Three New Iridoid Glycosides from the Fruit of *Gardenia jasminoides* var. *radicans*

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Three new iridoid glycosides, 6''-*O*-*trans*-feruloylgenipin gentiobioside (1), 2'-*O*-*trans*-*p*-coumaroylcardoside (2), 2'-*O*-*trans*-feruloylcardoside (3), were isolated from the fruit of *Gardenia jasminoides* var. *radicans* MAKINO (Rubiaceae). The structures of these compounds were elucidated on the basis of MS, NMR spectra analysis, glycoside hydrolysis, and sugar derivatization coupled with HPLC analysis.

Key words Rubiaceae; *Gardenia jasminoides* var. *radicans*; iridoid glycoside; 6''-*O*-*trans*-feruloylgenipin gentiobioside; 2'-*O*-*trans*-*p*-coumaroylcardoside; 2'-*O*-*trans*-feruloylcardoside

Gardenia jasminoides var. *radicans* (THUNB.) MAKINO (Rubiaceae), is extensively distributed in Guangxi, Sichuan, Jiangxi, Hubei provinces in China. Its fruit was used as a common folk medicine, or dye, and food additives for it contains high content of yellow pigments.^{1,2)} As a variant of the *Gardenia jasminoides* ELLIS, it was reported that there were no significant genetic diversity between it and *G. jasminoides*.³⁾ Moreover, it has the higher content of the main medicinal iridoid ingredients than *G. jasminoides*.⁴⁾ Recently, some of iridoid ingredients isolated from the *G. jasminoides* were reported to be of a potential antagonistic effect against the Alzheimer's disease.⁵⁾ However, there are few reports on the chemical constituents and pharmacological activity about the variant.^{2,6)} Still now, only five coumarins, three iridoid ingredients, four diterpenes, and other small molecule compounds have been isolated from this plant.^{6,7)} Herein, we report the isolation and identification of three new iridoid glycosides (compounds 1–3, Fig. 1) by the combination of spectroscopic analysis, acidic hydrolysis, and sugar derivatization. It is worth mentioning that the acyl moieties of iridoid glycosides from the variant most were *trans*-feruloyl, which was different from the *trans*-sinapoyl moiety found in *Gardenia jasminoides*.⁶⁾

Results and Discussion

The 90% EtOH extract of *G. jasminoides* var. *radicans* was suspended in water and partitioned with petroleum ether and ethyl acetate, respectively. The ethyl acetate extracts were subjected to silica gel, polyamide, octadecyl silica gel (ODS), Sephadex LH-20 column chromatography, and semipreparative reversed phase HPLC, affording three iridoid glycosides (compounds 1–3).

Compound 1 was obtained as a colorless transparent jelly. The molecular formula of C₃₃H₄₂O₁₈ for this compound was determined from the pseudo-molecular ion peak at *m/z* 749.2266 [M+Na]⁺ obtained by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). The ¹H- and ¹³C-NMR spectra of compound 1 (Table 1) showed four olefinic protons and six olefinic carbon signals, including a set of *trans* double bond signals at δ_H/δ_C 7.64 (d, *J*=15.9 Hz, H-3'')/

147.2 (C-3''), and 6.41 (d, *J*=15.9 Hz, H-2'')/115.2 (C-2''). Aromatic proton signals at δ_H 7.08 (dd, *J*=8.1, 1.3 Hz, H-9''), 6.81 (d, *J*=8.1 Hz, H-8''), and 7.22 (d, *J*=1.3 Hz, H-5'') and proton signal at δ_H 3.90 (brs, 6'''-OCH₃), together with carbon signals at δ_C 111.7 (C-5'''), 116.5 (C-8'''), 124.4 (C-9'''), 127.6 (C-4'''), 149.3 (C-7'''), and 150.5 (C-6'''), suggested the presence of a 1,3,4-trisubstituted benzene moiety. Furthermore, the heteronuclear multiple bond connectivity (HMBC) (Fig. 2) proton/carbons correlations from δ_H 7.64 (H-3'') to δ_C 127.6 (C-4'''), 169.2 (C-1'''), and from δ_H 7.22 (H-5'') to δ_C 149.3 (C-7'''), 147.2 (C-3'''), from δ_H 7.08 (H-9'') to δ_C 111.7 (C-5''') and 149.3 (C-7'''), from δ_H 6.81 (H-8'') to 127.6 (C-4''') and 150.6 (C-6'''), from δ_H 6.41 (H-2'') to δ_C 127.6 (C-4'''), 169.2 (C-1'''), and from δ_H 3.90 (6'''-OCH₃) to δ_C 150.5 (C-6''') revealed the presence of a *trans*-feruloyl moiety.

