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Rigenolide A, a new secoiridoid glucoside with a cyclobutane skeleton, and three new acylated secoiridoid glucosides from *Gentiana rigescens* Franch

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ABSTRACT

Rigenolide A (**1**), a new secoiridoid glucoside with a cyclobutane skeleton and three new acylated secoiridoid glucosides, 2′-(2,3-dihydroxybenzoyl)-gentiopicroside (**2**), 2′-(2,3-dihydroxybenzoyl)-swertiamarin (**3**), 3′-(2,3-dihydroxybenzoyl)-sweroside (**4**), along with two noriridoids (**7** and **8**) and two known secoiridoid glucosides (**5** and **6**), were isolated from *Gentiana rigescens* Franch. The structures of new compounds were elucidated by extensive spectroscopic analyses. The isolated compounds were evaluated for DPPH free-radical scavenging activity.

1. Introduction

Gentiana is the largest genus in the family Gentianaceae and contains about 400 species. A bitter taste of the plants belonging to this genus is well known, and herbal extracts from various species have been included in many herbal formulations as remedies for poor appetite and digestive problems worldwide [1]. *Gentiana rigescens* Franch. ex Hemsl. grows in southwest part of China, especially in the mountain areas of Yunnan province. The roots of this plant have been commonly used as a traditional Chinese medicine, for the treatment of inflammation, and for hepatitis, rheumatism, and cholecystitis [2]. In the studies on the constituents of this plant, iridoid glucosides, secoiridoid glucosides, and triterpenoids have been isolated [1,2]. As part of our investigation on the traditional herbal medicines used by ethnic minority groups in Yunnan Province, China, designed to identify natural products on which new therapeutic agents can be based, we have examined the MeOH extracts of the aerial parts and roots of *G. rigescens*, which has resulted in the isolation of a new secoiridoid glucoside with a cyclobutane skeleton (**1**) and three new acylated secoiridoid glucosides (**2–4**), along with two noriridoids (**7**, **8**) and two known secoiridoid glucosides, 2′-(2,3-dihidroxybenzoyl)-sweroside (**5**) and macrophylloside A (**6**) (Fig. 1). Here, we report the isolated and structure elucidation of these compounds and evaluation of their DPPH radical scavenging activities.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. MS were obtained on a Waters LCT PREMIER 2695. NMR spectra were measured on a Bruker AVANCE-500 instrument (^{1}H NMR: 500 MHz, ^{13}C NMR: 125 MHz) using tetramethylsilane as an internal standard.

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Column chromatography was performed with silica gel 60 N (63–210 μm, Kanto Kagaku, Japan), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical, Japan), and YMC-pack ODS-A (S-50 μm, YMC Co., Ltd., Japan). MPLC was performed on an Isorera One with SNAP KP-C18-HS (Biotage Japan). HPLC was performed on a JASCO apparatus consisting of a PU-980 prep pump, UV-970UV/VIS (at the wavelength of 254 nm) and COSMOSIL πNAP (250 × 20 mm; 5 μm; Nacalai Tesque). TLC was conducted on precoated silica gel 60 F_{254} (Merck, Germany), and spots were detected by UV illumination and by spraying cerium sulfate reagent followed by heating.

2.2. Plant material

G. rigescens Franch. ex Hemsl. was purchased in August, 2008, in Yunnan Province, China. The plant was identified by Professor Li-Shan Xie of the Kunming Institute of Botany, Chinese Academy of Sciences, China, and a voucher specimens (08JY0007) were deposited in the herbarium of the University of Tokushima.

2.3. Extraction and isolation

The dried aerial parts of *G. rigescens* (1.9 kg) were crushed and extracted with MeOH (16 L × 3) at room temperature for 3 days. The MeOH extracts were concentrated under reduced pressure to give a residue (354 g), which was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (88 g) was partitioned with *n*-hexane and 90% aq. MeOH, and the 90% aq. MeOH-soluble fraction (42 g) was subsequently partitioned with $CHCl_3$ and 50% aq. MeOH. The 50% aq. MeOH-soluble fraction (12 g) was subjected to chromatography over MCI gel CHP 20P [MeOH-H_2O (0:1 → 1:0)] to give 14 fractions. Fr. 7 was fractionated by SiO_2 CC [$CHCl_3$-MeOH (20:1 → 2:1)] to afford compound **1** (183 mg), along with 11 fractions (2.1–2.11).

