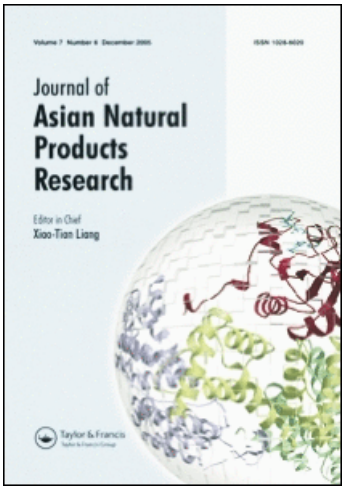

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New antioxidant phenylethanol glycosides from *Torenia concolor*

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Two new phenylethanol glycosides, phenylethyl-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (torenoside A, **1**) and 2′-*O*-3,4-dihydroxy-β-phenylethoxy-*O*-α-L-rhamnopyranosyl-(1″ → 3′)-(4′-*O*-caffeoyl)-β-D-glucopyranoside (torenoside B, **2**), along with the 17 known compounds (**3**–**19**) were isolated from *Torenia concolor*. Those structures were established on the basis of spectroscopic analysis including NMR spectroscopic techniques (^{13}C, ^{1}H, ^{1}H–^{1}H COSY, HMQC, HMBC, TOCSY, and NOESY). Moreover, phenylethanol glycosides **3**–**6** exhibited significant antioxidant activities in DPPH radical scavenging assay.

Keywords: *Torenia concolor*; Scrophulariaceae; phenylethanol glycosides; torenoside A; torenoside B

1. Introduction

Torenia concolor Lindl. (Scrophulariaceae) is a perennial prostrate herb found in sunny meadows at low elevation, which are widely distributed in India, Ryukyus, southern China, and Taiwan [1]. All parts of the plant have been used as folk medicine for expectorant, relieving cough, detoxification, defervescence, etc., in Taiwan [2]. Previous phytochemical studies of the family Scrophulariaceae have identified several different types of constituents, including flavonoids, iridoid glycosides, lignan glycosides, phenylethanol glycosides, and triterpenoids [3–6]. In continued studies on the bioactive constituents of this herb, we report herein the isolation and structural elucidation of two new phenylethanol glycosides, torenoside A (**1**) and torenoside B (**2**), along with the 17 known compounds, acetoside (**3**) [7], alphitolic acid (**9**) [8], augustic acid (**10**) [9], betulin (**11**), betulinic acid (**12**) [10], (2*R*,3*R*)-2,3-bis(3,4-dihydroxy)butyrolactone (**7**) [11], campneoside II (**5**) [7], 3-epimaslinic acid (**13**) [12], isoacetoside (**4**) [7], jionoside C (**6**) [13], lupeol (**14**) [10], maslinic acid (**15**) [14], oleanolic acid (**16**) [15], succinic acid (**8**) [16], as a mixture of stigmasterol (**17**) and β-sitosterol (**18**) [17], and β-sitosterol-3-*O*-D-glucoside (**19**) [18], which have not been described previously, from genus *Torenia.* Compounds **1**–**6** (Figure 1) were tested for their antioxidant activities. Structural elucidation of the new isolates was based on the spectroscopic analysis, including 1D and 2D NMR techniques (^{1}H–^{1}H COSY, HMQC, HMBC, TOCSY, and NOESY) and chemical hydrolysis.

2. Results and discussion

Two new phenylethanol glycosides named torenoside A (**1**) and torenoside B (**2**) were obtained from the MeOH extract of *T. concolor*. The molecular formula of **1** was determined to be $C_{20}H_{30}O_{10}$ by HR-FAB-MS,

