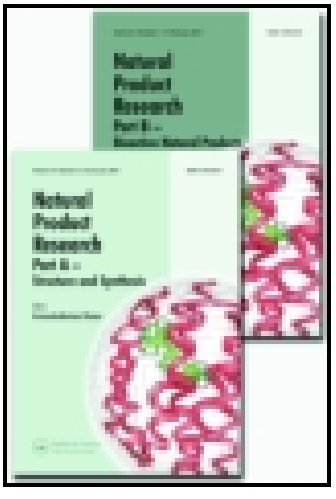

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Three new secoiridoid glycosides from the rhizomes and roots of Gentiana scabra and their anti-inflammatory activities

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Three new secoiridoid glycosides from the rhizomes and roots of *Gentiana scabra* and their anti-inflammatory activities

Wei Li[a], Wei Zhou[bc], Sohyun Kim[d], Jung-Eun Koo[d], Yuna Kim[a], Young-Sang Koh[d], Sang Hee Shim[e]*, Jin Yeul Ma[f]* and Young Ho Kim[b]*

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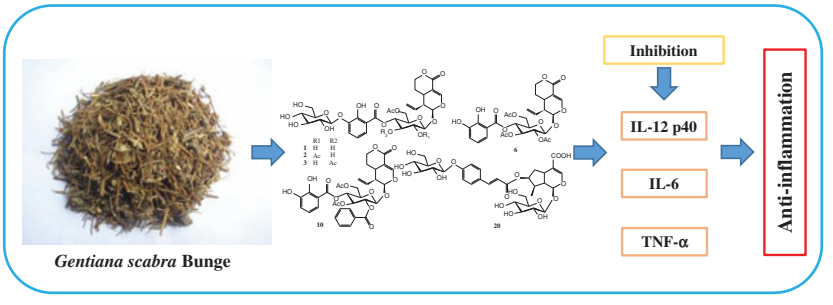

Three new (**1**–**3**) and 17 known (**4**–**20**) iridoid and secoiridoid glycosides were isolated from a methanol extract of the rhizomes and roots of *Gentiana scabra*. Their chemical structures were elucidated from 1D and 2D NMR, IR absorption, and HR-ESI-MS spectra, as well as comparisons of these data with reported values. The effects of the isolated compounds on lipopolysaccharide (LPS)-stimulated bone marrow-derived dendritic cells were investigated. Compounds **6**, **10** and **20** exhibited significant inhibitory effects on LPS-induced IL-12 p40 and IL-6 production with IC_{50} values of 1.62–14.29 μM. Compound **10** also showed a strong inhibitory effect on the LPS-stimulated production of TNF-α with an IC_{50} value of 10.45 μM.

Keywords: *Gentiana scabra*; Gentianaceae; secoiridoid glycoside; anti-inflammatory

1. Introduction

The rhizomes and roots of *Gentiana scabra* Bunge (Gentianaceae), commonly known as 'Longdan' in Chinese herbal medicine, have been used to treat inflammation, anorexia, indigestion and gastric infections (Ikeshiro & Tomita 1983). Pharmacological research has shown that *G. scabra* protects the liver, inhibits liver dysfunction and promotes gastric acid secretion, which makes it a popular ingredient in Chinese herbal medicines and health products (Zhang et al. 2010). The chemical constituents of *G. scabra* including iridoids, secoiridoids, iridoid glycosides, secoiridoid glycosides, triterpenoids, flavonoids, xanthones and alkaloids

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have been reported previously (Ikeshiro & Tomita 1983; Ikeshiro et al. 1990; Tan et al. 1996; Bergeron et al. 1997; Kakuda et al. 2001, 2002; Kim et al. 2009). Secoiridoid glycosides are primary functional components of *G. scabra* and exhibit smooth muscle relaxing, antibacterial and free radical-scavenging activities (Rojas et al. 2000; Kumarasamy et al. 2003). In this study, 20 iridoid and secoiridoid glycosides were isolated from the rhizomes and roots of *G. scabra*. The anti-inflammatory effects of isolated compounds on LPS-induced expression of the pro-inflammatory cytokines IL-6, IL-12 p40 and TNF-α in bone marrow-derived dendritic cells (BMDCs) were investigated.