The dried roots of *G. rigescens* (866 g) were cut into small pieces and were extracted with MeOH three times. The MeOH extracts were concentrated under reduced pressure to give a residue (234 g), which was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (35 g) was further partitioned with *n*-hexane and 90% aq. MeOH. The 90% aq. MeOH-soluble fraction (12 g) was subjected to chromatography over YMC ODS-A column [MeOH-H_2O (0:1 → 1:0)] to give 12 fractions. Fr. 2 was fractionated by MCI gel CHP 20P chromatography [MeOH-H_2O (0:1 → 1:0)] to afford fractions 2.1–2.7. Fr. 2.4 was applied to an MPLC ODS column (KP-C18-HS, 400 g) [MeOH-H_2O (0:1 → 1:0)] to yield fractions 2.4.1–2.4.11. SiO_2 CC [EtOAc-MeOH (30:1 → 5:1)] of fr. 2.4.2 followed by purification by HPLC on COSMOSIL πNAP [MeOH-H_2O (45:55)] afforded **3** (2 mg). Compound **2** (10 mg) was isolated from fr.2.4.8. by HPLC on COSMOSIL πNAP [MeOH-H_2O (45:55)]. Fr. 2.4.9 was chromatographed over a silica gel column [toluene-MeOH (10:1 → 1:1)], and purified by HPLC on COSMOSIL πNAP [MeOH-H_2O (45:55)] to afford **5** (7 mg). Fr. 2.5. was repeatedly chromatographed on SiO_2 CC [$CHCl_3$-acetone (30:1 → 2:1) and $CHCl_3$-EtOAc (5:1 → 0:1)], and then purified by HPLC on COSMOSIL πNAP [MeOH-H_2O (45:55)] to give **7** (4 mg) and **8** (2 mg). Repeated CC of fraction 3 on MCI gel CHP 20P [MeOH-H_2O (0:1 → 1:0)], silica gel CC [EtOAc-MeOH (10:1 → 0:1)], followed by HPLC on COSMOSIL πNAP [MeOH-H_2O (45:55)] afforded **4** (8 mg). Fr. 7 was separated by SiO_2 CC [$CHCl_3$-MeOH (30:1 → 1:1)], Sephadex LH-20 [MeOH-H_2O (0:1 → 1:0)] and purified by HPLC on COSMOSIL πNAP [MeOH-H_2O (70:30)] to give **6** (12 mg).

2.4. Rigenolide A (**1**)

Pale yellow amorphous powder; $[\alpha]_D^{17}$ -74.2 (c1.05, MeOH); HRESIMS: *m/z* 543.1462 $[M + Na]^+$ (calculated for $C_{25}H_{28}O_{12}Na$, 543.1478); ^{1}H-NMR (CD_3OD) (see Table 1); ^{13}C-NMR (CD_3OD) (see Table 1).

2.4.1. Acid hydrolysis of compound **1**

A solution of compound **1** (10 mg) in 2.5% H_2SO_4 (4 mL) was heated at 70 °C for 2 h. The reaction mixture was neutralized with Amberlite IRA-400 anion exchange resin (Organo, Tokyo), filtrated and concentrated under reduced pressure. The residue was partitioned between EtOAc and H_2O, and the H_2O-soluble fraction was analyzed by HPLC [column, Capcell Pak NH_2 SG80 (4.6 mm i.d. ×250 mm, 5 μm; Shiseido, Tokyo, Japan); solvent, CH_3CN-H_2O (17:3); flow rate, 0.75 mL/min; column temperature: 35 °C; detection, OR]. The sugar moiety was identified as D-glucose by comparison of its retention time and sign of optical rotation with those of an authentic sample [t_R: 16 min, OR (+)].

2.5. 2′-(2,3-Dihydroxybenzoyl)-gentiopicroside (**2**)

Off-white amorphous powder; $[\alpha]_D^{28}$ -169.0 (*c* 0.93, MeOH); HRESIMS: *m/z* 515.1163 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_{12}Na$, 515.1165); ^{1}H-NMR (CD_3OD) (see Table 2); ^{13}C-NMR (CD_3OD) (see Table 2).

Table 1
^{1}H and ^{13}C- NMR data for compound **1** in CD_3OD.