Signals at δ_H/δ_C 4.71 (d, *J*=7.8 Hz, H-1')/100.6 (C-1'), and 4.40 (d, *J*=7.7 Hz, H-1'')/104.9 (C-1'') and other some proton and carbon signals in δ_H/δ_C 4.52–3.20/77.9–64.7 revealed the presence of two sugar moieties. On acid hydrolysis, compound 1 afforded D-glucose, the structure of which was confirmed by HPLC method of Tanaka *et al.*^{8,9)} The anomeric centers

Fig. 1. The Structures of Compounds 1–3

The authors declare no conflict of interest.

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Table 1. NMR Data (in Methanol- d_4) of Compounds 1–3

Position	1		2		3	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	98.9	5.14 d ^{a)} (7.8)	96.4	5.53 d (2.8)	96.6	5.54 d (3.0)
3	153.4	7.47 brs	152.9	7.30 brs	152.8	7.30 brs
4	112.3		111.9		112.0	
5	36.6	3.14 m	30.7	2.97 m	30.9	2.99 m
6	39.7	2.79 dd (15.9, 8.0) 2.13 dd (15.9, 8.0)	39.5	2.18 m 1.79 m	39.8	2.20 m 1.80 m
7	129.1	5.83 brs	73.7	4.31 t (6.4)	73.8	4.31 t (6.5)
8	144.6		152.5		152.5	
9	46.8	2.69 t (7.6)	45.1	3.02 brs	45.3	3.03 brs
10	61.5	4.22 m 4.29 m	112.2	5.33 brs 5.28 brs	112.2	5.33 brs 5.29 brs
11	169.6		170.1		170.2	
11-OCH ₃	51.9	3.67 brs				
1'	100.6	4.71 d (7.8)	97.9	4.90 d (8.1)	98.2	4.89 ^{b)}
2'	74.8	3.28 m	74.6	4.86 dd ^{b)}	74.8	4.83 ^{b)}
3'	77.7	3.45 m	75.8	3.73 m	76.0	3.65 m
4'	71.5	3.40 m	71.7	3.47 m	71.9	3.41 m
5'	77.4	3.57 m	78.4	3.48 m	78.5	3.43 m
6'	70.1	4.10 ^{b)} 3.76 m	62.7	3.95 d (11.7) 3.68 m	62.9	3.72 m 3.95 m
1''	104.9	4.40 d (7.7)				
2''	75.0	3.26 m				
3''	77.9	3.42 m				
4''	71.5	3.39 m				
5''	75.3	3.57 m				
6''	64.7	4.52 dd (11.7, 1.2) 4.32 ^{b)}				
1'''	169.2		168.3		168.3	
2'''	115.2	6.41 d (15.9)	114.9	6.26 d (15.9)	115.4	6.29 d (15.9)
3'''	147.2	7.64 d (15.9)	146.9	7.60 d (15.9)	147.3	7.59 d (15.9)
4'''	127.6		127.4		128.1	
5'''	111.7	7.22 d (1.3)	131.4	7.45 d (8.3)	112.2	7.17 d (1.7)
6'''	150.5		116.9	6.81 d (8.3)	150.6	
7'''	149.3		160.9		149.4	
8'''	116.5	6.81 d (8.1)	116.9	6.81 brd (8.3)	116.5	6.79 d (8.4)
9'''	124.4	7.08 dd (8.1, 1.3)	131.4	7.45 brd (8.3)	124.2	7.06 dd (8.4, 1.7)
6'''-OCH ₃	56.6	3.90 brs			56.6	3.91 brs

Assignments were performed by means of DEPT, COSY, HSQC, HMBC experiments. a) Multiplicity: s, singlet; d, doublet; t, triplet; m, multiplet. b) Signals were overlapped.

Fig. 2. Key COSY (in Bold Lines) and HMBC (H→C) Correlations of 1

of the two glucopyranosyl moieties were determined to be β -configuration from the large coupling constant value at δ_H 4.71 (d, $J=7.8$ Hz, H-1'), and 4.40 (d, $J=7.7$ Hz, H-1''). The HMBC correlations of protons/ carbons at δ_H/δ_C 4.40 (H-1'')/70.1 (C-6'), and 3.76 (H-6'b)/104.9 (C-1'') suggested that

the sugar chain was glucopyranosyl-(1→6)-glucopyranoside, *i.e.*, a gentiobiosyl moiety.

