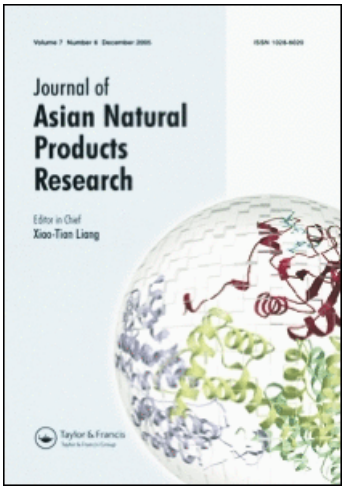

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Antioxidant phenolic glucosides from *Gentiana piasezkii*

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An extract of *Gentiana piasezkii* afforded a new arbutin derivative 6′-*O*-vanilloylarbutin (**1**) and a new flavone-*C*-glucoside 7-*O*-feruloylorientin (**2**), together with four known flavonoids lutonarin (**3**), saponarin (**4**), isoorient (**5**) and luteolin (**6**). Their structures were established based on spectroscopic methods including 2D NMR (COSY and gHMBC) techniques. Compounds **1**, **2**, **5** and **6** were evaluated for the antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay system.

Keywords: *Gentiana piasezkii*; Gentianaceae; Arbutin derivative; Flavone glucosides; Antioxidant activity

1. Introduction

The genus *Gentiana* (Gentianaceae) is well known to contain secoiridoid glucosides, flavone-*C*-glucosides and xanthone aglycones and its glucosides [1]. Flavonoids could be developed as antioxidant agents [2] and free radical scavengers, which are considered to be important as protective or therapeutic agents against atherosclerosis [3], ischemia–reperfusion [4], cancer, and aging [5].

Members of the genus *Gentiana* have been used as traditional Chinese herbal medicines to treat hepatitis [6]. In order to find active compounds as lead molecule for medicine, we have studied the constituents of *Gentiana piasezkii* Maxim and found two new natural products, 6′-*O*-vanilloylarbutin (**1**) and 7-*O*-feruloylorientin (**2**), together with four known flavonoids lutonarin (**3**), saponarin (**4**), isoorient (**5**) and luteolin (**6**) from *n*-BuOH soluble fraction of an ethanolic extract of the whole plant. Among these compounds, **2** and **6** exhibited significant activity as free radical scavengers in the 1,1-diphenyl-2-picryhydrazyl (DPPH) assay system [7,8].

2. Results and discussion

Compound **1** was obtained as yellow amorphous powder. Its molecular formula was assigned as $C_{20}H_{22}O_{10}$ on the basis of HRESI-MS (*m/z* 440.1540 $[M + NH_4]^+$) and ^{13}C NMR and

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DEPT spectral data. The IR spectrum showed absorption bands at 3367 (hydroxyl group), 1699 (ester carbonyl functions), 1647, 1558, 1509 and 1456 (aromatic ring) cm^{-1}. Both ^{1}H NMR and ^{13}C NMR spectra of compound **1** contained well-separated and easily assigned resonances and let us to believe that compound **1** was a phenolic glycoside. The ^{13}C NMR and DEPT experiments showed the presence of one methyl, one methylene, 12 methines, and six quaternary carbons. The ^{1}H NMR spectrum of **1** (table 1) indicated the presence of a *p*-disubstituted benzene unit (δ_H 6.58, 2H, *d*, $J = 8.4$ Hz, H-2, 6; 6.91, 2H, *d*, $J = 8.4$ Hz, H-3, 5 A_2B_2 spin system), a β-pyranoglucosyl moiety ($J_{1'2'} = 7.6$ Hz) [9], and a methoxyl group δ_H 3.85, *s*) attached to a 1,3,4-trisubstituted aromatic ring (δ_H 7.51, *s*, H-2″; 6.86, *d*, $J = 8.4$ Hz, H-5″; 7.56, *d*, $J = 8.4$ Hz, H-6″) [10]. The latter was identified as vanilloyl group by comparing its NMR spectral data (table 1) to those in literature [9,11]. The above structural fragments (figure 1) were supported by key correlations in a $^{1}H—^{1}H$ COSY experiment (table 1) and were put together by a gHMBC experiment (figure 2). gHMBC correlation peaks between H-1′, H-2, H-6 and H-3, H-5/C-1 indicated that the *p*-disubstituted benzene unit linked at C-1′ of the β-pyranoglucosyl moiety, that is compound **1** possessed an arbutin skeleton [10]. gHMBC correlations of H_3-OCH_3 and H-2″/ C-3″, as well as H_2-6′, H-2″ and H-6″/ C-7″ assembled the vanilloyl group attached at C-6′ of the arbutin skeleton. Hence, the compound **1** was elucidated as 6′-*O*-vanilloylarbutin.

