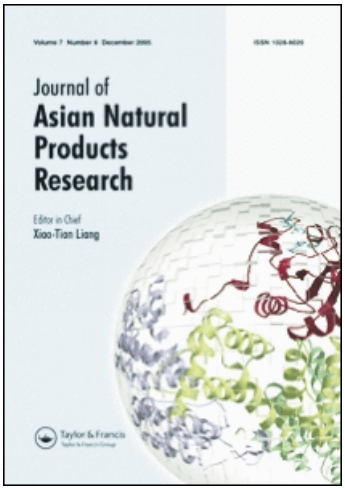

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Le Zhou[a]; Xing-Ke Li[a]; Fang Miao[b]; Ming-Li Yang[a]; Xin-Juan Yang[a]; Wei Sun[c]; Jian Yang[a]
[a] College of Science, Northwest A&F University, Shaanxi, Yangling, China [b] College of Life, Northwest A&F University, Shaanxi, Yangling, China [c] Department of Chemistry, Northwest University, Shaanxi, Xian, China

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Further studies on the chemical constituents of Chinese folk medicine *Gentiana apiata* N.E. Br.

Le Zhou[a]*, Xing-Ke Li[a], Fang Miao[b], Ming-Li Yang[a], Xin-Juan Yang[a], Wei Sun[c] and Jian Yang[a]

[a]*College of Science, Northwest A&F University, Shaanxi, Yangling 712100, China;* [b]*College of Life, Northwest A&F University, Shaanxi, Yangling 712100, China;* [c]*Department of Chemistry, Northwest University, Shaanxi, Xian 710068, China*

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One new secoiridoid glycoside with conjugated diene, named 3-*epi*-swertiajaposide C (**1**), was isolated from the whole plants of *Gentiana apiata* N.E. Br., together with 11 known compounds, 7-deoxyloganic acid (**2**), isoorientin (**3**), gentiopicroside (**4**), silybin B (**5**), swertiamarin (**6**), asystasioside A (**7**), 6′-*O*-β-D-glucopyranosylgentiopicroside (**8**), umbelliferone (**9**), oleanolic acid (**10**), kaempferol (**11**), and β-sitosterol (**12**). The structure of the new compound (**1**) was elucidated on the basis of spectroscopic evidence including UV, IR, MS, NMR, HMBC, HMQC, and NOESY. Compounds **1**, **2**, **5**, **6**, **8**, **9**, and **11** were found in this plant for the first time. Moreover, silybin B (**5**) was isolated from the other plants besides *Silybum marianum* (L.) Gaertn for the first time by the present study.

Keywords: *Gentiana apiata* N.E. Br.; iridoid; secoiridoid; 3-*epi*-swertiajaposide C; silybin B; 7-deoxyloganic acid

1. Introduction

Gentiana apiata N.E. Br. (Gentianaceae), commonly known as Qinlinglongdan or Zhulingcao in Chinese medicine, is a perennial herb that only grows in Qinba Mountain in China, and the whole plant has been used as Chinese folk medicine for the treatment of inappetence, hepatic injury disease, and gynaecopathia disease. The phytochemistry of Gentianaceae plants has been extensively studied, and they mainly contain iridoids, secoiridoids, flavonoids, and diterpenoids [1–7]. However, little phytochemical research on this plant has been found. In a previous paper, we reported the isolation and structural elucidation of asystasioside A, isoorientin, gentiopicroside, and D-sucrose from the whole plants of *G. apiata* N.E. Br. [8]. In the present study, we report the isolation and structural elucidation of a new secoiridoid, named 3-*epi*-swertiajaposide C (**1**), along with 11 known compounds, 7-deoxyloganic acid (**2**) [9], isoorientin (**3**) [8], gentiopicroside (**4**) [8], silybin B (**5**) [10], swertiamarin (**6**) [11,12], asystasioside A (**7**) [8], 6′-*O*-β-D-glucopyranosylgentiopicroside (**8**) [13], umbelliferone (**9**) [14,15], oleanolic acid (**10**) [16,17], kaempferol (**11**) [18,19], and β-sitosterol (**12**) [20,21], of which **1**, **2**, **5**, **6**, **8**, **9**, and **11** were found from this plant for the first time. Moreover, to the best of our knowledge, this is the first report on silybin B (**5**) from other plants besides *Silybum marianum* (L.) Gaertn by the present study.