The remaining ^1H - and ^{13}C -NMR signals were similar to those of genipin by comparing with the reported data.^{5,10} It suggested that 1 should be an iridoid glycoside with a *trans*-feruloyl, and gentiobiosyl moieties, which were confirmed by ^1H - ^1H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and HMBC spectra. The nuclear Overhauser effect spectroscopy (NOESY) spectrum showed the correlations between proton at δ_H 2.69 (H-9) and proton at δ_H 2.13 (H-6 β), and between two protons at δ_H 3.14 (H-5) and at δ_H 2.69 (H-9), confirming that the β -orientation of H-5 and H-9. There are no any nuclear Overhauser effect (NOE) correlations between protons at δ_H 5.14 (H-1) and 3.14 (H-5) or 2.69 (H-9), confirming the α -orientation of H-1. The gentiobiosyl moiety was attached at C-1 of genipin due to the HMBC correlations from proton at δ_H 5.14 (H-1) to carbon at δ_C 100.6 (C-1'), and proton at δ_H 4.71 (H-1') to carbon at δ_C 98.9 (C-1).

Fig. 3. Key COSY (in Bold Lines) and HMBC (H→C) Correlations of **2**

Furthermore, the correlation from proton at δ_{H} 4.32 (H-6''b) to carbon at δ_{C} 169.2 (C-1'') in HMBC spectrum suggested that the linkage of the feruloyl to the gentiobiosyl group be established to be at C-6''. Thus, the structure of **1** was deduced as 6''-*O*-*trans*-feruloylgenipin gentiobioside.

Compound **2** was obtained as a colorless transparent jelly. Its molecular formula $\text{C}_{25}\text{H}_{28}\text{O}_{12}$ was determined from the pseudo-molecular ion peak at m/z 543.1475 $[\text{M}+\text{Na}]^+$ in HR-ESI-MS. The ^1H - and ^{13}C -NMR spectra of **2** (Table 1) showed five olefinic protons and six olefinic carbons signals, including a set of *trans* double bond signals at δ_{H} 7.60 (d, $J=15.9\text{ Hz}$, H-3'''), and 6.26 (d, $J=15.9\text{ Hz}$, H-2'''). Proton/carbon signals at $\delta_{\text{H}}/\delta_{\text{C}}$ 6.81 (2H, brd, $J=8.3\text{ Hz}$, H-6''', 8''')/116.9 (C-6''', 8'''), and 7.45 (2H, brd, $J=8.3\text{ Hz}$, H-5''', 9''')/131.4 (C-5''', 9'''), together with carbon signals at δ_{C} 127.4 (C-4'''), and 160.9 (C-7'''), suggested the presence of a symmetrical 1,4-disubstituted benzene ring. Furthermore, the HMBC correlations (Fig. 3) observed between protons and carbons at $\delta_{\text{H}}/\delta_{\text{C}}$ 7.60 (H-3''')/168.3 (C-1'''), 7.45 (H-5''', 9''')/146.9 (C-3'''), 6.81 (H-6''', 8''')/127.4 (C-4'''), 6.26 (H-2''')/127.4 (C-4''') and 6.26 (H-2''')/168.3 (C-1'''), revealed the presence of a *trans*-*p*-coumaroyl-moiety. Signals at $\delta_{\text{H}}/\delta_{\text{C}}$ 4.90 (d, $J=8.1\text{ Hz}$, H-1')/97.9 (C-1') and other oxygenated methine signals revealed the presence of sugar residual. After acid hydrolysis and derivatization of **2** by the method of Tanaka *et al.*,^{8,9)} the D-glucose was detected in HPLC analysis. The β -configuration was established due to the coupling constant of the anomeric proton signal at δ_{H} 4.90 (d, $J=8.1\text{ Hz}$, H-1'). The remaining ^{13}C -NMR signals of compound **2** displayed the characteristic carbons at δ_{C} 96.4 (C-1), 111.9 (C-4), 152.9 (C-3), and 170.1 (C-11) of the iridoid nucleus. The ^1H -NMR spectrum of **2** showed the presence of exocyclic methylene protons at δ_{H} 5.33 (brs, H-10a) and 5.28 (brs, H-10b), and a signal at δ_{H} 4.31 (t, $J=6.4\text{ Hz}$, H-7) attributable to an allylic hydroxymethine proton. In the HMBC spectrum of **2**, the correlations of signals from δ_{H} 5.33 (H-10a) to δ_{C} 73.7 (C-7) and 45.1 (C-9), from δ_{H} 5.28 (H-10b) to δ_{C} 73.7 (C-7) and 45.1 (C-9), from δ_{H} 7.30 (H-3) to δ_{C} 30.7 (C-5), 96.4 (C-1), and 170.1 (C-11), and from δ_{H} 5.53 (H-1) to δ_{C} 30.7 (C-5) and 152.9 (C-3) further supported the structural elucidations. Thus, the structure of this iridoid moiety was assigned as a gardoside nucleus moiety.¹¹⁾ The NOESY spectrum of compound **2** showed the correlations between protons at δ_{H} 4.31 (H-7) and 2.18 (H-6 α), and between protons at δ_{H} 3.02 (H-9) and 1.79 (H-6 β), as well as between protons at δ_{H} 2.97 (H-5) and 1.79 (H-6 β). However, there are no any NOE correlations between proton at δ_{H} 5.57 (H-1) and protons at δ_{H} 2.97 (H-5) or δ_{H} 3.02 (H-9), confirming that the H-5 and H-9 were β -orientational, and H-1 and H-7 were α -orientational. The glucopyranosyl moiety was attached at C-1 of gardoside due to the HMBC correlations from proton at δ_{H} 5.53 (H-1) to car-

