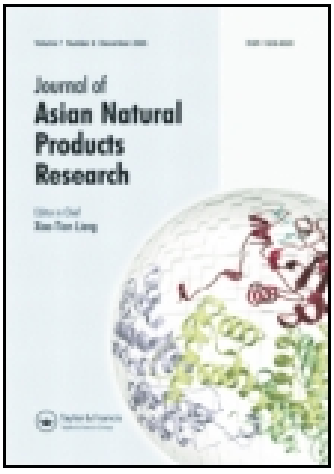

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Two new phenolic glycosides from the rhizome of *Gastrodia elata*

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Two new phenolic glycosides, named parishins F–G (**1–2**), together with known parishin E, were isolated from the rhizome of *Gastrodia elata*. The new structures were established as 1,3-di-[4-*O*-(β-D-glucopyranosyl) benzyl]-2-{4-*O*-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] benzyl} citrate (**1**) and 2-[4-*O*-(β-D-glucopyranosyl)benzyl] citrate (**2**), by means of MS, 1D, and 2D NMR spectral analyses, as well as chemical methods.

Keywords: *Gastrodia elata*; glycoside; parishin

1. Introduction

Gastrodia elata Blume (Tianma in Chinese), a popular Chinese herbal medicine, has been used for many years as an anticonvulsant, an analgesic, and a sedative agent against vertigo, general paralysis, epilepsy, and tetanus [1,2]. Abundant phenols including simple phenols [3–8] and phenolic conjugates such as parishins [9,10] have been reported from this plant. In our ongoing phytochemical investigation, some new peaks were found in the extract of *G. elata* through a rapid LC-ESI-MS screening [11]. As a result, three phenolic glycosides and one nucleoside had been successfully isolated by LC-MS guided separation on preparative HPLC. Among them, one phenolic compound parishin E (**3**) [10] and one nucleoside N^2-(*p*-hydroxybenzyl) guanosine [12] had been reported previously. This paper describes the isolation and structural elucidation of another two new phenolic glycosides, named as parishins F–G (**1–2**, Figure 1), by 1D and 2D NMR analyses and chemical methods.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The IR spectrum showed hydroxyl (3397 cm^{-1}), ester carbonyl (1733, 1232 cm^{-1}), and phenyl (1615, 1515 cm^{-1}) absorptions. The molecular formula was established as $C_{51}H_{66}O_{30}$ from the pseudo-molecular ion at *m/z* 1181.3535 $[M + Na]^+$ in the HR-ESI-MS. In ESI-MS/MS, the characteristic fragment ions of parishins at *m/z* 889 $[M - H\text{-}268]^-$, 727 $[M - H\text{-}268\text{-}162]^-$ [11], together with the molecular weight of 1158 being 162 mass units larger than 996 of parishin (tris[4-*O*-(β-D-glucopyranosyl)benzyl] citrate), suggested that compound **1** was hexose-substituted parishin derivative.

The NMR spectra of **1** showed four anomeric protons at δ_H 5.01 (d, $J = 5.6$ Hz), 4.97 (d, $J = 6.4$ Hz), 4.92 (d, $J = 5.6$ Hz), and 4.43 (d, $J = 7.6$ Hz), and the corre-