Compound **2,** a yellowish oil, was assigned a molecular formula of $C_{31}H_{28}O_{14}$ on the basis of HRESI-MS (*m/z* 625.1544 $[M + H]^+$) and ^{13}C NMR and DEPT spectral analysis. The IR spectrum showed absorption bands at 3264 (hydroxyl group), 1650 (α,β-unsaturated-γ-pyrone carbonyl) and 1706 (α,β-unsaturated ester carbonyl) cm^{-1} [12]. Its UV spectrum gave maximum absorptions at 202.6 and 329.4 nm. The above characteristic absorptions of the IR and UV spectra implied that compound **2** was a flavonoid [8]. Analysis of the ^{13}C NMR and DEPT spectra revealed the presence of one methyl, one methylene, 15 methines, and 14 quaternary carbons. In the ^{1}H NMR spectrum of **2,** there was a multiplet at δ 3.5–3.7

Table 1. NMR spectral data of compound **1**.

No.	δ_H[a]	δ_C[b] *(DEPT)*	*gCOSY*	*gHMBC (H to C)*
1	–	152.1 s	–	
2	6.58 d (8.4)	116.5 d	H-3	1, 3, 4
3	6.91 d (8.4)	119.4 d	H-2	1, 2, 4
4	–	153.6 s	–	
5	6.91 d (8.4)	119.4 d	H-6	1, 4, 6
6	6.58 d (8.4)	116.5 d	H-5	1, 4, 5
1′	4.76 d (7.6)	103.4 d	H-2′	1, 2′
2′	3.48 m	77.8 d	H-1′	1′, 3′
3′	3.48 m	74.9 d	–	2′, 4′
4′	3.48 m	72.0 d	H-5′	3′, 5′
5′	3.74 ddd (8, 8.8)	75.3 d	H-4′, 6′ab	4′, 6′
6′a	4.69 d (11.2)	65.1 t	H-5′, 6′b	5′, 7″
6′b	4.36 dd (11.2, 7.6)		H-5′, 6′a	
1″	–	122.3 s	–	
2″	7.51 s	113.6 d	–	1″, 3″, 4″, 6″, 7″
3″	–	148.6 s	–	
4″	–	152.7 s	–	
5″	6.86 d (8.4)	115.9 d	H-6″	4″, 7″
6″	7.56 d (8.4)	125.1 d	H-5″	1″, 2″, 4″, 7″
7″	–	167.8 s	–	
OMe	3.85 s	56.4 q	–	3″

[a]Recorded in CD_3OD at 400 MHz.
[b]Recorded in CD_3OD at 100 MHz.