Position	δ_H[a)]	δ_C[b)]
1	5.18 (1H, d, 4.5)	101.7
3	5.19 (1H, m)	72.0
4	—	57.0
5	—	69.3
6	2.06 (1H, ddd, 14.5, 12.0, 4.5) 1.92 (1H, br d, 14.5)	34.2
7	4.47 (1H, br t, 12.0) 4.17 (1H, dd, 12.0, 4.5)	65.5
8	5.74 (1H, m)	133.8
9	2.84 (1H, dd, 9.0, 4.5)	52.6
10	5.34 (1H, d, 17.0) 5.32 (1H, d, 10.5)	122.3
11	—	174.5
1′	4.84 (1H, d, 8.0)	99.2
2′	4.73 (1H, m)	81.0
3′	3.71 (1H, m)	74.5
4′	3.38 (1H, m)	71.8
5′	3.38 (1H, m)	79.7
6′	3.87 (1H, dd, 11.5, 1.5) 3.68 (1H, m)	62.5
1″	—	130.5
2″	7.09 (2H, d, 8.5)	129.7 (2C)
3″	6.69 (2H, d, 8.5)	116.0 (2C)
4″	—	157.3
7″	4.76 (1H, m)	42.3
8″	4.03 (1H, dd, 10.5, 8.5)	49.0
9″	—	175.5

a) δ ppm (mult., *J* in Hz), 500 MHz.
b) δ ppm, 125 MHz.

2.5.1. Methanolysis of compound **2**

To a solution of compound **2** (4.4 mg) in MeOH (500 μL) 0.05 M NaOMe/MeOH (200 μL) was added, and the mixture was left stand for 3 h at room temp. The reaction mixture was neutralized with Dowex 50WX8-100, filtered, and concentrated under reduced pressure. The residue was partitioned between $CHCl_3$ and 50% MeOH. A phenolcarboxylic acid derivative (0.7 mg) was obtained from the $CHCl_3$-soluble fraction, which was identified as methyl-2,3-dihydroxybenzoate. The 50% MeOH-soluble fraction was purified by silica gel column chromatography [$CHCl_3$-MeOH (20:1)] to give a secoiridoid glucoside (1.0 mg), which was identified as gentiopicroside by spectral analysis.

*2.6. 2′-(2,3-Dihydroxybenzoyl)-swertiamarin (***3***)*

Off-white amorphous powder; $[\alpha]_D^{18}$ -184.7 (*c* 0.77, MeOH); HRESIMS: *m/z* 517.1323 $[M + Na]^+$ (calculated for $C_{23}H_{26}O_{12}Na$, 517.1322); ^{1}H-NMR (CD_3OD) (see Table 2); ^{13}C-NMR (CD_3OD) (see Table 2).

2.6.1. Methanolysis of compound **3**

A solution of compound **3** (2.0 mg) in MeOH (500 μL) and 0.05 M NaOMe/MeOH (200 μL) was left stand for 3 h at room temperature. The reaction mixture was treated in the same way as described for **2** to give swertiamarin (0.8 mg) and methyl-2,3-dihydroxybenzoate (0.4 mg).

*2.7. 3′-(2,3-Dihydroxybenzoyl)-sweroside (***4***)*

Off-white amorphous powder; $[\alpha]_D^{28}$ -71.7 (*c* 0.19, MeOH); HRESIMS: *m/z* 533.1271 $[M + Na]^+$ (calculated for $C_{23}H_{24}O_{12}Na$, 533.1271); ^{1}H-NMR (CD_3OD) (see Table 2); ^{13}C-NMR (CD_3OD) (see Table 2).

2.7.1. Methanolysis of compound **4**

Compound **4** (4.0 mg) was hydrolyzed with 0.05 M NaOMe/MeOH, and worked up as described above to afford sweroside (2.8 mg) and methyl-2,3-dihydroxybenzoate (0.7 mg).

2.8. Compound **7**

Colorless oil; $[\alpha]_D^{27}$ +1.8 (*c* 0.04, $CHCl_3$); HRESIMS: *m/z* 207.0636 $[M + Na]^+$ (calculated for $C_9H_{12}O_4Na$, 207.0633); ^{1}H-NMR ($CDCl_3$) (see Table 3); ^{13}C-NMR ($CDCl_3$) (see Table 3).

2.9. Compound **8**

Colorless oil; $[\alpha]_D^{28}$ -7.6 (*c* 0.12, $CHCl_3$); HRESIMS: *m/z* 221.0789 $[M + Na]^+$ (calculated for $C_{10}H_{14}O_4Na$, 221.0790); ^{1}H-NMR ($CDCl_3$) (see Table 3); ^{13}C-NMR ($CDCl_3$) (see Table 3).

