}$ value of 13.9 µM, showing higher activity than the positive control, N$^\omega$-mono-methyl-$\omega$-arginine (l-NMMA, IC$_{50}$ 20.7 µM), without substantial cell toxicity. Although compounds 6 and 7 also showed strong inhibitory activity (IC$_{50}$ value of 6.2 and 6.5 µM, respectively), they reduced the cell viability of BV-2 cells ($46.7 ± 0.2$% and $57.6 ± 5.2$%). Also compounds 2 and 3 were structurally similar, but compound 2 (IC$_{50}$ value of 23.3 µM) displayed higher inhibitory activity than compound 3 (IC$_{50}$ value of 42.4 µM).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter (JASCO, Easton, MD, USA). Infrared (IR) spectra were recorded on a Bruker IFS-66/S Fourier-transform IR spectrometer (Bruker, Karlsruhe, Germany). Ultraviolet (UV) spectra were recorded with a Shimadzu UV-1601 UV-vis spectrometer (Shimadzu, Tokyo, Japan). NMR spectra were recorded on a Varian Unity INOVA 500 NMR spectrometer (Varian Palo Alto, CA, USA) operating at 500 MHz ($^1$H) and 125 MHz ($^{13}$C) and a Bruker AVANCE III 700 NMR spectrometer operating at 700 MHz ($^1$H) and 175 MHz ($^{13}$C) with chemical shifts given in ppm (δ). HRESI mass spectra were obtained on a Waters SYNAPT G2 Q-TOF mass spectrometer (Waters, MA, USA). Preparative HPLC was performed using a Gilson 306 pump (Middleton, WI, USA) with a Shodex Refractive Index Detector (New York, NY, USA) and a Phenomenex Luna 10 µm column ($250 × 10$ mm) (Phenomenex, Torrance, CA, USA).

Silica gel 60 (Merck, Darmstadt, 70–230 mesh, and 230–400 mesh; Merck, Darmstadt, Germany) and RP-C$_{18}$ silica gel (Merck, 230–400 mesh) were used for column chromatography. LPLC was performed using a LiChroprep Lobar-A RP-C$_{18}$ column (Merck, 240 mm × 10 mm i.d.) equipped with a FMI QSY-0 pump. Thin-layer chromatography (TLC) was performed using Merck pre-coated silica gel F$_{254}$ plates and RP-18 F$_{254a}$ plates (Merck). Spots were detected on TLC under UV light or by heating after spraying the samples with anisaldehyde-sulfuric acid.

3.2. Plant material

The twigs of S. oblatavar. 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.

3.3. Extraction and isolation

Twigs of S. oblatavar. dilatata (6.9 kg) were extracted three times using 80% aqueous MeOH for 1 day under reflux, and were then filtered. The resulting MeOH extract (450 g) was suspended in distilled water (2.4 L) and then successively partitioned with n-hexane, CHCl$_3$, EtOAc and n-ButOH, yielding 15, 25, 48, and 213 g, respectively. The n-hexane-soluble fraction (15 g) was separated over a silica gel column (n-Hexane–EtOAc, 8:1 → 1:1) to yield seven fractions (H1–H7). Fraction H6 (0.7 g) was chromatographed on an RP-C$_{18}$ silica gel column with 60% aqueous MeOH to give 14 subfractions (H61–H64). Fraction H65 (19 mg) was purified by semi-preparative HPLC (80% aqueous MeOH, flow rate of 2.0 ml/min) to acquire compound 4 (7 mg). Fraction H7 (0.6 g) was fractionated into 12 subfractions (H71–D712) using an RP-C$_{18}$ silica gel open column eluting with 60% aqueous MeOH. Compound 5 (21 mg) was obtained by purification of fraction H72 (41 mg) using semi-preparative HPLC (55% aqueous CH$_3$CN, flow rate of 2.0 ml/min). Fraction H75 (20 mg) was purified by semi-preparative HPLC (75% aqueous CH$_3$CN, flow rate of 2.0 ml/min) to yield compound 6 (8 mg). The CHCl$_3$-soluble fraction (25 g) was chromatographed on a silica gel column (CHCl$_3$–MeOH, 60:1 → 1:1). Ten fractions (C1–C10) were collected after TLC analysis. Fraction C6 (2.0 g) was separated on an RP-C$_{18}$ silica gel open column, eluted with a gradient solvent system of 50 → 80% aqueous MeOH, to give six subfractions (C61–C66). Compounds 2 (6 mg) and 3 (9 mg) were afforded from fraction C61 (239 mg) using a Lobar-A RP-C$_{18}$ column (40% aqueous MeOH) followed by semi-preparative HPLC (22% aqueous CH$_3$CN, flow rate of 2.0 ml/min). Compound C72 (200 mg) was separated by a Lobar-A RP-C$_{18}$ column with 40% aqueous MeOH and further purified by semi-preparative HPLC (23% aqueous CH$_3$CN, flow rate of 2.0 ml/min) to yield compound 1 (4 mg). Fraction C8 (3.0 g) was chromatographed on an RP-C$_{18}$ silica gel open column with 50% aqueous MeOH to give eight subfractions (C81–C88). Fraction C88 (426 mg) was purified by semi-preparative HPLC (50% aqueous CH$_3$CN, flow rate of 2.0 ml/min) to acquire compound 7 (14 mg).
3.3.1. Dilatanone (1)

Colorless amorphous gum; [α]_D−73.3 (c 0.03, MeOH); IR (KBr) \( \nu_{\text{max}} \) cm\(^{-1}\): 3398, 3225, 1664; UV (MeOH) \( \lambda_{\text{max}} \) (log e) 202 (0.9), 231 (0.7) nm; \(^1\)H (700 MHz, CD\(_3\)OD) and \(^{13}\)C (175 MHz, CD\(_3\)OD) NMR data, see Table 1; HR-ESI-MS (positive-ion mode) \( m/z \) 225.1109 [M + Na] \(^+\) (calcd. for C\(_{10}\)H\(_{18}\)O\(_4\)Na, 225.1103).