*Corresponding author. Email: zhoulechem@yahoo.com.cn

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2. Results and discussion

The 70% ethanolic extract of the whole plants of *G. apiata* N.E. Br. was suspended in H_2O and defatted with petroleum ether extraction. The water layer was adsorbed on a macroporous resin LSA-30 (∅ 9.5 × *L* 70 cm), eluted with 30% aqueous methanol, 80% ethanol in water, and acetone successively, after washing with H_2O. Repeated silica gel column chromatography with $CHCl_3$—MeOH of the 30% aqueous methanol eluate, followed by silica gel 60 column chromatography eluted with $CHCl_3$—MeOH, afford a new secoiridoid glycoside, 3-*epi*-swertiajaposide C (**1**), and seven known compounds, 7-deoxyloganic acid (**2**), isoorientin (**3**), gentiopicroside (**4**), silybin B (**5**), swertiamarin (**6**), asystasioside A (**7**), and 6′-*O*-β-D-glucosylgentiopicroside (**8**); the 80% aqueous EtOH eluate was repeatedly chromatographed on silica gel column using $CHCl_3$—MeOH as eluant to yield umbelliferone (**9**), oleanolic acid (**10**), and kaempferol (**11**); and the acetone eluate was subjected over silica gel column with petroleum ether–AcOEt (100:1) to obtain β-sitosterol (**12**).

Compounds **3**, **4**, and **7** were identified as isoorientin, gentiopicroside, asystasioside A by co-TLC with reference standards isolated from the same plant in our lab before [8]. Identification of the known compounds **2**, **5**, **6**, **8**–**12** was accomplished by comparison of their spectral data with those reported in the literature.

Compound **1** as white amorphous powder showed a quasi-molecular ion peak at *m/z* 411.02 $[M+Na]^+$ in ESI-MS and a quasi-molecular ion peak at *m/z* 411.1269 $[M+Na]^+$ in HR-ESI-MS corresponding to the molecular formula $C_{17}H_{24}NaO_{10}$; IR spectrum showed the presence of carbonyl group (1701 cm^{-1}) and double bond (1639 cm^{-1}); UV spectrum exhibited absorption maximum at λ_{max} 273 nm ($\log \varepsilon$ 4.26) due to the presence of a conjugated system. The six signals among δ 77–62 in the ^{13}C NMR spectrum, in conjunction with the signal at δ 98.7, suggested that one sugar moiety existed in the molecule of **1**. Furthermore, the co-TLC analysis of the acid hydrolysate of **1** with authentic sugar samples demonstrated that the sugar moiety was glucose. The coupling constant (8.4 Hz) of H-1′ of glucose pointed out to the β-configuration of the sugar [22]. In addition to the signals of the glucosyl group, ^{13}C NMR and DEPT spectra (Table 1) showed the presence of one signal (δ_C 165.1) for carbonyl carbon, four signals (δ_C 119–147) for sp^2 carbon due to two double bonds, one signal (δ_C 57.7) for OCH_3 carbon, one signal (δ_C 13.9) for CH_3 carbon, one signal (δ_C 23.3) for CH_2 carbon, one signal (δ_C 66.3) for OCH_2 carbon, and two signals (δ_C 90.7, 95.3) for two acetal carbons. These structural features were further supported by the proton signals at δ_H 1.69 (d, $J = 6.8$ Hz, 3H) for CH_3, 3.30 (s, 3H) for MeO, 5.94 (s, 1H) and 5.10 (s, 1H) for two acetal hydrogens, 2.40–2.45 (m, 2H) for CH_2, and 4.09–4.20 (m, 2H) for OCH_2 in ^1H NMR spectrum. Moreover, DEPT and HMQC spectra disclosed the presence of one olefinic carbon (δ_C 136.4) with one hydrogen (δ_H 6.14). Based on the multiplicities and coupling constants of the signals at δ_H 1.69 and 6.14, the occurrence of one $=CHCH_3$ moiety was established. Comparison of the ^{13}C NMR spectral data with those of swertiajaposide C more recently isolated from *Swertia japonica* [23] revealed that **1** contained the same secoiridoid framework as swertiajaposide C. In the ^1H NMR spectrum, the presence of only one olefinic proton signal at δ_H 6.14 (q, $J = 6.8$ Hz, 1H) disclosed the existence of a five substituted diene unit. The assignment of all proton signals in ^1H NMR spectrum was carried out by HMQC and HMBC correlations. In HMBC spectrum (Figure 1), the correlations between the proton of methoxy and C-3 and between H-3 and C-1, C-4, C-5 suggested that OCH_3 was attached to C-3, the correlations of