2.10. DPPH free-radical scavenging assay

One hundred microliters of each sample in EtOH was added to 100 μL of a DPPH solution (60 μM, in EtOH). After

Table 2
^{1}H and ^{13}C-NMR data for compounds **2**–**4** in CD_3OD.

	2		**3**		**4**	
Position	δ_H[a)]	δ_C[b)]	δ_H[a)]	δ_C[b)]	δ_H[a)]	δ_C[b)]
1	5.69 (1H, m)	97.3	5.65 (1H, d, 1.0)	99.9	5.57 (1H, m)	98.1
3	7.12 (1H, s)	149.1	7.08 (1H, s)	153.5	7.60 (1H, d, 2.5)	154.0
4	—	104.9	—	109.3	—	105.5
5	—	126.4	—	64.3	3.11 (1H, m)	28.4
6	5.53 (1H, br s)	117.9	1.76 (1H, ddd, 14.0, 11.0, 5.0)	35.5	1.76 (1H, ddd, 13.0, 4.0, 2.0)	25.9
			1.69 (1H, ddd, 14.0, 3.0, 1.5)		1.69 (1H, ddd, 13.0, 12.0, 4.0)	
7	4.73 (1H, dd, 17.5, 3.5)	70.3	4.67 (1H, ddd, 11.0, 11.0, 3.0)	65.6	4.43 (1H, ddd, 12.0, 4.0, 2.0)	69.7
	4.60 (1H, d, 17.5)		4.23 (1H, ddd, 11.0, 5.0, 1.5)		4.34 (1H, ddd, 12.0, 12.0, 2.0)	
8	5.65 (1H, m)	134.7	5.32 (1H, m)	133.2	5.54 (1H, m)	133.2
9	3.23 (1H, br d, 6.0)	46.0	3.23 (1H, br d, 6.0)	51.9	2.72 (1H, dd, 9.0, 5.5)	43.8
10	5.15 (1H, d, 16.5)	118.2	5.35 (1H, m)	121.5	5.31 (1H, dd, 17.0, 1.5)	121.0
	5.12 (1H, d, 10.0)		5.26 (1H, d, 9.0)		5.28 (1H, dd, 10.5, 1.5)	
11	—	165.4	—	166.9	—	168.5
1′	4.94 (1H, d, 8.0)	96.8	4.94 (1H, m)	99.0	4.85 (1H, d, 8.0)	99.6
2′	4.98 (1H, t, 8.0)	75.2	4.98 (1H, m)	75.7	3.51 (1H, m)	72.9
3′	3.74 (1H, m)	75.3	3.79 (1H, m)	75.2	5.26 (1H, t, 9.5)	79.6
4′	3.42 (1H, m)	71.8	3.44 (1H, m)	71.5	3.65 (1H, t, 9.5)	69.5
5′	3.46 (1H, m)	78.7	3.47 (1H, ddd, 10.0, 6.0, 2.0)	78.8	3.49 (1H, m)	78.1
6′	3.95 (1H, d, 11.5)	62.6	3.94 (1H, dd, 12.0, 2.0)	62.5	3.92 (1H, dd, 11.5, 2.5)	62.3
	3.72 (1H, m)		3.72 (1H, dd, 12.0, 6.0)		3.73 (1H, dd, 11.5, 6.0)	
1″	—	113.5	—	113.3	—	114.1
2″	—	151.3	—	151.8	—	151.4
3″	—	147.0	—	147.4	—	147.0
4″	7.02 (1H, d, 8.0)	122.2	7.02 (1H, dd, 8.0, 1.5)	122.4	7.02 (1H, dd, 8.0, 1.5)	121.8
5″	6.72 (1H, t, 8.0)	120.6	6.76 (1H, t, 8.0)	120.2	6.76 (1H, t, 8.0)	119.9
6″	7.27 (1H, d, 8.0)	121.0	7.38 (1H, dd, 8.0, 1.5)	121.1	7.43 (1H, dd, 8.0, 1.5)	121.4
7″	—	170.8	—	171.9	—	171.4

[a)] δ ppm (mult., *J* in Hz), 500 MHz.
[b)] δ ppm, 125 MHz.

Table 3
^{1}H and ^{13}C-NMR data for compounds **7** and **8** in $CDCl_3$.