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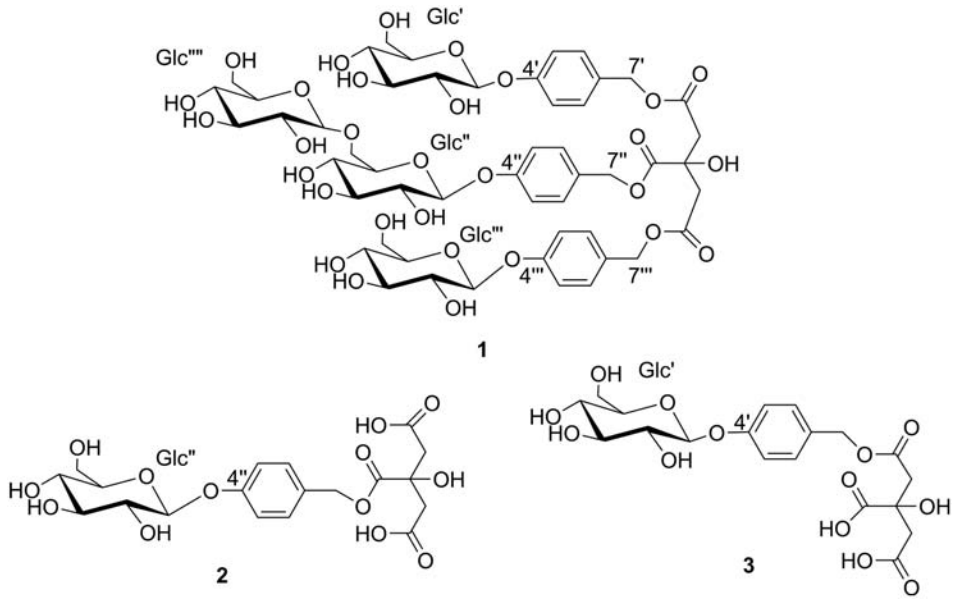

Figure 1. Structures of compounds **1**–**3**.

sponding carbon signals at δ_C 102.9 (three carbons) and 105.2 (Table 2), instead of three glucose signals in parishin. The downfield signal at δ_C 70.7 (Glc C-6″) and the corresponding protons at δ 4.14 (Glc $H_{6a''}$) and 3.88–3.92 (Glc $H_{6b''}$) suggested that the fourth glucose (identified by comparison with authentic glucose on TLC and GC analysis) was located at Glc C-6″ to form a gentiobiose residue [13]. The HMBC correlation of Glc H-1′ at δ_H 5.01, Glc H-1″ at δ_H 4.97, Glc H-1‴ at δ_H

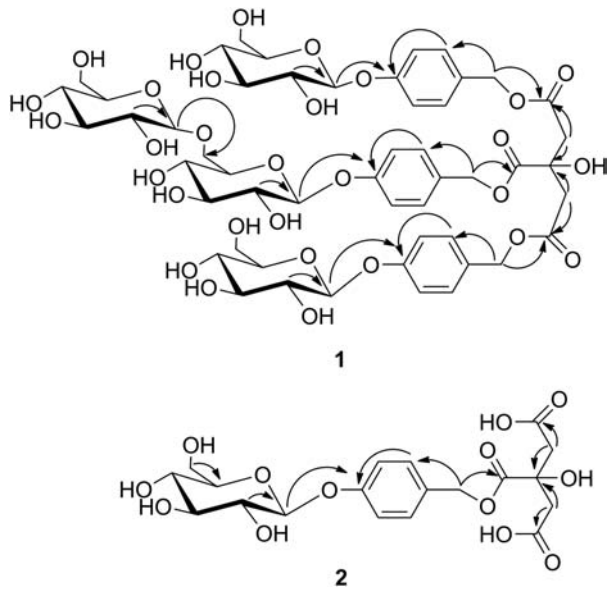

Figure 2. Key HMBC correlations of compounds **1** with **2**.

4.92 with C-4′, 4″, 4‴ at δ_C 159.4, and Glc H-1⁗ at δ_H 4.43 (Glc H-1⁗) with Glc C-6″ at δ_C 70.7 further confirmed the glycosylation sequence and position (Figure 2). The larger coupling constants of $^3J_{H\text{-}1,H\text{-}2}$ confirmed β-orientation at the anomeric centers for four glucoses. Furthermore, the two doublets of two methylene protons and two carbon signals of three carbonyl groups at δ 173.0 (two carbons) and 176.0 in citrate moiety further indicated that the fourth glucose was located at Glc″, which formed a symmetrical situation. ^{1}H—^{1}H COSY, HSQC data and hydrolysis results were all consistent with the results deduced above. Thus, the structure of **1** was elucidated as 1,3-di-[4-*O*-(β-D-glucopyranosyl)benzyl]-2-{4-*O*-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]benzyl} citrate, named as parishin G.