bon at δ_{C} 97.9 (C-1'), and proton at δ_{H} 4.90 (H-1') to carbon at δ_{C} 96.4 (C-1). Furthermore, the linkage of the *trans*-*p*-coumaroyl-moiety to the gardoside group was located at C-2' by the HMBC correlations from proton at δ_{H} 4.86 (H-2') to carbon at δ_{C} 168.3 (C-1'''). Therefore, the structure of **2** was elucidated as 2'-*O*-*trans*-*p*-coumaroylgardoside.

Compound **3** was also obtained as a colorless transparent jelly. Its molecular formula $\text{C}_{26}\text{H}_{30}\text{O}_{13}$ was determined from the pseudo-molecular ion peak at m/z 573.1590 $[\text{M}+\text{Na}]^+$ in HR-ESI-MS. The ^1H - and ^{13}C -NMR data of **3** were analogous to those of **2**, but showing the presence of one extra methoxyl groups (δ_{H} 3.91 and δ_{C} 56.6). The methoxyl group were located at C-6''' on the basis of aromatic proton signals at δ_{H} 7.06 (dd, $J=8.4, 1.7\text{ Hz}$, H-9'''), 6.79 (d, $J=8.4\text{ Hz}$, H-8'''), and 7.17 (d, $J=1.7\text{ Hz}$, H-5''') as well as all comparable aromatic carbon signals, suggesting that **3** had a *trans*-feruloyl substituent at C-2', same as compound **1**. Therefore, the structure of **3** was elucidated as 2'-*O*-*trans*-feruloylgardoside.

Experimental

General Experimental Procedures Melting points were determined on an X-5 micro-melting point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China). Optical rotations were carried out using a JASCO P-1020 automatic digital polarimeter (JASCO Corporation, Tokyo, Japan). UV spectra were recorded on a JASCO V-550 UV/VIS spectrometer (JASCO Corporation, Tokyo, Japan). IR spectra were obtained using a Fourier Transform infrared spectrometer (Bruker Instrument, Inc., German). 1D- and 2D-NMR spectra were recorded in CD_3OD using Bruker AV-300 spectrometer (Bruker Instrument, Inc., German) with tetramethylsilane (TMS) as the internal standard, and the chemical shifts were expressed in δ values (ppm). Analytical reversed-phase HPLCs were performed on an UltimateTM XB-C18 column (5 μm , 4.6 $\times$ 250mm, Welch, Potomac, MA, U.S.A.) and Materials XB-C18 (5 μm , 10 $\times$ 250mm) for semipreparative HPLC purification. Open column chromatography was performed by silica gel (300–400 mesh, Qingdao, Haiyang Chemical Group Corporation, Qingdao, China), and ODS (50 μm , YMC, Tokyo, Japan). All the reagents were purchased from Tianjin Damao Chemical Company (Tianjin, China). Thin-layer chromatography was performed by precoated aluminum gel plate (aluminum gel HSAF254, 1 mm, Yantan, China). The spraying reagent used for TLC was 10% H_2SO_4 in EtOH. Standard sugars D-glucose and L-glucose, and L-cysteine methyl ester were purchased from Adamas-beta Company (Basel, Switzerland). *o*-Tolyl isothiocyanate was purchased from Sigma Company (Santa Clara, CA, U.S.A.).