	7		8	
Position	δ_H [a]	δ_C [b]	δ_H [a]	δ_C [b]
3	5.83 (1H, s)	88.0	5.25 (1H, s)	94.1
4	—	123.2	—	123.7
5	—	154.1	—	153.0
6	2.48 (1H, m) 2.33 (1H, ddd, 18.0, 5.5, 5.5)	28.5	2.54 (1H, m) 2.28 (1H, ddd, 17.5, 4.5, 4.5)	28.5
7	4.38 (2H, m)	65.3	4.38 (2H, m)	65.2
8	4.46 (1H, m)	62.2	4.20 (1H, m)	61.3
9	2.23 (1H, dd, 19.0, 10.5) 2.15 (1H, dd, 19.0, 4.0)	36.8	2.21 (1H, dd, 19.0, 10.0) 2.15 (1H, dd, 19.0, 4.5)	36.8
10	1.35 (3H, d, 6.5)	20.5	1.30 (3H, d, 6.5)	20.7
11	—	162.4	—	162.4
3-OMe	—		3.49 (3H, s)	55.7

[a] δ ppm (mult., *J* in Hz), 500 MHz.
[b] δ ppm, 125 MHz.

mixing gently and stand for 30 min at room temperature, optical densities were measured at 540 nm using microplate reader. L-Ascorbic acid was used as a positive control.

3. Results and discussion

Rigenolide A (**1**) was obtained as a pale yellow amorphous powder. The molecular formula of compound **1** was assigned as $C_{25}H_{28}O_{12}$ by the HRESIMS (*m/z* 543.1462 $[M + Na]^+$). The ^{1}H NMR spectrum showed signals due to one vinyl group, one acetal proton, and an oxymethylene, along with signals arising from a sugar moiety, which were similar to those of swertiamarin. However, an olefinic proton signal assignable to H-3 was absent, whereas signals due to a 1,4-disubstituted benzene ring were observed. The ^{13}C NMR spectrum was also similar to that of swertiamarin except for the absence of olefinic carbon resonances due to C-3 and C-4, but instead it showed signals due to an ester carbonyl carbon, three sp^3 methine, and one sp^3 quaternary carbon, along with six sp^2 carbons ascribable to a 1,4-disubstituted benzene ring. The sugar moiety was identified as D-glucose by direct HPLC analysis of the acid hydrolysate of compound **1** using an optical rotation detector. The ^{1}H-^{1}H COSY correlation of H-1–H-9–H-8–H_2-10, and of H_2-6–H_2-7, together with the HMBC cross peaks of H-1 with C-3 (δ_C 72.0), C-5 and C-1′; H_2-6 with C-4 (δ_C 69.3) and C-5; H_2-7 with C-11 suggested the presence of a substructure corresponding to swertiamarin, in which C-3 and C-4 were found to be sp^3 methine carbon and sp^3 quaternary carbon, respectively. In contrast, the existence of a 7,8-dihydro-*p*-coumaroyl unit was indicated by the HMBC correlations of H-7″ with C-2″ and the ester carbonyl resonance (δ_C 175.5, C-9″) and of H-2″ with C-4″. This unit was shown to be bound to the glucosyl C-2 from the HMBC correlation of H-2′ with C-9″. Furthermore, the ^{1}H-^{1}H COSY correlation of H-3 and H-8″, together with the HMBC correlations of H-7″ with C-4 and C-5 indicated that the swertiamarin unit and the 7,8-dihydro-*p*-coumaroyl unit were connected at C-7″–C-4 and C-8″–C-3 (Fig. 2). Thus, the planar structure of compound **1** was established. The relative configuration of compound **1** was