Compound **2** was also obtained as a white amorphous powder. The IR spectrum showed absorptions for hydroxyl groups at 3402 cm^{-1}, ester groups at 1732 and 1231 cm^{-1}, as well as aromatic ring at 1614 and 1513 cm^{-1}. The molecular formula was established as $C_{19}H_{24}O_{13}$ from the pseudo-molecular ion at *m/z* 483.1107 $[M + Na]^+$ in the HR-FTMS. The negative ESI-MS/MS showed fragment ions at *m/z* 173 $[M - H\text{-}268\text{-}H_2O]^-$, which was in accordance with mono-substituted parishin [11].

The NMR spectra of **2** showed similar patterns with that of mono-substituted parishin E (1-[4-*O*-(β-D-glucopyranosyl) benzyl] citrate) [10], except for the citric acid moiety. In the citric acid moiety of **2**, the two doublets at δ_H 2.94 (2H, d, $J = 15.9$ Hz) and 2.74 (2H, d, $J = 15.9$ Hz) for two methylenes, and two carbon signals for three carbonyl groups at δ_C 175.7 (two carbons) 176.9 indicated that the citrate moiety existed in a symmetrical situation, rather than four doublets for two methylene protons and three separated carbon signals for three carbonyl groups in parishin E. Thus, compound **2** was confirmed as 2-[4-*O*-(β-D-glucopyranosyl) benzyl] citrate, named as parishin H. The HMBC correlations of Glc H-1″ at δ_H 5.07 with C-4″ at δ_C 159.2, H-7″ at δ_H 5.12 with the carbonyls at δ_C 176.9, together with ^{1}H–^{1}H COSY and HSQC data, further confirmed the above structure (Figure 2).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 spectrometer (Jasco, Tokyo, Japan). IR spectra were recorded on a fourier transform infrared (FT-IR) spectrometer (Perkin-Elmer, Tucson, AZ, USA) as KBr pellets with absorption given in cm^{-1}. UV spectra were acquired on a SP-1901 spectrometer (Shanghai Spectrum, Shanghai, China). ESI-MS were obtained with a Finnigan TSQ MS/MS spectrometer (Thermo, San Jose, CA, USA) in *m/z* (rel. %). HR-FTMS were recorded on a Bruker Apexiii 7.0 Tesla FTMS (Bruker Daltonics, Billerica, MA, USA). 1D and 2D NMR spectra were measured on a Bruker DRX400 spectrometer (Bruker, Fallanden, Switzerland) in D_2O with 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt as an internal standard. Diaion HP-20 was purchased from Mitsubishi Kagaku (Tokyo, Japan). Preparative HPLC (Waters, Milford, MA, USA): Waters Delta Prep 4000 with a Waters 996 PDA detector; Nova-Pak C18 column (200 × 40 mm i.d., 6 μ; Waters) with a flow rate of 60 ml/min; Superiorex ODS column (250 × 20 mm i.d., 5 μ; Shiseido, Tokyo, Japan) with a flow rate of 15 ml/min; UV detection at 270 nm.

3.2. Plant material

The rhizome of *G. elata* was collected from Guangyuan city, Sichuan Province, China, and identified by Professor Sui-Qing Chen (Henan College of Traditional Chinese Medicine). The voucher specimen (No. 050528) is deposited in the Laboratory of Medicinal Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences.