Plant Material The fruit material of *G. jasminoides* var. *radicans* was purchased in Bozhou, Anhui province, China, and identified by Professor GX Zhou, college of pharmacy, Jinan University. A specimen was deposited in the Pharmacognosy Department of the college.

Extraction and Isolation The dried fruit of *G. jasminoides* var. *radicans* were grounded, and extracted three times with 85–95% alcohol under soaking at room temperature for 24h. The extract solution were combined and concentrated under reduced pressure to about 3.5kg and then partitioned successively with petroleum ether, ethyl acetate, and *n*-butanol, to afford 500 g, 270 g, and 950 g of extracts, respectively, and 840 g water-soluble residue. The ethyl acetate extract

(270 g) was subjected to silica gel chromatography eluting with gradient CHCl_3 -MeOH systems (1:0→20:1→18:1→15:1→12:1→10:1→8:1→5:1→5:2→1:1→0:1) to afford 24 fractions (Fr. A–W) after combination of fractions by TLC pattern. Fraction U (17.8 g) was further separated by silica gel chromatography eluting with CHCl_3 -MeOH systems (20:1→15:1→10:1→5:1→5:2→1:1→0:1) to obtain 9 fractions (Fr. 1–9) by TLC. Fraction 7 (1.2 g) was subjected to sephadex LH-20 column eluting with MeOH to obtain compound **1** (180 mg). Fraction 8 was subjected to ODS column chromatography eluting with MeOH- H_2O (20→30→40→60→80→100%) to afford 3 subfractions (SFr. 8-1–3). Subfraction 8-1 (580 mg) was purified on sephadex LH-20 column eluting with MeOH to obtain compounds **2** (120 mg) and **3** (15 mg).

6''-O-trans-Feruloylgenipin Gentiobioside (1): Colorless transparent jelly; mp 175–177°C; $[\alpha]_{\text{D}}^{25}$ –20.1 ($c=0.04$, MeOH); UV λ_{max} (MeOH): 288 nm; IR ν_{max} (KBr): 3428, 2931, 1694, 1632, 1083 cm^{-1} ; ^1H - and ^{13}C -NMR spectral data, see Table 1. HR-ESI-MS m/z : 749.2266 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{33}\text{H}_{42}\text{O}_{18}\text{Na}$, 749.2269).

2'-O-trans-p-Coumaroylgardoside (2): Colorless transparent jelly; mp 179–180°C; $[\alpha]_{\text{D}}^{25}$ –15.0 ($c=0.02$, MeOH); UV λ_{max} (MeOH): 312 nm; IR ν_{max} (KBr): 3406, 1695, 1634, 1604, 1173 cm^{-1} ; ^1H - and ^{13}C -NMR spectral data, see Table 1. HR-ESI-MS m/z : 543.1475 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{25}\text{H}_{28}\text{O}_{12}\text{Na}$, 543.1479).

2'-O-trans-Feruloylgardoside (3): Colorless transparent jelly; mp 181–182°C; $[\alpha]_{\text{D}}^{25}$ –45.2 ($c=0.02$, MeOH); UV λ_{max} (MeOH): 232 nm; IR ν_{max} (KBr): 3458, 1633, 1518, 1385, 1275 cm^{-1} ; ^1H - and ^{13}C -NMR spectral data, see Table 1. HR-ESI-MS m/z : 573.1590 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{26}\text{H}_{30}\text{O}_{13}\text{Na}$, 573.1484).

Acid Hydrolysis and HPLC Analysis The absolute configuration of the sugar moieties in the structures was determined by the method of Tanaka *et al.*^{8,9)} Compound **1** (4 mg) was hydrolyzed with 4 mL of 2 M HCl for 6 h at 80°C. The mixture was evaporated to dryness under vacuum. The residue was dissolved in pyridine (2 mL) containing L-cysteine methyl ester (2 mg) and heated at 60°C for 1 h. Then, *o*-tolyl

isothiocyanate (10 μL) was added to the mixture, which was heated at 60°C for 1 h. The reaction mixture was directly analyzed by reversed-phase HPLC. Analytical HPLC was performed on the column at 28°C with isocratic elution of 25% CH_3CN containing 0.08% formic acid for 40 min and subsequent washing the column with MeOH at a flow rate of 1 mL/min. Peaks were detected by UV detector at 250 nm. The peak of the derivative of **1** was observed at t_{R} 15.95 (D-Glu) min. The standard sugars D-glucose was subjected to the same method. The peak of the standard was at t_{R} 16.05 min. Following the above procedure, the derivatives of **2** gave peaks at t_{R} 16.13 (D-Glu) min.

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