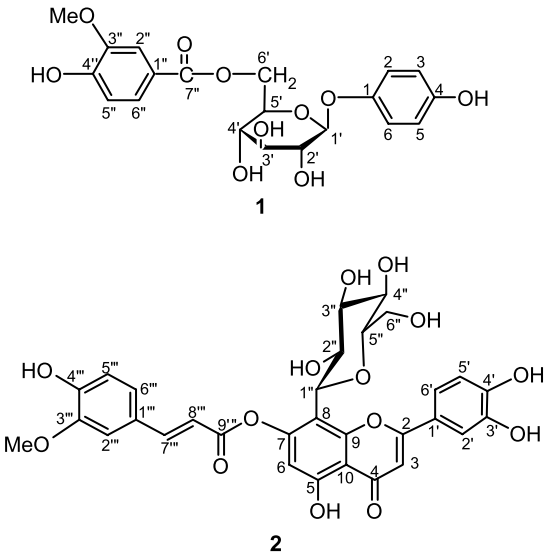

Figure 1. Structures of compounds **1** and **2**.

(5H) and a doublet at δ 4.84 (H-1″, *d*, $J = 9.6$ Hz) ascribable to a β-pyranoglucosyl moiety [13–15]; in addition the ^{1}H NMR spectrum showed some characteristic signals for a luteolin nucleus [13], at δ 6.63 (H-3), 6.43 (H-6), 7.36 (H-2′), 6.86 (*d*, $J = 8$ Hz, H-5′) and 7.38 (*d*, $J = 8$ Hz, H-6′). Furthermore, in the ^{1}H NMR spectrum, the signals of δ 7.19 (br *s*, H-2‴), 6.73 (*d*, $J = 8$ Hz, H-5‴) and 6.99 (*d*, $J = 8$ Hz, H-6‴), together with olefinic signals at δ 7.21 (1H, *d*, $J = 16$ Hz, H-7‴), 6.24 (1H, *d*, $J = 16$ Hz, H-8‴) and 3.76 (3H—OCH_3) showed the presence of a feruloyl moiety in the molecule of **2** [12]. The ^{13}C NMR spectral data of **2** were very similar to those of orientin [13], except for the additional feruloyl moiety. Extensive

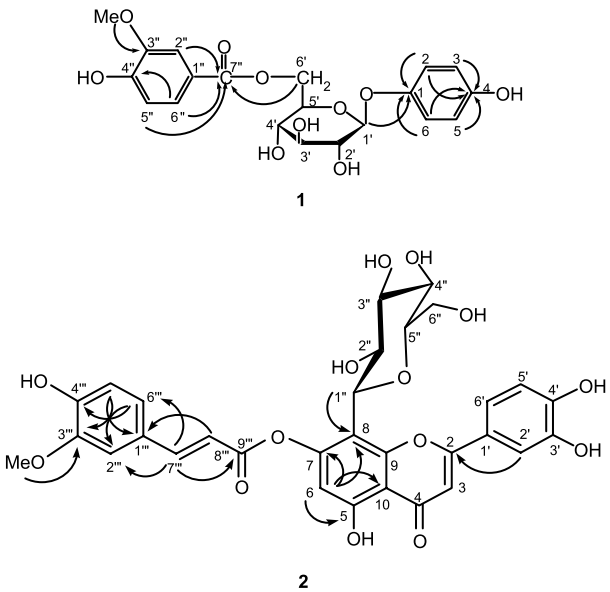

Figure 2. Key gHMBC correlations of **1** and **2**.

Table 2. Free radical scavenging activity of **1**, **2**, **5**, **6** and Trolox in the DPPH assay.

Samples	*Free radical scavenging activity IC_{20} (μM)*
1	49.66 ± 2.30
2	5.20 ± 0.10
5	78.64 ± 5.42
6	6.64 ± 0.39
Trolox	7.14 ± 0.47

Results are given as mean ± SD ($n = 3$). Statistical analyses were performed using Student's *t*-test.

analyses of gHMBC data confirmed above elucidation, although the attachment of feruloyl could not be confirmed by the gHMBC (figure 2). However, it was most possible that the feruloyl moiety was linked at O-7 by comparison of the NMR spectral data of **2** and those of orientin [13]. Consequently, compound **2** was identified as 7-*O*-feruloylorientin.