3.3.2. Dilationate (2)

Colorless amorphous gum; [α]_D−2.5 (c 0.04, MeOH); IR (KBr) \( \nu_{\text{max}} \) cm\(^{-1}\): 3367, 2945, 2831, 1453, 1032; UV (MeOH) \( \lambda_{\text{max}} \) (log e) 202 (1.1); \(^1\)H (500 MHz, CD\(_3\)OD) and \(^{13}\)C (125 MHz, CD\(_3\)OD) NMR data, see Table 1; HR-ESI-MS (positive-ion mode) \( m/z \) 263.1264 [M + Na] \(^+\) (calcd. for C\(_{13}\)H\(_{20}\)O\(_4\)Na, 263.1259).

3.4. Preparation of Mosher ester derivatives 1R and 1S

Compound 1 (0.5 mg) in deuterated pyridine (0.6 ml) was transferred to a clean NMR tube. (S)-(+)–α-Methoxy–α-(trifluoromethyl) phenylacetyl (MTPA) chloride (10 μL) Sigma, St. Louis, MO) was added immediately into the NMR tube under a N\(_2\) gas stream. The NMR tube was then shaken carefully to mix the sample and the MTPA chloride evenly. The NMR reaction tube was left at room temperature overnight. The reaction was then completed to afford the (R)-MTPA ester derivative (1R) of 1. The (S)-MTPA ester derivative of 1 (1S) was obtained in the same method as 1R. The \(^1\)H NMR spectra of 1R and 1S were measured directly in the NMR reaction tubes.

3.4.1. 1-(R)-MTPA ester (1R)

\(^1\)H NMR (pyridine-\( d_5\), 700 MHz) \( \delta \): 6.179 (1H, m, H′-4), 5.944 (1H, s, H-1), 2.714 (1H, m, H′-3′a), 2.252 (1H, m, H-5′a), 2.222 (1H, m, H-3′b), 1.828 (1H, m, H-5′b), 1.811 (3H, s, H-4), 1.629 (3H, s, H-9′), 1.575 (3H, s, H-7′), 1.130 (3H, s, H-8′).

3.4.2. 1-(S)-MTPA ester (1S)

\(^1\)H NMR (pyridine-\( d_5\), 700 MHz) \( \delta \): 6.143 (1H, m, H′-4), 5.911 (1H, s, H-1), 2.591 (1H, m, H′-3′a), 2.279 (1H, m, H-5′a), 2.189 (1H, m, H-3′b), 1.844 (1H, overlap, H-5′b), 1.844 (3H, s, H-4), 1.601 (3H, s, H-9′), 1.501 (3H, s, H-7′), 1.133 (3H, s, H-8′).

3.5. Assessment of NO production and cell viability

BV-2 cells, originally developed by Dr. V. Bocchini at the University of Perugia (Perugia, Italy), were used for this study. The cells were seeded into a 96-well plate at 4 × 10⁴ cells/well and treated with/without the purified compounds at different concentrations. LPS (100 ng/ml) was added to BV-2 cells and grown for 1d. The produced levels of nitrite (NO\(_2\)) \(^\fast\), a soluble oxidized product of NO, were evaluated with the Griess reagent (1% sulfanilamide and 0.1% N-1-naphthylethenediamine dihydrochloride in 5% phosphoric acid). The supernatant (50 μL) was harvested and mixed with an equal volume of Griess reagent. After 10 min the absorbance was gauged at wavelength 570 nm. Cell viability was measured using the MTT assay. \( N^\circ \)-monomethyl-L-arginine (NMMA, Sigma, St. Louis, USA), a well-known NO synthase inhibitor, was tested as a positive control.

4. Conclusions

In this study, seven chemical constituents, including two new compounds (1-2), were isolated and characterized from the twigs of S. oblata var. dilatata. The chemical structures of the compounds (1-2) were determined through NMR (\(^1\)H and \(^{13}\)C NMR, COSY, HSQC, and HMBC), HRMS, and chemical methods. All isolated compounds (1-7) were evaluated for their anti-inflammatory activities. Of them, compounds 6 and 7 exhibited strong anti-inflammatory activities, but the cell viability was relatively diminished. Compounds 2 and 4 showed significant inhibition on NO production in the LPS-stimulated murine microglia BV-2 cells. Although the compound 2 could be formed during MeOH extraction, but this compound might play an important role in the anti-inflammatory activity of the MeOH extract of S. oblata var. dilatata twigs. The other compounds displayed moderate activities. These results indicate that S. oblata var. dilatata and its active constituents might be a potentially valuable source for new anti-neuroinflammatory agents.

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References