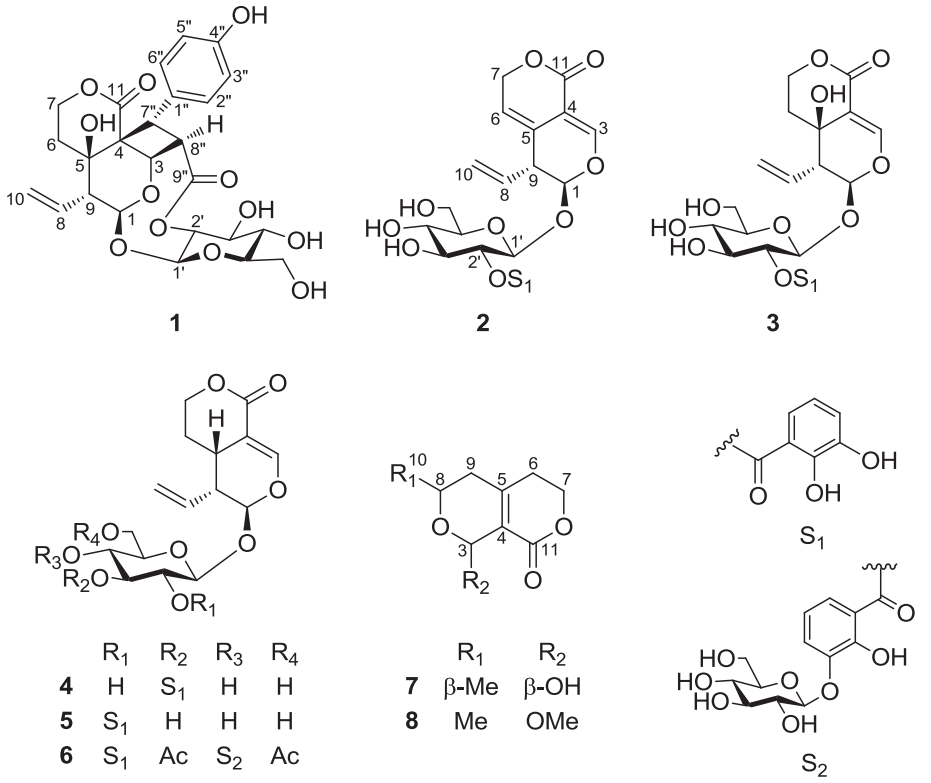


Fig. 1. Isolated compounds from *G. rigescens*.

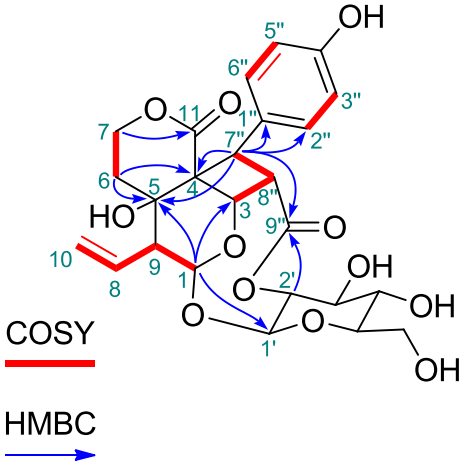


Fig. 2. Key COSY and HMBC correlations of compound **1**.

elucidated by the NOESY experiment. The NOESY correlation of H-1 with H-6α and H-8 suggested that they were located at α-side, while the dihydro-*p*-coumaroyl unit was shown to be connected to the β-side of the swertiamarin unit from the NOESY correlation of H-9 and H-7″, and therefore, the configuration of H-3 was assigned to be α. The α-configurations of H-8″ and *p*-hydroxyphenyl group were also assigned from the NOESY cross peaks of H-2″(6″) with H-3 and H-8″ (Fig. 3). Since the sugar moiety was confirmed to be D-glucose, the absolute configuration of compound **1** was thus characterized as shown in Fig. 1.

The HRESIMS of compound **2** gave an $[M + Na]^+$ ion peak at m/z 515.1163, indicating the molecular formula of $C_{23}H_{24}O_{12}$. The ^1H and ^{13}C NMR spectra were similar to those of gentiopicroside except for the observation of the signals due to a 1,2,3-trisubstituted benzene ring and an ester carbonyl resonance (δ_C 170.8). The HMBC cross peaks of H-6″ with C-7″ and C-2″ and of H-5″ with C-3″ suggested the existence of a 2,3-dihydroxybenzoyl moiety. Methanolysis of compound **2** with 0.05 M NaOMe gave gentiopicroside [3] and methyl-2,3-dihydroxybenzoate [4]. The location of the 2,3-dihydroxybenzoyl moiety was confirmed to be at C-2′ from the HMBC correlation of H-2′ with C-7″. On the basis of these data, compound **2** was characterized as 2′-(2,3-dihidroxybenzoyl)-gentiopicroside.