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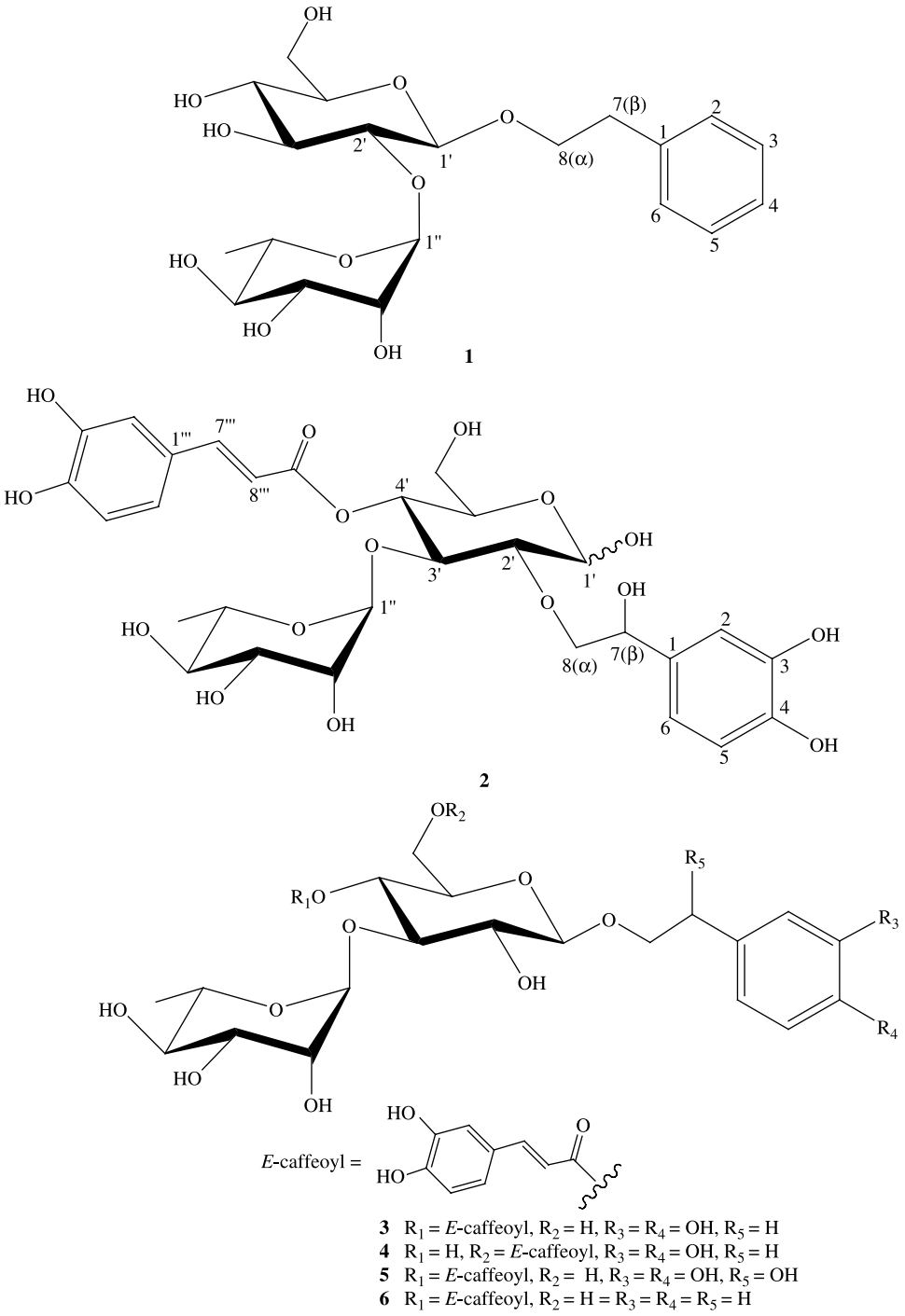

Figure 1. Structures of compounds **1**–**6**.

which exhibited a *quasi*-molecular ion peak at *m/z* 453.1733 $[M+Na]^+$. The IR spectrum showed absorptions at 3398 (OH), 1458 (C═C), and 1085 (C—O—C) cm^{-1}. The ^1H, ^{13}C NMR, and DEPT spectra showed two anomeric signals at δ_H 4.38 (1H, d, $J = 7.5$ Hz) and 5.22 (1H, br s); δ_C 100.8 and 101.7, which were identified as β-D-glucopyranose and α-L-rhamnopyranose, in addition to eight carbon signals for the aglycone of phenylethanol, suggesting that **1** is a phenylethanol glycoside [19] (Table 1). Acid hydrolysis of **1** with 10% HCl gave glucose and rhamnose as the component sugars, which were identified by TLC comparison with authentic samples. Inspection of the HMBC spectrum of **1**, the correlations of H-1′ of Glc and C-8(α) of the aglycone and H-1″ of Rha and C-2′ of Glc were conspicuously observed (Figure 2). On the basis of these results, **1** was assigned as phenylethyl-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside and named as torenoside A.

Compound **2** was determined to have an elemental composition of $C_{29}H_{36}O_{16}$, based

Table 1. ^{13}C and ^1H NMR spectral data of torenoside A (**1**) and torenoside B (**2**).