2. Results and discussion

Three new (**1**–**3**) secoiridoid glycosides and 17 known (**4**–**20**) compounds were isolated from a methanol extract of the rhizomes and roots of *G. scabra*. The isolated compounds were identified as 2,3-deacetyltrifloroside (**1**), 3-deacetyltrifloroside (**2**), 2-deacetyltrifloroside (**3**), trifloroside (**4**; Kim et al. 2009), 4‴-*O*-β-D-glucopyranosyltrifloroside (**5**; Kim et al. 2009), deglucosyltrifloroside (**6**; Wang et al. 2013), scabraside (**7**; Kim et al. 2009), 4‴-*O*-β-D-glucopyranosylscabraside (**8**; Kim et al. 2009), gelidoside (**9**; Tan & Kong 1997), deglucosylscabraside (**10**; Ikeshiro & Tomita 1983), gentiopicroside (**11**; Kim et al. 2009), 6′-*O*-β-D-glucopyranosylgentiopicroside (**12**; Kakuda et al. 2001), 3′-*O*-β-D-glucopyranosylgentiopicroside (**13**; Takeda et al. 1999), 4′-*O*-β-DD-glucopyranosylgentiopicroside (**14**; Kakuda et al. 2001), 8-epikingiside (**15**; Garcia et al. 1989), loganin (**16**; Ikeshiro & Tomita 1983), caryoptoside (**17**; Damtoft 1992), secologanoside (**18**; Basaran et al. 1988), gentianaside (**19**; Kyoya et al. 2005) and 4″-*O*-β-D-glucopyranosyllinearoside (**20**; Bergeron et al. 1997). The chemical structures of these compounds were elucidated based on 1D and 2D NMR spectroscopic analyses, MS spectra and comparisons of these data with reported values. Notably, compounds **6**, **9**, **15**, **17**, **18** and **20** were isolated from *G. scabra* for the first time.

Compound **1** was isolated as a white powder. The molecular formula was established as $C_{31}H_{38}O_{18}$ by a quasimolecular-ion peak $[M + Na]^+$ at *m/z* 721.1925 (calcd for 721.1950) in the HR-ESI-MS spectrum. The IR spectrum of **1** contained absorption bands characteristic of a hydroxyl group (3412 cm^{-1}), an ester group (1760 cm^{-1}) and an aromatic moiety (1641 and 1588 cm^{-1}). The ^1H NMR spectrum contained three signals corresponding to aromatic protons at δ_H 6.68 (t, $J = 8.1$ Hz, H-5″), 7.55 (dd, $J = 8.1$, 1.2 Hz, H-6″) and 7.65 (dd, $J = 8.1$, 1.2 Hz, H-4″); double bonds at δ_H 5.07 (m, H-10), 5.36 (ddd, $J = 16.0$, 9.1, 7.7 Hz, H-8), and 7.88 (d, $J = 2.2$ Hz, H-3); an acetal proton at δ_H 5.67 (brs, H-1); two anomeric protons at δ_H 5.30 (d, $J = 7.5$ Hz, H-1′) and 5.56 (d, $J = 7.5$ Hz, H-1‴) and methyl protons at δ_H 1.91 (s, 6′-$COCH_3$). The ^{13}C NMR and DEPT spectra of **1** contained signals corresponding to three carbonyl groups [δ_C 164.7 (C-11), 169.3 (C-7″), 170.3 (6′-$COCH_3$)], six aromatic carbon atoms [δ_C 114.2 (C-1″), 118.8 (C-5″), 122.3 (C-4″), 123.0 (C-6″), 147.0 (C-3″), 152.5 (C-2″)], two double bonds [δ_C 105.2 (C-4), 120.0 (C-10), 132.3 (C-8), 152.1 (C-3)], two anomeric carbon atoms [δ_C 100.5 (C-1′), 102.6 (C-1‴)], ten sugar moiety carbon atoms [δ_C 62.1–78.8] and a methyl carbon atom at δ_C 20.4. The above-mentioned data are consistent with compound **1** as a secoiridoid glycoside similar to trifloroside (**4**). However, compound **1** lacks the two acetyl groups at C-2′ and 3′ of compound **4**. HMBC correlations between δ_H 4.42/4.66 (H-6′) and δ_C 170.3 (6′-$COCH_3$) indicated that the acetyl group was connected to C-6′ of glucose. Enzymatic hydrolysis of **1** produced D-glucose as a sugar residue, identified by GC and consistent with reported results. The β-D-configurations of the glucose residue were determined from the coupling constant (7.5 Hz) of the anomeric proton. Basis on the structure determined from these data, compound **1** was named 2,3-deacetyltrifloroside.