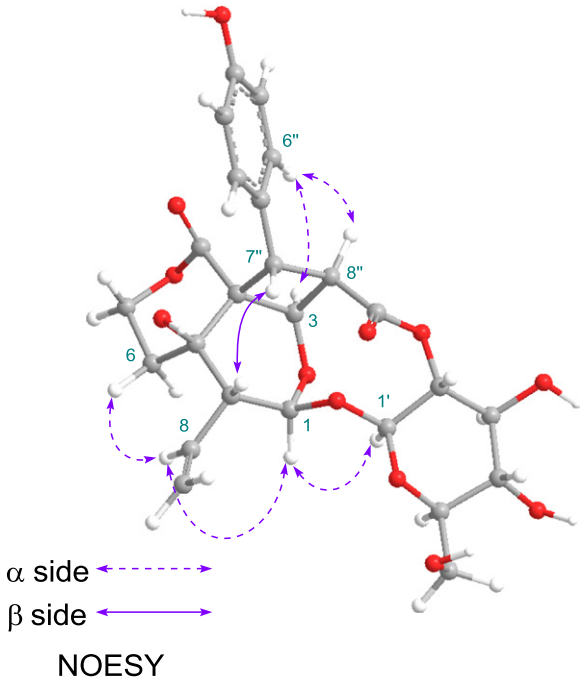


Fig. 3. Key NOESY correlations of compound **1**.

The molecular formula of compound **3** was established as $C_{23}H_{26}O_{13}$ by the HRESIMS. The ^1H and ^{13}C NMR spectra were well correlated with those of compound **2** except for the appearance of one methylene [δ_H 1.76 (1H, ddd, J = 14.0, 11.0, 5.0 Hz), 1.69 (1H, ddd, J = 14.0, 3.0, 1.5 Hz); δ_C 35.5] and one oxygenated quaternary carbon (δ_C 64.3) instead of one of trisubstituted olefins seen in **2**, indicating that **3** was a 2,3-dihydroxybenzoyl derivative of swertiamarin. This was further confirmed by alkaline methanolysis with 0.05 M NaOMe, which liberated methyl-2,3-dihydroxybenzoate and swertiamarin [5]. The location of the 2,3-dihydroxybenzoyl moiety was assigned to be at C-2′ from the HMBC correlation of H-2′ with C-7″. Therefore, the structure of compound **3** was determined as shown (Fig. 1).

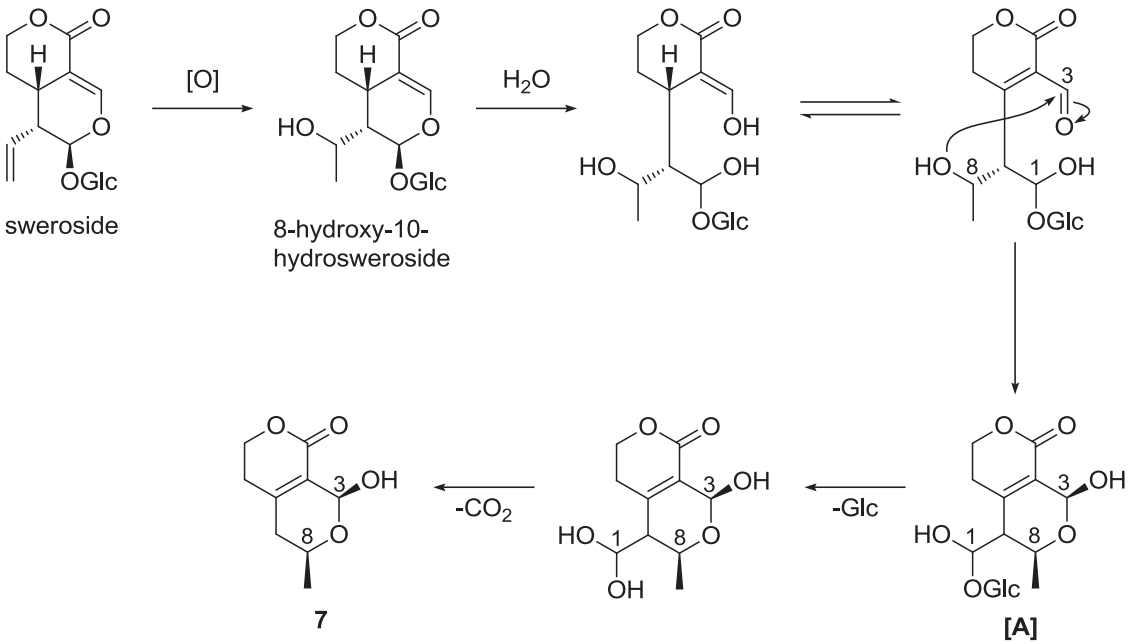
The molecular formula of compound **4** was assigned to be $C_{23}H_{26}O_{12}$ by the HRESIMS. The ^1H and ^{13}C NMR spectra were similar to those of compound **3**, except for the observation of an sp^3 methine [δ_H 3.11 (1H, m); δ_C 28.4] instead of the oxygen-bearing sp^3 quaternary carbon. Compound **4** yielded on methanolysis with 0.05 M NaOMe methyl-2,3-dihydroxybenzoate and secoiridoid glucoside, which was identified as sweroside [6,7]. The 2,3-dihydroxybenzoyl moiety was confirmed to be bound to glucosyl C-3 from the HMBC cross peak of H-3′ with C-7″. On the basis of these data, the structure of compound **4** was determined as shown in Fig. 3.