3.3. Extraction and isolation

Air-dried rhizome (5.0 kg) was ground and extracted with 70% EtOH (3 × 10 liters) under reflux. The combined extracts were concentrated under reduced pressure, and defatted with petroleum ether. Then the remaining aqueous fraction was subjected to Diaion HP-20 column (118 × 10.8 cm, i.d.) eluted with a stepwise gradient mixture of EtOH/H_2O (0:10, 1:9, 2:8, 3:7, 5:5, and 7:3) to give six fractions (Frs 1–6). Fr. 1 (10 g) was separated by Nova-Pak column under gradient MeOH/H_2O (10:90 → 60:40 in 45 min) to give three sub-fractions Frs 1.1–1.3. Fr. 1.2 (200 mg) was further purified over the same Nova-Pak column under an isocratic MeOH/H_2O (35:65; V/V) to yield compound **1** (55 mg; t_R 6.4 min). Fr. 3 (20 g) was separated on Nova-Pak column under MeOH/H_2O/HOAc (22:77.5:0.5; V/V) to give Frs 3.1–3.5. Fr. 3.1 (1 g) was further purified with Nova-Pak column under MeOH/H_2O/HOAc (10:89.5:0.5; V/V) to yield compounds **2** (69 mg; t_R 10.9 min) and **3** (100 mg; t_R 13.0 min).

*3.3.1. Parishin F (**1**)*

White powder; $[\alpha]_D^{24}$ − 56.4 ($c = 0.11$, MeOH). UV (H_2O) λ_{max} nm (log ε): 220 (4.50), 272 (3.46). IR (KBr)υ_{max} (cm^{-1}): 3397, 1733, 1615, 1515, 1400, 1232, 1075. ^1H and ^{13}C NMR spectral data: Tables 1 and 2. ESI-MS (neg.), 35 eV, *m/z*: 1157 (11), 889 (100), 727 (34). HR-ESI-MS (pos.): *m/z* 1181.3535 $[M + Na]^+$ (calcd for $C_{51}H_{66}O_{30}Na$, 1181.3531).

Table 1. ^{13}C and ^1H NMR spectral data of the aglycone portion of compounds **1**–**2** in D_2O.

	1		**2**	
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
Citrate				
1	46.4	2.90 (d, 15.8)	45.9	2.94 (d, 15.9)
		2.72 (d, 15.8)		2.74 (d, 15.9)
2	76.1		75.9	
3	46.4	2.90 (d, 15.8)	45.9	2.94 (d, 15.9)
		2.72 (d, 15.8)		2.74 (d, 15.9)
1-CO	173.0		175.7	
2-CO	176.0		176.9	
3-CO	173.0		175.7	
Benzyl′				
1′	132.3			
2′,6′	132.8	7.23 (d, 8.4)		
3′,5′	119.3	7.04 (d, 8.4)		
4′	159.4			
7′	69.3	4.93 (s)		
Benzyl″				
1″	131.9		132.1	
2″,6″	132.7	7.10 (d, 8.4)	133.0	7.34 (d, 8.6)
3″,5″	119.3	7.04 (d, 8.4)	119.0	7.07 (d, 8.6)
4″	159.4		159.2	
7″	70.7	4.77 (s)	70.2	5.12 (s)
Benzyl‴				
1‴	132.3			
2‴,6‴	132.8	7.23 (d, 8.4)		
3‴,5‴	119.3	7.04 (d, 8.4)		
4‴	159.4			
7‴	69.3	4.93 (s)		

Table 2. ^{13}C and ^{1}H NMR spectral data of the glycosidic portion of compounds **1**–**2** in D_2O.