Compounds **2** and **3** were obtained as white powder. The molecular formulae of **2** and **3** were $C_{33}H_{40}O_{19}$ based on a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 763.2024 in their HR-ESI-MS spectra (calcd for $C_{33}H_{40}O_{19}Na$, 763.2056). The ^1H and ^{13}C NMR spectra of **2** and **3** were

similar to those of **1**. However, **2** and **3** both contained two acetyl groups attached to the glucose moiety. The difference between compounds **2** and **3** was revealed in their ^{13}C NMR and DEPT spectra. Compound **2** contains a glucose moiety indicated by peaks at δ_C 64.0 (C-6′), 68.5 (C-3′), 71.8 (C-4′), 74.5 (C-5′), 75.5 (C-2′) and 96.9 (C-1′). In contrast, the ^{13}C NMR spectra of compound **3** contained peaks at δ_C 62.6 (C-6′), 72.1 (C-3′), 72.3 (C-2′), 72.5 (C-5′), 73.9 (C-4′) and 96.9 (C-1′). These data show that the acetyl groups of **2** and **3** are located at different positions on the glucose moiety. The HMBC spectrum of **2** showed correlations between δ_H 4.40/4.95 (H-6′) and δ_C 170.1 (6′-$COCH_3$), and 5.80 (H-2′) and δ_C 169.7 (2′-$COCH_3$), indicating acetyl groups on the C-2′ and C-6′ atoms of glucose. The HMBC spectrum of **3** showed correlations between δ_H 4.44/4.62 (H-6′) and δ_C 170.3 (6′-$COCH_3$), and 5.83 (H-3′) and δ_C 168.9 (3′-$COCH_3$), indicating acetyl groups on the C-3′ and C-6′ atoms of glucose. Thus, based on the structures elucidated from these data, compounds **2** and **3** were named as 3-deacetyltrifloroside and 2-deacetyltrifloroside, respectively (Figure 1).

The effects of various concentrations (1, 2, 5 and 10 μM) of compounds **1**–**20** on the secretion of cytokines IL-6, IL-12 p40 and TNF-α were evaluated. BMDCs were incubated in 48-well plates and treated for 1 h with the isolated compounds prior to stimulation with LPS (10 ng/mL). Supernatants were harvested 18 h after stimulation. SB203580, an inhibitor of p38 kinase, was used as a positive control (Lee et al. 1994) and inhibited IL-6, IL-12 p40 and TNF-α production with IC_{50} values of 3.5, 5.0 and 7.2 μM, respectively. Compounds **6**, **10** and **20** significantly inhibited the production of IL-6 and IL-12 p40 production, with IC_{50} values ranging from 1.62 to 14.29 μM (Figure 2). In particular, compound **10** was the most effective for IL-12 p40 inhibition and was more potent than the positive control. Compound **10** also showed a strong inhibitory effect on LPS-stimulated production of TNF-α with an IC_{50} value of 10.45 μM. However, several of the isolated compounds had no effects at the concentrations tested ($IC_{50} > 100$ μM). The anti-inflammatory effects of secoiridoid glycosides from the *Gentiana*