The molecular formula of compound **7** was established as $C_9H_{12}O_4$ by the HRESIMS. The ^1H and ^{13}C NMR spectra showed the presence of one secondary methyl, one oxymethylene, one oxymethine, one ester carbonyl carbon, and one acetal carbon. The ^1H-^1H COSY spectrum showed partial structures corresponding to H_2-6–H_2-7 and H_2-9–H_2-8–Me-10. Furthermore, the HMBC correlations of H-3 with C-4, C-5, and C-8; H-6 with C-4 and C-5; H-7 with C-11; and H-9 with C-4, C-5, and C-6 were observed. The planar structure, thus obtained, was the same as swercinctolide B reported by

Table 4
DPPH radical scavenging activity (IC_{50}[a] in μM) of compounds **1**–**6**.

Compound	$IC_{50} \pm SE$
1	>100
2	48.2 ± 1.2
3	36.7 ± 2.4
4	36.4 ± 1.1
5	44.6 ± 0.8
6	16.2 ± 0.3
Methyl 2,3-dihydroxybenzoate	85.6 ± 2.6
L-Ascorbic acid	30.1 ± 0.3

[a] Data are mean ± SE from four experiments.



Scheme 1. Plausible biogenetic pathway of compound **7**.

Yang et al. [8]. However, the observation of the NOESY correlation of H-3 with H-8, indicative of the orientation of H-3 and H-8 to be the same side, was different from that described in the literature. Therefore, the structure of compound **7** was concluded to be a epimer of swercinctolide B as shown (Fig. 3). Considering its small optical rotation value, compound **7** might be partially racemic.

The molecular formula of compound **8** was established as $C_{10}H_{14}O_4$ by the HRESIMS. The ^1H and ^{13}C NMR spectra were correlated with those of compound **7** except for the observation of a methoxy signal [δ_H 3.49 (3H, s); δ_C 55.7]. The location of the methoxy group was assigned to be at C-3 from the HMBC correlation of the methoxy proton signal with the acetal carbon resonance. Accordingly, the structure of compound **8** was elucidated as shown in Fig. 1. The stereostructure of compound **8** was not clear, since neither H-8 nor OMe has NOESY correlation with H-3 in the NOESY experiment.

Two known compounds were identified as 2′-(2,3-dihidroxybenzoyl)-sweroside (**5**) [9] and macrophylloside A (**6**) [10] by comparison of their spectral data with those described in the literature.

Compounds **1**–**6** were evaluated for their DPPH free-radical scavenging activities. All of the tested compounds except for **1** showed activities as shown in Table 4. Compound **6** showed potent DPPH free-radical scavenging activity with an IC_{50} value of 16.2 μM, which was more potent than that of L-ascorbic acid. The compounds possessing a 2,3-dihydroxybenzoyl moiety showed DPPH free-radical scavenging activity, whereas methyl 2,3-dihydroxybenzoate only showed a weak activity (IC_{50} 85.6 μM). This result suggested that the 2,3-dihydroxybenzoyl moiety play an important role for this activity.

Rigenolide A (**1**) is a structurally unique secoiridoid glucoside derivative possessing a cyclobutane skeleton, which was formed by intramolecular [2 + 2] cycloaddition between swertiamarin moiety and *p*-coumaroyl group bound to C-2′ of swertiamarin. Although similar iridoid derivative has been isolated from *Verbena littoralis* [11], this is the first example of a secoiridoid glucoside derivative having a cyclobutane skeleton. Compounds **2**–**4** are new acylated secoiridoid glucosides with a 2,3-dihydroxybenzoyl group. The presence of this class of compounds is considered to be characteristic of the *Gentiana* plants, since they were only found in the plant of this genus [1,9,10,12–15], despite the fact that there have been several reports of acylated iridoid and secoiridoid glucosides from various plants [16–19]. In contrast, compounds **7** and **8** are considered to be noriridoids derived from a secoiridoid glucoside. A plausible biogenetic pathway for compound **7** is proposed as shown in Scheme 1.

Conflict of Interest

The authors hereby state that there is no conflict of interest.

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