	1		**2**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
Glc′				
1′	102.9	5.01 (d, 5.6)		
2′	75.4	3.52–3.60 (overlap)		
3′	78.6	3.49–3.55 (overlap)		
4′	71.9	3.53–3.60 (overlap)		
5′	78.1	3.60–3.65 (overlap)		
6′	63.1	3.83–3.90 (overlap)		
		3.72–3.75 (overlap)		
Glc″				
1″	102.9	4.97 (d, 6.4)	102.5	5.07 (d, 7.6)
2″	75.4	3.52–3.60 (overlap)	75.4	3.52 (dd, 9.2, 7.6)
3″	78.4	3.27 (m)	78.6	3.55 (m)
4″	72.1	3.41–3.45 (overlap)	71.9	3.43 (t, 9.2)
5″	77.1	3.70–3.74 (overlap)	78.0	3.58 (m)
6″	70.7	4.14 (dd, 10.8,5.6)	63.0	3.87 (dd, 12.4,1.6)
		3.88–3.92 (overlap)		3.69 (dd, 12.4,5.6)
Glc‴				
1‴	102.9	4.92 (d, 5.6)		
2‴	75.4	3.52–3.60 (overlap)		
3‴	78.6	3.49–3.55 (overlap)		
4‴	71.9	3.53–3.60 (overlap)		
5‴	78.1	3.60–3.65 (overlap)		
6‴	63.1	3.83–3.90 (overlap)		
		3.72–3.75 (overlap)		
Glc⁗				
1⁗	105.2	4.43 (d, 7.6)		
2⁗	75.6	3.31 (dd, 8.4,7.6)		
3⁗	78.6	3.49–3.55 (overlap)		
4⁗	71.7	3.53–3.60 (overlap)		
5⁗	78.1	3.60–3.65 (overlap)		
6⁗	63.3	3.83–3.90 (overlap)		
		3.72–3.75 (overlap)		

3.3.2. *Parishin G (**2**)*

White powder; $[\alpha]_D^{15} - 55.1$ ($c = 0.21$, MeOH). UV (H_2O) λ_{max} nm (log ε): 220 (3.85), 270 (2.52). IR (KBr)υ_{max} (cm^{-1}): 3402, 1732, 1614, 1513, 1400, 1231, 1076. ^{1}H and ^{13}C NMR spectral data: Tables 1 and 2. ESI-MS (neg.), 15 eV, *m/z*: 459 (100), 173 (50), 111 (5). HR-ESI-MS (pos.): *m/z* 483.1107 $[M + Na]^+$ (calcd for $C_{19}H_{24}O_{13}Na$, 483.1109).

3.3.3. *Acid hydrolysis*

Compounds **1** and **2** (2 mg) were heated at 100°C for 4 h in 2 ml of 1 M H_2SO_4. The residue was neutralized with $BaCO_3$ and centrifuged, and then the supernatant was analyzed by thin layer chromatography (TLC) co-chromatography with authentic samples using $CHCl_3$/MeOH/HOAc (3:2:0.3) as developing solvents. Glucose (brown spot, R_f 0.67) was detected from **1** and **2** by spraying with *p*-anisidinephtalate reagent.

3.3.4. *Alkaline hydrolysis*

A solution of **1** or **2** (2 mg) in 0.5 M KOH (2 ml) was reacted for 4 h at rt, and the mixture was neutralized with 2 M HCl and freeze-dried. By dissolving in 1 ml MeOH, the residue was analyzed by TLC co-

chromatography with authentic samples using $CHCl_3$/MeOH/HOAc (3:2:0.3) as developing solvents. Citric acid (yellow spot, R_f 0.54) was detected from **1** and **2** by spraying with bromophenol blue reagent, while gastrodin (blue spot, R_f 0.89) was detected by spraying with phosphomolybdic acid reagent and heating for 10 min at 105°C.

3.3.5. Determination of sugar configuration

The sugar residue was reacted with L-leucine methyl ester hydrochloride, and then silanized by trimethylchlorosilane to obtain the glucose derivatives for GC analysis [Agilent Technologies 6890; DB-5 cap. column (60 m × 0.25 mm × 0.25 μm); column temp.: 180°/255°; programed increase: 5°/min; injection volume: 0.1 μl, split ratio: 1:50] [14]. The retention time of the derivate for D-glucose was t_R: 22.84 min.

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