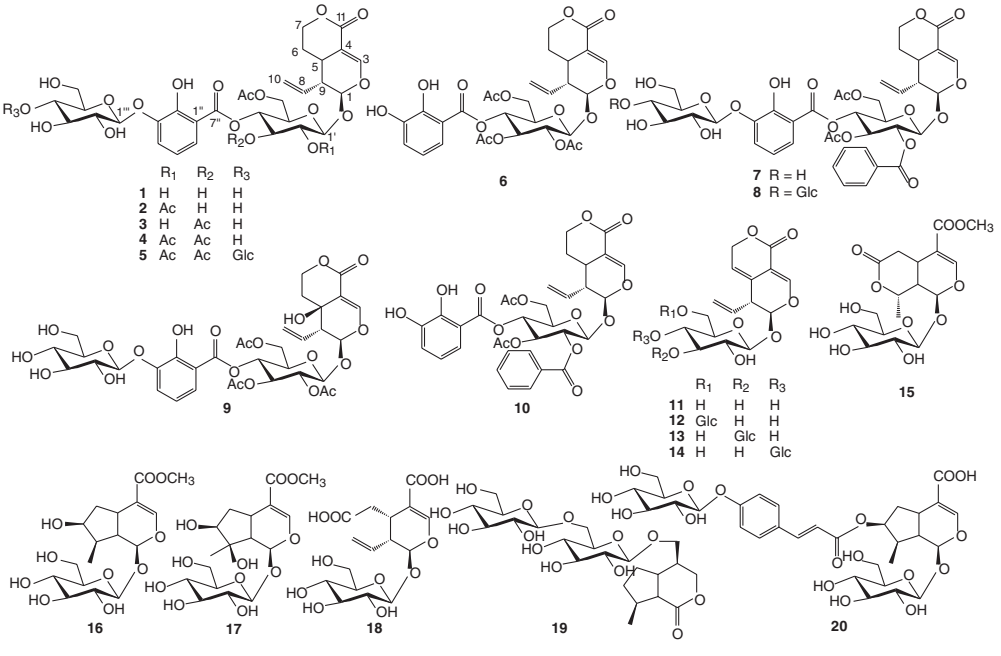

Figure 1. Structures of compounds **1**–**20** from the rhizomes and roots of *G. scabra*.

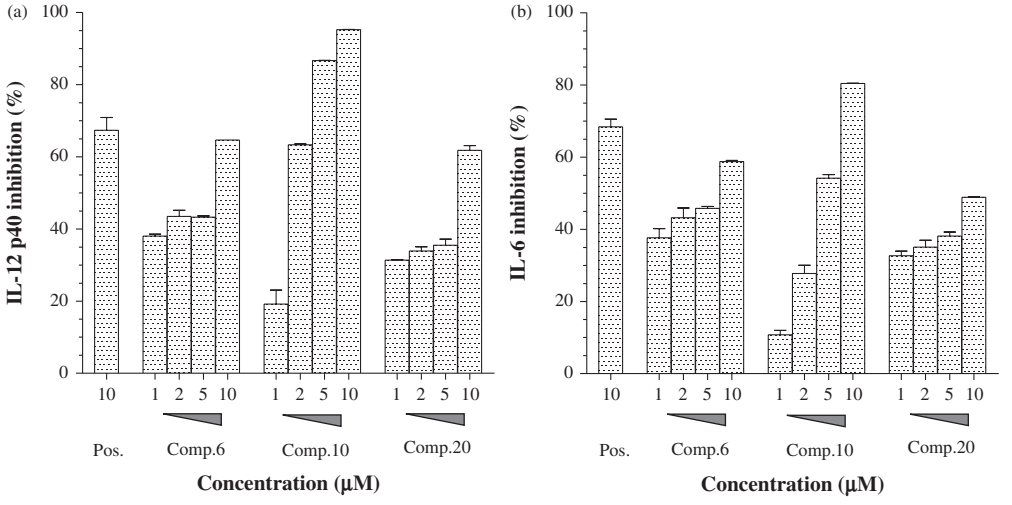

Figure 2. Effect of compounds **6**, **10** and **20** on IL-12 p40 (A) and IL-6 (B) production by LPS-stimulated BMDCs at the concentration of 1.0, 2.0, 5.0 and 10.0 μM. The data were presented as inhibition rate (%) compared with the value of vehicle-treated DCs. SB203580 was used as positive control (Pos.).

genus have been reported recently, and compound **10** significantly suppress the LPS-induced production of nitric oxide (Wang et al. 2013).

The observed anti-inflammatory activities and structural features of compounds **1**–**10** provide information regarding structure–function relationships. Compounds **6** and **10** showed stronger activities than compounds **1**–**5** and **7**–**9**. This may be attributed to the fact that C-3″ was not bound to a glucosyl group. This suggests that the 2,3-dihydroxy benzoyl group on the C-4′ atom of the glucose moiety plays an important role in the anti-inflammatory activity. These also provide information that can be used to evaluate the structure–function relationship of other secoiridoid glycosides. This study is the first to show that iridoid and secoiridoid glycosides from *G. scabra* inhibit the production of pro-inflammatory cytokines IL-12 p40, IL-6 and TNF-α. The data presented herein suggest that the various iridoid and secoiridoid glycosides in *G. scabra* contribute to its observed anti-inflammatory effect.