Compounds **3–6** were identified as lutonarin [12], saponarin [16,17], isoorient [14,18] and luteolin [19], respectively, by comparing their physical and spectral data with those reported in literature and from chemical evidence.

For the evaluation of antioxidant activity of pure compounds, a DPPH assay was adopted with Trolox as positive control [7,8]. In the DPPH system, the free radical scavenging activity of tested samples was expressed as IC_{20}. The scavenger activities of **1**, **2**, **5**, **6**, and Trolox are listed in table 2; compound **2** was most active ($IC_{20} = 5.20 \pm 0.10\,\mu M$) in the tested compounds.

3. Experimental

3.1 General experimental procedures

Melting points were determined with an X-4 Digital Display Micro-Melting point apparatus and are uncorrected. Optical rotations were recorded by using a Perkin-Elmer 241 polarimeter. UV spectra were measured on a Spect 50-UV/Vis instrument (Analytic Jena AG). IR spectra were obtained with an FTS165-IR instrument (Bio-Rad, USA). ^{1}H NMR (400.13 Hz), ^{13}C NMR (100.62 Hz) and 2D NMR spectra were recorded on a Varian INOVA-400 FT-NMR spectrometer (USA) in CD_3OD or DMSO with TMS as internal standard. HRESI-MS was recorded on a Bruker APEX II. Column chromatography (CC) was performed over silica gel (Qingdao Marine Chemical Group, China; 200–300 mesh), RP-18 (Analteck, Newark, DE, USA, 35–75 μm), resin (Heibei Cangzhou Bon Chemical, HPD100), Sephadex LH-20 (Shanghai Chemical Reagent Factory, China) and polyamide (Shanghai Reagent Factory No. 4, China). Pre-coated silica gel plates (GF_{254}) and polyamide layer sheets (Shanghai Reagent Factory No. 4) were used for TLC analysis. Trolox was purchased from Aldrich, USA and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) from Sigma, USA.

3.2 Plant material

The whole plants of *Gentiana piasezkii* Maxim were collected in Qingshui County, Gansu province, P.R. China in September 2001 and were identified by Professor Ji Ma, Faculty of Pharmacy, First Military Medical University of PLA, Guangzhou, P.R. China. A voucher specimen (No. 2001–01) has been deposited at our laboratory.

3.3 Extraction and isolation

The air-dried whole plant (2.8 kg) was powdered and extracted with 95% EtOH at room temperature (20 L × 4, each extraction lasted 7 days). The combined extracts were evaporated to dryness under reduced pressure. The residue (300 g) was then suspended in H_2O (1.5 L), extracted with $CHCl_3$ (1.5 L × 3) and *n*-BuOH (1.5 L × 3), successively. The *n*-BuOH extract (97 g) was subjected to column chromatography (CC) on resin using H_2O and 95% EtOH, respectively. The concentrated 95% EtOH eluent (85 g) was subjected to CC on silica gel (1.0 kg) using $CHCl_3$ with increasing volume of CH_3OH (v/v = 50:1, 40:1, 15:1, 8:1, 5:1, 3:1, 1:1, each about 3.0 L) as eluent. Fractions were examined by TLC and combined to afford seven fractions (Fr. A to G). Fr. B (4.2 g) was isolated by CC on silica gel firstly using $CHCl_3$/EtOAc (3:1), then $CHCl_3$/CH_3OH (30:1), and lastly by CC on RP-18 using CH_3OH/H_2O (7:3) to yield pure **6** (50 mg). Fr. C (5.0 g) was repeatedly separated over silica gel columns eluting with $CHCl_3$/CH_3OH (15:1) and EtOAc/CH_3OH (40:1) to afford pure **1** (60 mg). Fr. D (3.4 g) was isolated by CC on silica gel with $CHCl_3$/CH_3OH (5:1), $CHCl_3$/CH_3OH/H_2O (12:4:1), EtOAc/CH_3OH/H_2O (20:1:1), further by CC on polyamide with CH_3OH/H_2O (1:1) to yield pure **2** (50 mg). Fr. E (5.0 g) was isolated by CC on silica gel with $CHCl_3$/CH_3OH (5:1), $CHCl_3$/acetone/H_2O (9:45:7) and EtOAc/CH_3OH/H_2O (20:2:1) to yield pure **5** (80 mg). Fr. F (8.2 g) was isolated by CC on silica gel with $CHCl_3$/CH_3OH/H_2O (12:5:1) and EtOAc/CH_3OH/H_2O (20:4:1), further isolated by CC Sephadex LH-20 with CH_3OH and by CC on polyamide with CH_3OH/H_2O (v/v = 4:5) to obtain pure **4** (53 mg). Fr. G (5.6 g) was isolated by CC on silica gel with $CHCl_3$/CH_3OH/H_2O (12:6:1) and EtOAc/CH_3OH/H_2O (20:5:1), further isolated by CC on polyamide with CH_3OH/H_2O (1:1) to yield pure **3** (47 mg).