Table 1. ^{1}H and ^{13}C NMR spectral data (CD_3OD) for **1**.

Position	δ_H (mult., *J* in Hz)	δ_C (mult.[a])	Position	δ_H (mult., *J* in Hz)	δ_C (mult.[a])
1	5.94 (s, 1H)	90.7 (d)	10	1.69 (d, 6.8, 3H)	13.9 (q)
3	5.10 (s, 1H)	95.3 (d)	11	–	165.1 (s)
4	–	119.1 (s)	OCH_3	3.30 (s, 3H)	57.7 (q)
5	–	146.8 (s)	1′	4.56 (d, 8.4, 1H)	98.7 (d)
6	2.45 (br dddd, 5.6, 11.2, 17.6, $1H_\alpha$), 2.40 (tt, 4.0, 4.4, 17.6, $1H_\beta$)	23.3 (t)	2′	3.17 (br t, 8.4, 9.2, 1H)	74.5 (d)
			3′	2.93 (br t, 8.0, 9.2, 1H)	77.5 (d)
7	4.20 (ddd, 4.0, 5.6, 10.8, $1H_\alpha$), 4.09 (ddd, 4.4, 10.8, 11.2, $1H_\beta$)	66.3 (t)	4′	3.25 (dd, 6.4, 8.0, 1H)	71.3 (d)
			5′	3.11(m, 1H)	78.0 (d)
8	6.14 (q, 6.8, 1H)	136.4 (d)	6′	3.39 (dd, 6.0, 11.6, 1H), 3.65 (dd, 1.6, 11.6, 1H)	62.5 (t)
9	–	130.1 (s)			

[a]Multiplet was determined by DEPT.

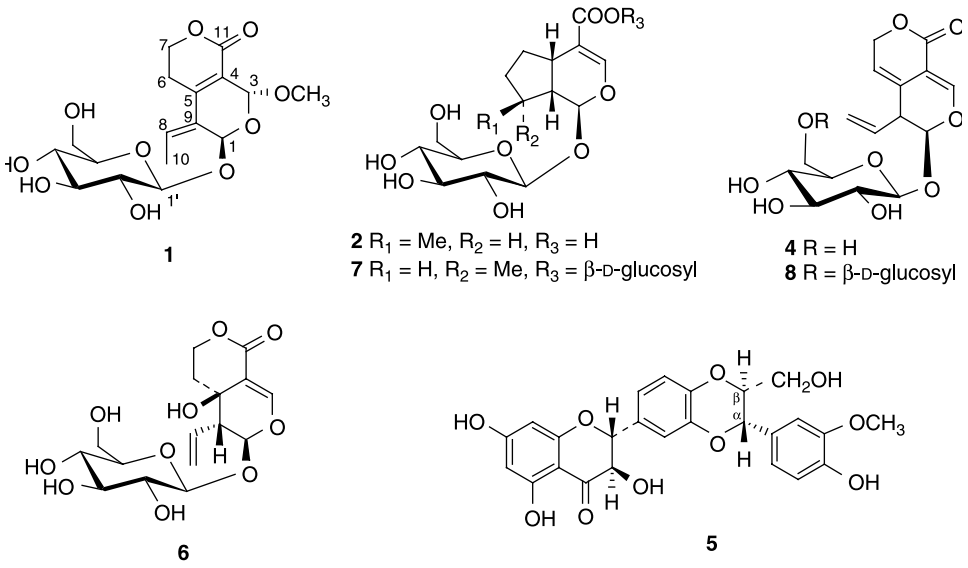

Figure 1. The structures of **1**, **2**, and **4**–**8**.