3. Experimental

3.1. *General experimental procedures*

Optical rotations were determined using a Jasco DIP-370 automatic polarimeter (Easton, USA). The FT-IR spectra were measured using a Jasco Report-100 infrared spectrometer. The NMR spectra were recorded using a JEOL ECA 600 spectrometer (^{1}H, 600 MHz; ^{13}C, 150 MHz). High-resolution electrospray ionisation mass spectra (HR-ESI-MS) were obtained using an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Santa Clara, USA). Column chromatography was performed using a silica gel (Kieselgel 60, 70–230, and 230–400 mesh, Merck, Darmstadt, Germany), YMC RP-18 resins, and thin-layer chromatography was performed using pre-coated silica gel 60 F_{254} and RP-18 $F_{254}S$ plates (both 0.25 mm, Merck).

3.2. *Plant material*

Dried rhizomes and roots of *G. scabra* were purchased from herbal company, Naemome Dah, Ulsan, Korea in December 2013 and identified by one of the authors (Prof. Young Ho Kim).

A voucher specimen (CNU 13109) was deposited at the Herbarium of College of Pharmacy, Chungnam National University.

3.3. *Extraction and isolation*

Dried rhizomes and roots (2.5 kg) of *G. scabra* were extracted with MeOH (10 L × 3) under reflux. The MeOH extract (670.0 g) was suspended in water and partitioned with $CHCl_3$ and *n*-BuOH. The *n*-BuOH fraction (190.0 g) was subjected to silica gel (8 cm × 30 cm) column chromatography with $CHCl_3$–MeOH–H_2O (12:1:0, 6.5:1:0, 4:1:0, 2.5:1:0.1) to give four fractions (Fr. 1A–1D). The fraction 1B (15.0 g) was subjected to YMC (3 cm × 80 cm) column chromatography with a MeOH–H_2O (0.05:1 to 0.95:1) elution solvent to give eight sub-fractions (Fr. 1B-1–1B-8). The fraction 1B-5 was subjected to silica gel (2.5 cm × 80 cm) column chromatography with $CHCl_3$–MeOH (10:1 to 7:1) elution solvent to give compounds **6** (1.1 g), **10** (127.0 mg) and **11** (2.2 g). The fraction 1C (17.0 g) was subjected to YMC (3 cm × 80 cm) column chromatography with a MeOH–H_2O (0.05:1 to 1:1) elution solvent to give 12 sub-fractions (Fr. 1C-1–1C-12). The fraction 1C-1 was separated using a silica gel (1.5 cm × 80 cm) column chromatography with $CHCl_3$–MeOH (8.5:1 to 8:1) elution solvent to give compounds **1** (13.0 mg), **15** (22.0 mg) and **16** (1.5 g). The fraction 1C-3 was separated using an YMC (1 cm × 80 cm) column chromatography with a MeOH–acetone–H_2O (0.25:0.25:1 to 0.4:0.4:1) elution solvent to give compounds **4** (60.0 mg) and **17** (15.0 mg). The fraction 1C-5 was separated using an YMC (1 cm × 80 cm) column chromatography with a MeOH–acetone–H_2O (0.2:0.2:1 to 0.3:0.3:1) elution solvent to give compounds **2** (11.0 mg) and **3** (5.0 mg). The fraction 1C-6 was separated using a silica gel (1.5 cm × 80 cm) column chromatography with $CHCl_3$–MeOH (14:1 to 10:1) elution solvent to give compounds **5** (100.0 mg) and **8** (420.0 mg). The fraction 1D (10.0 g) was subjected to YMC (3 cm × 80 cm) column chromatography with a MeOH–H_2O (0:1 to 1:1) elution solvent to give 9 sub-fractions (Fr. 1D-1–1D-9). The fraction 1D-4 was separated using a silica gel (1 cm × 80 cm) column chromatography with $CHCl_3$–MeOH–H_2O (6.5:1:0.05 to 4:1:0.1) elution solvent to give compounds **12** (70.0 mg), **13** (20.0 mg) and **14** (2.5 mg). The fraction 1D-5 was separated using a silica gel (1 cm × 80 cm) column chromatography with $CHCl_3$–MeOH–H_2O (6.5:1:0.1 to 4:1:0.1) elution solvent to give compounds **18** (6.0 mg), **19** (8.5 mg) and **20** (15.0 mg). The fraction 1D-6 was separated using an YMC (2 cm × 80 cm) column chromatography with a MeOH–acetone–H_2O (0.4:0.4:1) elution solvent to give compounds **7** (360.0 mg) and **9** (280.0 mg).