3.3.1 6′-*O*-Vanilloylarbutin (1). Yellow amorphous powder, mp 143–144°C. $[\alpha]_D^{25}$ −25 (*c* 0.12, CH_3OH). IR (KBr) cm^{-1}: ν_{max} 3367, 2921, 2847, 1699, 1647, 1602, 1558, 1509, 1489, 1456, 1430, 1371, 1287, 1223, 1071, 1032, 978, 876, 831, 780, 762, 624, 516. UV (CH_3OH): λ_{max} (log ε) nm 215.2 (2.3), 261.0 (6.4), 293.1 (4.4). HRESI-MS: *m/z* 440.1540 $[M + NH_4]^+$ (calcd for $C_{20}H_{22}O_{10} + NH_4$, 440.1551). ^{1}H NMR and ^{13}C NMR: see table 1.

3.3.2 7-*O*-Feruloylorientin (2). Yellowish oil. $[\alpha]_D^{20}$ −157 (c 0.25, CH_3OH). IR (KBr): ν_{max} cm^{-1} 3264, 2935, 2257, 2128, 1706, 1650, 1606, 1516, 1489, 1445, 1357, 1273, 1227, 1176, 1124, 1085, 1027, 998, 823, 765, 688, 563. UV (CH_3OH): λ_{max} (log ε) nm 202.6 (41.4), 329.4 (23.8). ^{1}H NMR (DMSO-d_6): 13.63 (1H, *s*, H-5-OH), 6.63 (1H, *s*, H-3), 6.43 (1H, *s*, H-6), 7.36 (1H, *s*, H-2′), 6.86 (1H, *d*, *J* = 8 Hz, H-5′), 7.38 (1H, *d*, *J* = 8 Hz, H-6′), 4.84 (1H, *d*, *J* = 9.6 Hz, H-1″), 7.19 (1H, br *s*, H-2‴), 6.73 (1H, *d*, *J* = 8 Hz, H-5‴), 6.99 (1H, *d*, *J* = 8 Hz, H-6‴), 7.21 (1H, *d*, *J* = 16 Hz, H-7‴), 6.24 (1H, *d*, *J* = 16 Hz, H-8‴), 3.76 (3H, *s*, H_3-OCH_3). ^{13}C NMR(DMSO-d_6): 163.8 s (C-2, 7), 102.9 d (C-3, 6), 181.8 s (C-4), 156.4 s (C-5, 9), 107.0 s (C-8, 10), 121.4 s (C-1′), 119.0 d (C-2′), 145.7 s (C-3′), 149.7 s (C-4′), 116.1 d (C-5′), 113.3 d (C-6′), 76.4 d (C-1″), 71.9 d (C-2″), 80.1 d (C-3″), 70.7 d (C-4″), 81.8 d (C-5″), 61.4 t (C-6″), 125.6 s (C-1‴), 111.0 d (C-2‴), 147.9 s (C-3‴), 149.2 s (C-4‴), 115.5 d (C-5‴), 123.0 d (C-6‴), 144.5 d (C-7‴), 114.8 d (C-8‴), 165.4 s (C-9‴), 55.7 q (C-OCH_3). HRESI-MS: *m/z* 625.1544 $[M + H]^+$ (calcd for $C_{31}H_{28}O_{14}$ + H, 625.1552).