H-1/C-1′, H-1/C-3, H-1/C-8, and H-1/C-5 showed that the glucosyl group was linked to C-1. Table 1 summarizes the NMR spectral data, which support the assignment of **1** as shown in Figure 1. The stereochemistries of 8, 9-double bond, C-1 and C-3 were assigned on the basis of the NOESY spectrum (Figure 2). In the NOESY spectrum, the correlations of H-1/H-10 and H-6/H-8 showed the *Z* configuration of 8, 9-double bond, and the correlations of H-1/H-1′ implied that the Glc moiety at C-1 occurred on the β face of the ring system, and no NOESY interaction between H-1 and H-3 showed that the MeO group at C-3 was located on the α face of the ring system, indicating that the GlcO group and the MeO group are *trans*-orientation to each other. Based on these spectral data and the comparison of the optical rotation of **1** with that of swertiajaposide C [23], the structure of **1** was elucidated as (5*Z*,6*S**,8*R**)-5-ethylidene-6-(β-D-glucopyranosyloxy)-4,5,6,8-tetrahydro-8-methoxy-1*H*,3*H*-pyrano[3,4-*c*]pyran-1-one, the absolute configuration of which remains to be established.

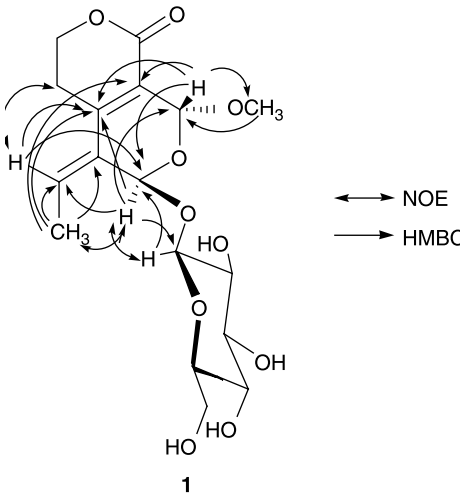

Figure 2. The main HMBC and NOESY correlations of **1**.

The ^{13}C and 2D NMR experiments supported the assignment of **1** to the new compound named 3-*epi*-swertiajaposide C.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a J-20C digital polarimeter. Melting points

were taken on a XT-4 microscopic melting point apparatus and are uncorrected. UV and IR spectra were measured on SP-2100 UV/VIS spectrometer and a NEXUS FT-IR 400 infrared spectrometer (KBr disk), respectively. NMR spectra were recorded on a Bruker AM-400 FT-NMR spectrometer using TMS as internal standard at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, respectively. EI-MS and FAB-MS were obtained with a Trace DSQ spectrometer and UGZAB spectrometer, respectively. TLC was performed on silica gel GF_{254} (0–40 μm, Qingdao Haiyang Chemical Group Co., Qingdao, China) activated at 110°C for 1 h. Column chromatography was carried out on silica gel (200–300 mesh, Qingdao Haiyang) and silica gel 60 (0.015–0.04 μm, Merck, Darmstadt, Germany). Compounds were visualized on UV light.

3.2 Plant material

The whole plants of *G. apiata* N.E. Br. were collected in Qingling Mountain, Shaanxi Province, China, in August 2006, and identified by Vice Prof. Fang Miao, one of the authors in this paper. A voucher specimen is deposited in botanic specimen center of Northwest A&F University, Yangling, China.