	1		**2**	
	^1H	^{13}C	^1H	^{13}C
Aglycone				
1		138.4		129.8
2	7.27 (1H, d, $J = 8.0$ Hz)	128.6	6.82 (1H, br s)	114.4
3	7.28 (1H, d, $J = 8.0$ Hz)	127.9		146.2
4	7.19 (1H, t, $J = 8.0$ Hz)	125.8		146.3
5	7.28 (1H, d, $J = 8.0$ Hz)	127.9	6.72 (1H, d, $J = 6.5$ Hz)	116.2
6	7.27 (1H, d, $J = 8.0$ Hz)	128.6	6.69 (1H, dd, $J = 6.5$, 1.5 Hz)	118.8
7β	2.96 (2H, m)	35.9	4.60 (1H, dd, $J = 8.5$, 2.0 Hz)	78.4
8α	4.10 (1H, dd, $J = 17.5$, 9.0 Hz)	70.3	3.98 (1H, m)	72.9
	3.76 (1H, dd, $J = 17.5$, 9.0 Hz)		3.65 (1H, m)	
Glc				
1′	4.38 (1H, d, $J = 7.5$ Hz)	101.7	4.55 (1H, d, $J = 6.5$ Hz)	99.0
2′	3.43 (1H, dd, $J = 9.0$, 7.5 Hz)	79.2	3.45 (1H, dd, $J = 8.5$, 6.5 Hz)	81.9
3′	3.49 (1H, t, $J = 9.0$ Hz)	77.8	4.13 (1H, t, $J = 8.5$ Hz)	77.3
4′	3.39 (1H, t, $J = 9.0$ Hz)	70.3	5.09 (1H, t, $J = 8.5$ Hz)	70.4
5′	3.27 (1H, m)	76.4	3.76 (1H, m)	77.8
6′	3.88 (1H, dd, $J = 12.0$, 2.0 Hz)	61.3	3.62 (1H, m)	62.0
	3.67 (1H, dd, $J = 12.0$, 5.5 Hz)		3.58 (1H, m)	
Rha				
1″	5.22 (1H, br s)	100.8	5.17 (1H, br s)	102.1
2″	3.95 (1H, dd, $J = 3.0$, 2.0 Hz)	70.8	3.77 (1H, m)	72.0
3″	3.70 (1H, dd, $J = 9.0$, 3.0 Hz)	70.9	3.52 (1H, dd, $J = 8.0$, 2.5 Hz)	71.9
4″	3.39 (1H, t, $J = 9.0$ Hz)	72.6	3.28 (1H, m)	73.5
5″	4.08 (1H, dd, $J = 9.0$, 6.0 Hz)	68.4	3.58 (1H, m)	70.4
6″	1.25 (1H, d, $J = 6.0$ Hz)	16.6	1.21 (1H, d, $J = 6.0$ Hz)	18.3
Caffeoyl				
1‴				127.6
2‴			7.06 (1H, d, $J = 1.5$ Hz)	115.2
3‴				146.8
4‴				148.2
5‴			6.78 (1H, d, $J = 6.5$ Hz)	116.4
6‴			6.96 (1H, dd, $J = 6.5$, 1.5 Hz)	123.2
7‴			7.60 (1H, d, $J = 13.5$ Hz)	149.8
8‴			6.29 (1H, d, $J = 13.5$ Hz)	114.4
C═O				167.9