3.3.3 Evaluation of DPPH radical scavenging activity. The antioxidant activities of the pure compounds were assessed on the basis of the radical scavenging effect of the stable DPPH free radical [7,8]. One milliliter of a 100 μM DPPH ethanol solution was added to 10 μL of sample solutions of different concentrations and allowed to react at room temperature. After 30 min the absorbance values were measured at 517 nm using a spectrophotometer and converted into the percentage antioxidant activity (AA) using the following formula:

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100]/Abs_{control}\}$$

Ethanol (1.0 mL) plus sample solution (10 μL) was used as a blank. DPPH solution plus ethanol was used as a negative control. The IC_{20} value is the concentration of test sample required to scavenge 20% DPPH free radicals. Trolox was used as positive control.

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References

[1] M. Hostetmann-Kaldas, K. Hostettmann, O. Sticher. *Phytochemistry*, **20**, 443 (1981).
[2] Y.L. Huang, P.Y. Yeh, C.C. Shen, C.C. Chen. *Phytochemistry*, **64**, 1277 (2003).
[3] G.M. Chisolm, D. Steinberg. *Free Radic. Biol. Med.*, **28**, 1815 (2000).
[4] M. Okuda, I. Ikai, B. Chance, C. Kumar. *Biochim. Biophys. Res. Commun.*, **174**, 217 (1991).
[5] M.F. Beal. *Ann. Neurol.*, **38**, 357 (1995).
[6] Jiangsu College of New Medicine. *A Dictionary of the Traditional Chinese Medicines*, pp. 627–629, Shanghai People's Press, Shanghai (1977).
[7] C.H. Chen, C.Y. Shaw, C.C. Chen, Y.C. Tsai. *J. Nat. Prod.*, **65**, 740 (2002).
[8] Y.L. Huang, P.E. Yeh, C.C. Shen, C.C. Chen. *Phytochemistry*, **64**, 1277 (2003).
[9] F. Khatoon, M. Khabiruddin, W.H. Ansari. *Phytochemistry*, **27**, 3010 (1988).
[10] J. Garcia, E.M. Mpondo, G. Cartier. *J. Nat. Prod.*, **52**, 996 (1989).
[11] J. Garcia, E.M. Mpondo, G. Cartier. *J. Nat. Prod.*, **52**, 858 (1989).
[12] H. Abougazar, E. Bedir, I.A. Khan, I. Calis. *Planta Med.*, **69**, 814 (2003).
[13] S.G. Leitao, F.D. Monache. *Phytochemistry*, **49**, 2167 (1998).
[14] S.H. Kuo, M.H. Yen, M.I. Chung, C.N. Lin. *Phytochemistry*, **41**, 309 (1996).
[15] C. Hirobe, Z.S. Qiao, K. Takeya, H. Itokawa. *Phytochemistry*, **46**, 521 (1997).
[16] A.J. Chulia, A.M. Mariotie. *J. Nat. Prod.*, **48**, 480 (1985).
[17] S.J. Bloor. *Phytochemistry*, **50**, 1395 (1999).
[18] C.N. Lin, S.H. Kuo, M.I. Chung, F.N. Ko, C.M. Teng. *J. Nat. Prod.*, **60**, 851 (1997).
[19] D.Q. Yu, J.S. Yang. *Analytical Chemistry Manual, VII Analysis of Nuclear Magnetic Resonance*, p. 818, Chemistry Industry Press, Beijing (2002).