3.3.1. *2,3-Deacetyltrifloroside (**1**)*

White powder; $C_{31}H_{38}O_{18}$; $[\alpha]_D^{25}$: −13.02 ($c = 0.1$, MeOH); IR (KBr): ν_{max} 3412, 1760, 1641, 1588 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz): 5.67 (1H, brs, H-1), 7.88 (1H, d, $J = 2.2$ Hz, H-3), 2.87 (1H, m, H-5), 1.42 (1H, m, H-6a), 1.30 (1H, m, H-6b), 4.16 (1H, ddd, $J = 12.5$, 11.0, 3.0 Hz, H-7a), 3.76 (1H, t, $J = 10.6$ Hz, H-7b), 5.36 (1H, ddd, $J = 16.0$, 9.1, 7.7 Hz, H-8), 2.61 (1H, m, H-9), 5.07 (2H, m, H-10), 5.30 (1H, $J = 7.5$ Hz, H-1′), 4.39 (1H, t, $J = 9.0$ Hz, H-2′), 4.20 (1H, m, H-3′), 5.80 (1H, t, $J = 9.6$ Hz, H-4′), 4.25 (1H, m, H-5′), 4.66 (1H, dd, $J = 12.3$, 4.8 Hz, H-6′a), 4.42 (1H, m, H-6′b), 7.65 (1H, dd, $J = 8.1$, 1.2 Hz, H-4″), 6.68 (1H, t, $J = 8.1$ Hz, H-5″), 7.55 (1H, dd, $J = 8.1$, 1.2 Hz, H-6″), 5.56 (1H, $J = 7.5$ Hz, H-1‴), 4.10 (1H, m, H-2‴), 4.28 (1H, m, H-3‴), 4.26 (1H, m, H-4‴), 4.06 (1H, m, H-5‴), 4.48 (1H, m, H-6‴a), 4.32 (1H, m, H-6‴b), 1.91 (3H, s, 6′-OCO*CH*$_3$). ^{13}C NMR (pyridine-d_5, 150 MHz): 97.6 (C-1), 152.1 (C-3), 105.2 (C-4), 27.5 (C-5), 24.8 (C-6), 67.7 (C-7), 132.3 (C-8), 42.7 (C-9), 120.0 (C-10), 164.7 (C-11), 100.5 (C-1′), 74.8 (C-2′), 72.6 (C-3′), 72.4 (C-4′), 74.6 (C-5′), 62.9 (C-6′), 114.2 (C-1″), 152.5 (C-2″), 147.0 (C-3″), 122.3 (C-4″), 118.8 (C-5″), 123.0 (C-6″), 169.3 (C-7″), 102.6 (C-1‴),

74.6 (C-2‴), 78.3 (C-3‴), 70.9 (C-4‴), 78.8 (C-5‴), 62.1 (C-6‴), 170.3 (6′-O*C*OCH$_3$), 20.4 (6′-OCO*CH*$_3$); HR-ESI-MS: *m/z* 721.1925 [M + Na]$^+$ (calcd for $C_{31}H_{38}O_{18}Na$, 721.1950).