3.3 Extraction and isolation

The dried whole plant of *G. apiata* N.E. Br. (1500 g) was powdered and exhaustively extracted with 70% ethanol at room temperature under ultrasound-assistance (2 times, 45 min each), and the combined extract was concentrated to dryness under reduced pressure at 40°C to afford 445.40 g of a gum residue. The residue was partitioned between petroleum ether and H_2O (1:4). The water phase passed through a column (∅ 9.5 × *L* 70 cm) packed with LSA-30 macroporous resin with a rate of 0.5 ml/s, then the column was eluted with water to remove saccharide (detected by α-naphthol test), and subsequently eluted with 30% aqueous methanol, 80% aqueous ethanol and acetone to afford 30% methanol portion (70.1 g), 80% ethanol portion (12.7 g), and acetone portion (8.4 g).

The 30% methanol portion (25 g) was subjected to a silica gel column, eluted with $CHCl_3$—MeOH (stepwise, 15:1, 10:1, 8:1, 5:1, and 2:1) to afford three fractions. Fraction 1 (5.3 g), eluted with $CHCl_3$—MeOH (15:1), was purified over silica gel column and eluted with $CHCl_3$—MeOH—H_2O (stepwise, 40:2:1, 20:2:1, 10:2:1), followed by silica gel 60 column, eluted with $CHCl_3$—MeOH (stepwise, 15:1, 10:1), to afford **2** (200 mg, R_f 0.78, *n*-butanol—acetic acid—H_2O; R_f 0.58, AcOEt—MeOH—H_2O, 5:2:1), **3** (200 mg, R_f 0.64, *n*-butanol—AcOH—H_2O, 4:2:1; R_f 0.60, AcOEt—acetone—AcOH—H_2O, 5:3:1:1), **4** (100 mg, R_f 0.53, *n*-butanol—AcOH—H_2O, 4:2:1; R_f 0.64, AcOEt—acetone—AcOH—H_2O, 5:3:1:1); Fraction 2 (9.6 g), eluted with $CHCl_3$—MeOH (10:1; 8:1), was purified over silica gel 60 column and eluted with $CHCl_3$—MeOH (stepwise, 15:1, 10:1), to afford **5** (110 mg, R_f 0.50, $CHCl_3$—MeOH, 9:1), **4** (2.9 g), **6** (20 mg, R_f 0.50, *n*-butanol—AcOH—H_2O, 4:2:1; R_f 0.69, AcOEt—MeOH—H_2O, 5:2:1), and **1** (16 mg, R_f 0.42, *n*-butanol—AcOH—H_2O, 4:2:1; R_f 0.55, AcOEt—acetone—AcOH—H_2O, 5:3:1:1); fraction 3 (5.5 g), eluted with $CHCl_3$—MeOH (5:1, 2:1), was purified over silica gel 60 column, eluted with $CHCl_3$—MeOH (stepwise, 15:1, 10:1), to afford **7** (920 mg, R_f 0.32, *n*-butanol—AcOH—H_2O, 4:2:1; R_f 0.33, AcOEt—acetone—AcOH—H_2O, 5:3:1:1) and **8** (17 mg, R_f 0.54, AcOEt—MeOH—H_2O, 5:2:1; R_f 0.28, AcOEt—acetone—AcOH—H_2O, 5:3:1:1).

The 80% ethanol portion was chromatographed on silica gel column using $CHCl_3$—MeOH (stepwise, 100:1, 97:3, 95:1, 80:1) as eluant to yield **9** (20 mg, R_f 0.54, $CHCl_3$—MeOH, 10:1; R_f 0.45,

petroleum ether—AcOEt, 1:1), **10** (42 mg, R_f 0.66, cyclohexane—acetone—AcOEt, 4:2:1; R_f 0.25, $CHCl_3$—MeOH, 30:1), and **11** (150 mg, R_f 0.72, petroleum ether—AcOEt, 1:1; R_f 0.48, $CHCl_3$—MeOH, 30:1).

The acetone portion was subjected over silica gel column with petroleum ether—AcOEt (100:1) to obtain **12** (40 mg, R_f 0.46, $CHCl_3$; R_f 0.69, petroleum ether—AcOEt, 1:1).