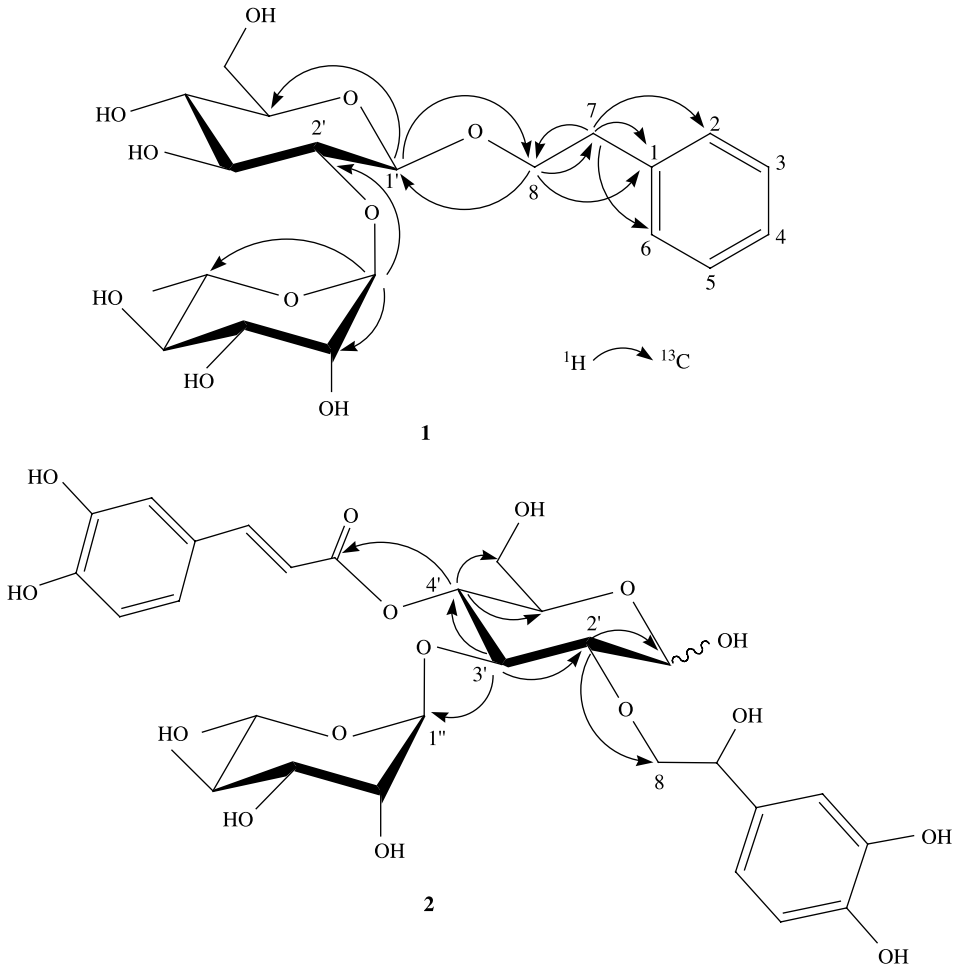

Figure 2. The key HMBC correlations of **1** and **2**.

on the results of HR-FAB-MS at *m/z* 663.1901 $[M + Na]^+$. The IR spectrum showed absorptions at 3397 (OH) and 1690 (C═O of COOH), 1450 (C═C), and 1048 (C—O—C) cm^{-1}. The ^{1}H, ^{13}C NMR, COSY, and HMQC spectra of **2** showed the presence of two ABX system signals at δ_H 6.72 (1H, d, $J = 6.5$ Hz), 6.82 (1H, br s), and 6.69 (1H, dd, $J = 6.5$, 1.5 Hz) for the 3,4-dihydroxy-β-phenyl moiety; and 6.78 (1H, d, $J = 6.5$ Hz), 6.96 (1H, dd, $J = 6.5$, 1.5 Hz), and 7.06 (1H, d, $J = 1.5$ Hz) for the caffeoyl moiety, two *trans* olefinic protons as AB-type signals at δ_H 6.29 (1H, d, $J = 13.5$ Hz) and 7.60 (1H, d, $J = 13.5$ Hz), together with two anomeric protons at δ_H 4.55 (1H, d, $J = 6.5$ Hz)/δ_C 99.0 and δ_H 5.17 (1H, br s)/δ_C 102.1. As in **1**, glucose and rhamnose in **2** were verified by TLC analysis after acid hydrolysis. The HMBC spectrum of **2** showed correlations between H-4′ and C-3′, C-5′, C-6′, C═O; between H-3′ and C-2′, C-4′, C-1″; and between H-2′ and C-1′, C-4′, C-8α; which suggested that the linkage of C-2′, C-3′, and C-4′ of glucose were directly connected to C-8 of aglycone moiety, C-1″ of rhamnose, and C═O group of caffeoyl moiety, respectively (Figure 2). Thus, the structure of **2** was elucidated to be 2′-*O*-3,4-dihydroxy-β-phenylethoxy-*O*-α-L-rhamno-pyranosyl-(1″ → 3′)-(4′-*O*-caffeoyl)-β-D-glucopyranoside and named as torenoside B.

Compounds **1**–**6** were tested for their antioxidant scavenging effects on DPPH. Our results demonstrated that **3**–**6** exhibited significant antioxidant effects with IC_{50} values of 5.34, 9.98, 10.81, and 5.97 μg/ml, respectively, whereas compounds **1** and **2** were found inactive. It was noted that the compounds **3** and **6** had profound potent effect among these isolates. However, since **3** and **6** were more effective than those of **5**, the methene group at C-7 seemed to play a crucial role for the antioxidant activity.