3.3.2. *3-Deacetyltrifloroside (**2**)*

White powder; $C_{33}H_{40}O_{19}$; $[\alpha]_D^{25}$: −12.85 (*c* 0.1, MeOH); IR (KBr): ν_{max} 3410, 1755, 1631, 1579 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz): 5.56 (1H, brs, H-1), 7.80 (1H, d, *J* = 2.2 Hz, H-3), 2.89 (1H, m, H-5), 1.38 (1H, m, H-6a), 1.28 (1H, m, H-6b), 4.20 (1H, ddd, *J* = 12.5, 11.0, 3.0 Hz, H-7a), 4.06 (1H, m, H-7b), 5.28 (1H, ddd, *J* = 16.0, 9.1, 7.7 Hz, H-8), 2.49 (1H, m, H-9), 5.00 (2H, m, H-10), 5.40 (1H, *J* = 7.5 Hz, H-1′), 5.80 (1H, t, *J* = 9.0 Hz, H-2′), 4.31 (1H, m, H-3′), 5.41 (1H, m, H-4′), 4.26 (1H, m, H-5′), 4.95 (1H, m, H-6′a), 4.40 (1H, m, H-6′b), 7.64 (1H, dd, *J* = 8.1, 1.2 Hz, H-4″), 6.62 (1H, t, *J* = 8.1 Hz, H-5″), 7.51 (1H, dd, *J* = 8.1, 1.2 Hz, H-6″), 5.56 (1H, *J* = 7.5 Hz, H-1‴), 4.19 (1H, m, H-2‴), 4.28 (1H, m, H-3‴), 4.26 (1H, m, H-4‴), 4.04 (1H, m, H-5‴), 4.48 (1H, m, H-6‴a), 4.32 (1H, m, H-6‴b), 2.00 (3H, s, 2′-OCO*CH*$_3$), 1.91 (3H, s, 6′-OCO*CH*$_3$). ^{13}C NMR (pyridine-d_5, 150 MHz): 97.2 (C-1), 151.4 (C-3), 105.9 (C-4), 27.7 (C-5), 24.6 (C-6), 68.1 (C-7), 131.7 (C-8), 42.0 (C-9), 120.3 (C-10), 164.7 (C-11), 96.9 (C-1′), 75.5 (C-2′), 68.5 (C-3′), 71.8 (C-4′), 74.5 (C-5′), 64.0 (C-6′), 114.0 (C-1″), 152.5 (C-2″), 147.1 (C-3″), 122.2 (C-4″), 118.7 (C-5″), 122.9 (C-6″), 169.6 (C-7″), 102.8 (C-1‴), 74.9 (C-2‴), 78.3 (C-3‴), 70.9 (C-4‴), 78.8 (C-5‴), 62.1 (C-6‴), 169.7 (2′-O*C*OCH$_3$), 20.4 (2′-OCO*CH*$_3$), 170.1 (6′-O*C*OCH$_3$), 20.5 (6′-OCO*CH*$_3$); HR-ESI-MS: *m/z* 763.2024 [M + Na]$^+$ (calcd for $C_{33}H_{40}O_{19}Na$, 763.2056).

3.3.3. *2-Deacetyltrifloroside (**3**)*

White powder; $C_{33}H_{40}O_{19}$; $[\alpha]_D^{25}$: −12.02 (*c* = 0.1, MeOH); IR (KBr): ν_{max} 3427, 1763, 1633, 1581 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz): 5.54 (1H, brs, H-1), 7.84 (1H, d, *J* = 2.2 Hz, H-3), 2.95 (1H, m, H-5), 1.41 (1H, m, H-6a), 1.29 (1H, m, H-6b), 4.22 (1H, ddd, *J* = 12.5, 11.0, 3.0 Hz, H-7a), 4.10 (1H, m, H-7b), 5.30 (1H, ddd, *J* = 16.0, 9.1, 7.7 Hz, H-8), 2.50 (1H, m, H-9), 5.00 (2H, m, H-10), 5.35 (1H, *J* = 7.5 Hz, H-1′), 4.47 (1H, m, H-2′), 5.83 (1H, t, *J* = 9.6 Hz, H-3′), 5.50 (1H, t, *J* = 9.0 Hz, H-4′), 4.28 (1H, m, H-5′), 4.62 (1H, dd, *J* = 12.3, 4.8 Hz, H-6′a), 4.44 (1H, m, H-6′b), 7.63 (1H, dd, *J* = 8.1, 1.2 Hz, H-4″), 6.66 (1H, t, *J* = 8.1 Hz, H-5″), 7.51 (1H, dd, *J* = 8.1, 1.2 Hz, H-6″), 5.54 (1H, *J* = 7.5 Hz, H-1‴), 4.24 (1H, m, H-2‴), 4.28 (1H, m, H-3‴), 4.25 (1H, m, H-4‴), 4.03 (1H, m, H-5‴), 4.44 (1H, m, H-6‴a), 4.32 (1H, m, H-6‴b), 1.98 (3H, s, 3′-OCO*CH*$_3$), 1.89 (3H, s, 6′-OCO*CH*$_3$). ^{13}C NMR (pyridine-d_5, 150 MHz): 97.0 (C-1), 151.3 (C-3), 106.1 (C-4), 27.8 (C-5), 24.6 (C-6), 68.1 (C-7), 131.7 (C-8), 42.0 (C-9), 120.3 (C-10), 164.8 (C-11), 96.9 (C-1′), 72.3 (C-2′), 72.1 (C-3′), 73.9 (C-4′), 72.5 (C-5′), 62.6 (C-6′), 114.2 (C-1″), 152.4 (C-2″), 147.1 (C-3″), 122.3 (C-4″), 118.7 (C-5″), 122.9 (C-6″), 169.7 (C-7″), 102.7 (C-1‴), 74.5 (C-2‴), 78.2 (C-3‴), 70.9 (C-4‴), 78.9 (C-5‴), 62.1 (C-6‴), 168.9 (3′-O*C*OCH$_3$), 20.6 (3′-OCO*CH*$_3$), 170.3 (6′-O*C*OCH$_3$), 20.3 (6′-OCO*CH*$_3$); HR-ESI-MS: *m/z* 763.2024 [M + Na]$^+$ (calcd for $C_{33}H_{40}O_{19}Na$, 763.2056).