3.3.1 3-Epi-swertiajaposide C (*1*)

White amorphous powder; m.p. 107–108°C; $[\alpha]_D^{21}$ − 36.67 ($c = 0.003$, MeOH); UV (MeOH) λ_{max} (log ε) 273 (4.26) nm; IR ν_{max} (KBr) cm^{-1}: 3424 (O—H), 2922, 1701 (C═O), 1639 (C═C), 1433, 1302, 1248, 1136, 1067 (C—O), 987; ^1H NMR (CD_3OD) and ^{13}C NMR spectral data are given in Table 1, and HMBC and NOESY correlations are given in Figure 2; HR-ESI-MS (positive mode) *m/z*: 411.1269 (calcd for $C_{17}H_{24}NaO_{10}$, 411.1267); ESI-MS (positive mode) *m/z*: 411.02 $[M+Na]^+$.

3.3.2 7-Deoxyloganic acid (*2*)

White powder; m.p. 95–97°C; $[\alpha]_D^{21}$ − 79.7 ($c = 0.0055$, MeOH); ^1H and ^{13}C NMR spectral data were consistent with the values reported in the literature [9]; FAB-MS *m/z* (rel. int.): 359 [M-1] (100), 197 [M-Glc] (22), 153 [M-Glc-CO_2] (5).

3.3.3 Silybin B (*5*)

Yellowish-white powder; m.p. 158–159°C; $[\alpha]_D^{21}$ + 0.68 ($c = 0.3$, MeOH); UV (MeOH) λ_{max}: 208, 217(sh), 230(sh), 288, 321(inf.) nm; IR ν_{max} (KBr) cm^{-1}: 3455, 3120, 3087, 1639, 1594, 1512, 1467, 1435, 1366, 1279, 1238, 1189, 1166, 1080; ^1H, ^{13}C, and 2D NMR spectral data were consistent with the literatural values [10]; FAB-MS (*m/z*): 482 $[M^+]$, 483 $[M+H]^+$, 489 $[M+Li]^+$, 505.1 $[M+Na]^+$. EI-MS (*m/z*): 481.6 $[M^+]$, 482.8 $[M+H]^+$, 463.6 $[M-H_2O]^+$, 301.8, 179.8, 152.8, 151.8, 137.8, 123.8.

3.3.4 6′-O-β-D-glucopyranosylgentiopicroside (*8*)

White amorphous powder; ESI-MS *m/z*: 540.98 $[M+Na]^+$; ^1H and ^{13}C NMR spectral data were consistent with the literature values [13].

3.3.5 Umbelliferone (*9*)

Yellowish-white needle crystal; UV ($CHCl_3$) λ_{max}: 215, 326; ^1H NMR (acetone-d_6) and ^{13}C NMR spectral data were in agreement with the literature values [14,15]; CI-MS *m/z*: 163 $[M+1]^+$, 134 $[M-CO]^+$.

3.3.6 Oleanolic acid (*10*)

Colorless amorphous powder; IR ν_{max} (KBr) cm^{-1}: 3455(OH), 2942(CH_3), 1693(C═O), 1462(C—H), 1385, 1274, 1182, 1036; ^1H NMR spectral data were consistent with those reported in the literature [16,17]; CI-MS *m/z*: 457.0 $[M+1]^+$, 438.0 $[M-H_2O]^+$, 248.0, 208.0, 203.0, 133.1.

3.3.7 Kaempferol (*11*)

Yellow amorphous powder; m.p. 276–278°C. UV (MeOH) λ_{max}: 265, 323, 367 nm; IR ν_{max} (KBr) cm^{-1}: 3417 (—OH), 1661(C—O), 1612, 1506(—Ar), 1382, 1311, 1180, 972, 877, 821; ^1H and ^{13}C NMR spectral data were in agreement with the literature values [18,19]; CI-MS *m/z*: 287.0 $[M+1]^+$, 259.0 $[M-CO]^+$, 121.0, 69.0.

3.3.8 β-Sitosterol (*12*)

White needle crystal; m.p. 137–138°C. ^1H and ^{13}C NMR spectral data were in agreement with the literature values [20,21]; EI-MS *m/z*: 414.29 $[M]^+$, 329.25, 255.15, 145.15.

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