3. Experimental

3.1 General experimental procedures

The IR spectra were measured on a Mattson Genesis II spectrophotometer using a KBr matrix. The UV spectra were obtained on a Shimadzu UV-160A instrument. The ^{1}H and ^{13}C NMR spectra were performed on a Bruker NMR (Avance 400 MHz) and Varian NMR spectrometers (Unity Plus 500 MHz) using $CDCl_3$, CD_3OD, and C_5D_5N as the solvent for measurement. FAB-MS data were performed on a Jeol SX-102A instrument. High-resolution FAB-MS was measured on a Finnigan/Thermo Quest MAT mass spectrometer. Silica gels (70–230 and 230–400 mesh, Merck, Darmstadt, Germany) were used for column chromatography and precoated silica gel (60 F-254, Merck) plates were used for TLC. The spots on TLC were detected by spraying with 5% H_2SO_4 and then heating at 100°C. Preparative HPLC was performed using a reverse-phase column (Cosmosil 5SL-II column, 250 mm × 20 mm i.d., Kyoto, Japan) on a Shimadzu LC-6AD series apparatus with a RID-10A refractive index detector.

3.2 Plant material

The whole *T. concolor* plants were collected in July 2006 in the mountain of Nantou County, Taiwan, and identified by Prof. Chung-Chuan Chen, School of Chinese Medicine Resources, China Medical University. A voucher specimen (No. CMU200607B1) had been deposited in the Graduate Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung, Taiwan.

3.3 Extraction and isolation

The air-dried *T. concolor* (3.89 kg) was extracted with methanol thrice at 50°C. The combined extracts were evaporated under a reduced pressure and yielded a residue (744.14 g), which was suspended in water and successively extracted with *n*-hexane, $CHCl_3$, and ethyl acetate (EtOAc), yielding *n*-hexane (129.07 g), $CHCl_3$ (71.25 g), EtOAc (41.59 g), and aqueous (502.23 g) fractions. The $CHCl_3$ layer was chromatographed over a silica gel column and eluted with $CHCl_3$–MeOH (1:0 → 0:1) to get 10 fractions, C1–C10. Fraction C5 (8 g) was rechromatographed over Sephadex LH-20, eluted with $CHCl_3$–MeOH (1:2) to give six fractions, C5.1–C5.6. Fraction C5.3 was further separated and purified by silica gel, eluted with CH_2Cl_2–MeOH (1:0 → 0:1) to give **14** (14.8 mg), **15** (7.9 mg), **16** (12.1 mg), and a mixture of **17** and **18** (30 mg). Fraction C5.4 (3 g) was chromatographed on silica gel, eluting with CH_2Cl_2–MeOH and then purified by HPLC (Cosmosil $5C_{18}$-AR II) to obtain **9** (2.3 mg), **10** (5.4 mg), **11** (6.9 mg), and **19** (19.6 mg) eluting with 65% MeOH and **12** (22.2 mg), **13** (14.0 mg), **14** (10.2 mg), **15** (9.0 mg) with 70% MeOH. The EtOAc layer was subjected to silica gel column chromatography eluting with increasing amount of EtOAc and then MeOH in *n*-hexane to give fractions E1–E9. Fraction E5 (5 g) was further separated by chromatography on a Sephadex LH-20 with MeOH to yield five fractions, E5.1–E5.5. Fraction E5.2 (750 mg) was further purified by HPLC (Cosmosil $5C_{18}$-AR II) with 55% MeOH to afford **2** (6.0 mg), **3** (7.0 mg), **4** (20.0 mg), and **5** (22.0 mg). Fraction E5.3 (300 mg) was chromatographed using HPLC (Cosmosil $5C_{18}$-AR II) with 45% MeOH to afford **1** (9.8 mg), **6** (3.6 mg), and **7** (17.0 mg). By using HPLC with 70% MeOH, **8** (23.0 mg) was obtained from fraction E5.5 (100 mg).

3.3.1 Torenoside A (1)

Brown solid (7.6 mg); UV (MeOH) λ_{max} (nm): 229, 260, 290, and 330; IR (KBr) ν_{max} (cm^{-1}): 3398, 1672, 1460, and 1085; ^1H and ^{13}C NMR spectral data: see Table 1; HR-FAB-MS *m/z*: 453.1733 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_{10}Na$, 453.1737).

3.3.2 Torenoside B (2)

Brown solid (5.3 mg); UV (MeOH) λ_{max} (nm): 204, 266, 298, and 331; IR (KBr) ν_{max} (cm^{-1}): 3397, 1690, 1450, and 1048; ^1H and ^{13}C NMR spectral data: see Table 1; HR-FAB-MS *m/z*: 663.1901 $[M + Na]^+$ (calcd for $C_{29}H_{36}O_{16}Na$, 663.1907).

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