3.4. *Enzymatic hydrolysis*

Compounds **1**–**3** (each 3.0 mg) was mixed with β-glucosidase (3.0 mg) in water (1.0 mL) and was shaken in a water bath at 37°C for 12 h. After this, the reaction mixture was concentrated and then subjected to silica gel (1.0 cm × 15.0 cm, 40–63 μm) column chromatography with $CHCl_3$–MeOH (15:1, 60 mL) and $CHCl_3$–MeOH–H_2O (7:3:0.5, 60 mL) to afford aglycone and a sugar fraction. The sugar fraction was concentrated to dryness using N_2 gas. The resulting residue was dissolved in dry pyridine (0.1 mL), and then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL) was added to the solution. After heating the reaction mixtures at 60°C for 2 h, 0.1 mL of trimethylsilylimidazole solution was added. Heating at 60°C was

continued for a further 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL each), and the organic layer was analysed using gas chromatography (GC): DB-5 capillary column (0.32 mm × 30 m); FID detector; column temp., 210°C; injector temp., 270°C; detector temp., 300°C; carrier gas He (2 mL/min). Under these conditions, standard sugars gave peaks at t_R (min) = 14.12 and 12.24 for L- and D-glucose, respectively. The peaks of the hydrolysate of **1**–**3** were detected at t_R (min) = 12.21, 12.20 and 12.23, respectively, which was identified as D-glucose by comparison with the retention time of the authentic samples after treatment with trimethylsilylimidazole in pyridine.

3.5. *Cell culture*

BMDCs were grown from wild-type C57BL/6 mice (Orient Bio Inc., Seongnam-si, South Korea) as previously described. All animal procedures were approved and performed according to the guidelines of the Institutional Animal Care and Use Committee of Jeju National University (#2010-0028). Briefly, the mouse tibia and femur was obtained by flushing with Dulbecco's modified Eagle's medium to yield bone marrow cells. The cells were cultured in RPMI 1640 medium containing 10% heat-inactivated foetal bovine serum (FBS; Gibco, New York, NY, USA), 50 μM β-ME, 2 mM glutamine supplemented with 3% J558L hybridoma cell culture supernatant containing granulocyte–macrophage colony stimulating factor. The culture medium was replaced with fresh medium every second day. At day 6 of culture, non-adherent cells and loosely adherent DC aggregates were harvested, washed and resuspended in RPMI 1640 supplemented with 5% FBS.

3.6. *Cytokine production measurements*

BMDCs were incubated in 48-well plates in 0.5 mL containing 1×10^5 cells per well, and then treated with the isolated compounds **1**–**20** at the indicated concentration for 1 h before stimulation with 10 ng/mL LPS from *Salmonella minnesota* (Alexis, New York, NY, USA). Supernatants were harvested 18 h after stimulation. Concentrations of murine TNF-α, IL-6, IL-12 p40 in the culture supernatants were determined by using ELISA (BD PharMingen, San Diego, CA, USA) according to the manufacture's instructions. The data are presented as means ± SD of at least three independent experiments performed in triplicate.

4. Conclusion

In our study, 20 iridoid and secoiridoid glycosides were isolated from a methanol extract of the rhizomes and roots of *G. scabra*. Compounds **6**, **9**, **15**, **17**, **18** and **20** were isolated from *G. scabra* for the first time. Moreover, this study is the first to show that iridoid and secoiridoid glycosides from *G. scabra* inhibit the production of pro-inflammatory cytokines IL-12 p40, IL-6 and TNF-α. The data presented herein suggest that the various iridoid and secoiridoid glycosides in *G. scabra* contribute to its observed anti-inflammatory effect.

Supplementary material

Supplementary material relating to this paper is available online, alongside Figures S1–S19 and Table S1.

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