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Chemical constituents from the rhizomes of *Gastrodia elata* f. glauca and their potential neuroprotective effects

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ABSTRACT

A phytochemical study on the rhizomes of *Gastrodia elata* f. glauca resulted in the isolation of three new parishin derivatives and one new adenosine derivative, together with six known compounds. Their structures were elucidated mainly by spectroscopic analysis and compared to published data. All these isolates were evaluated for their neuroprotective effects against 6-hydroxydopamine-induced cell death, and new compound **4** showed potent activity with an EC_{50} value of 12.0 μM.

1. Introduction

Gastrodia elata has long been used as a prominent traditional Chinese herbal medicine for the treatment of neurological disorders such as general paralysis, vertigo, and epilepsy (Chen and Sheen, 2011; Li et al., 2015; Matias et al., 2016; Zhan et al., 2016). Five varieties of *G. elata* are cultivated in China, including *G. elata* f. glauca S. Chow, *G. elata* f. elata, *G. elata* f. viridis Makino, *G. elata* f. flavida S. Chow, and *G. elata* f. alba S. Chow, which are considered as the original plants of the traditional Chinese medicine "Tian-ma" (Chen et al., 2015). Among these varieties, *G. elata* f. glauca S. Chow is the most widely cultivated. Recently, the neuroprotective activity of *G. elata* has attracted widespread attention. Indeed, pharmacological studies have demonstrated that gastrodin, parishin, vanillyl alcohol, N^6-(4-hydroxybenzyl) adenosine, and *G. elata* extract could protect neuronal cells by correction of neurotransmitter imbalance and inhibition of oxidative response in models of neurodegenerative disorders (Liu and Mori, 1992; Kim et al., 2011; Ng et al., 2016; Tang et al., 2017). Over 80 constituents have been isolated from *G. elata*, but only a few of them have been evaluated for their neuroprotective activity (Chen et al., 2016; Huang et al., 2007; Jang et al., 2015; Li et al., 2016). These results encouraged us to investigate the potential active components of *G. elata* f. glauca.

In this study, a detailed chemical investigation of the rhizomes of *G. elata* f. glauca was conducted, together with the evaluation of their neuroprotective effects against 6-hydroxydopamine-induced cell death. This resulted in the isolation and characterization of four new compounds, along with six known compounds.

2. Results and discussion

Compound **1** was obtained as a light yellow powder. Its molecular formula was assigned as $C_{41}H_{46}O_{20}$ determined from HR-ESI-MS at *m/z* 881.2462 $[M+Na]^+$ (calcd. 881.2475 $[M+Na]^+$). The IR spectrum showed absorptions at 3427 cm^{-1} (−OH), 1735–1721 cm^{-1} (ester), 1613 and 1513 cm^{-1} (aromatic ring). The ^1H NMR spectrum of **1** showed the citrate moiety signals at δ_H 2.90, 2.77 (each 1H, d, J = 15.0 Hz, H-2) and δ_H 2.76, 2.62 (each 1H, d, J = 15.6 Hz, H-4), the *trans*-cinnamoyl group signals at δ_H 7.75 (2H, m, H-2‴, 6‴), 7.68 (1H, d, J = 16.2 Hz, H-7‴), 7.44 (3H, m, H-3‴, 4‴, 5‴), and 6.70 (1H, d, J = 16.2 Hz, H-8‴), and the *p*-hydroxybenzyl alcohol moiety signals at δ_H 7.30, 7.28 (each 2H, H-3″, 5″), 7.04, 7.01 (each 2H, H-2″, 6″), 5.00, 4.97 (each 2H, H-7″), with a molar ratio of 1:1:2 (citrate moiety: *trans*-cinnamoyl group: *p*-hydroxybenzyl alcohol moiety). In addition, two sugar anomeric carbons were detected at δ_C 100.7 and 100.3 in the ^{13}C NMR spectrum, respectively attached to proton signals at δ_H 4.86 (1H, d, J = 7.2 Hz) and 5.09 (1H, d, J = 7.8 Hz) in the HMQC experiment. The NMR data of **1** were similar to those of parishin except for the existence of signals for the *trans*-cinnamoyl group mentioned above (Lin et al., 1996). The HMBC correlations (Fig. 2) from δ_H 4.97 (d, J = 4.2 Hz) to C-1 (δ_C 169.7), and from δ_H 5.00 (s) to C-6 (δ_C 173.2)

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Table 1
^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for **1**-**3** in DMSO-d_6.

unit	Position	**1**		**2**		**3**	
		δ_H (J_{HZ})	δ_C	δ_H (J_{HZ})	δ_C	δ_H (J_{HZ})	δ_C
	1		169.7		169.6		169.6
	2	2.90 (d, 15.0)	43.5	2.93 (d, 15.0)	43.4	2.93 (d, 15.0)	43.4
		2.77 (d, 15.0)		2.80 (d, 15.6)		2.80 (d, 15.6)	
	3		73.5		73.5		73.5
	4	2.76 (d, 15.6)	43.4	2.93 (d, 15.0)	43.4	2.93 (d, 15.0)	43.4
		2.62 (d, 15.6)		2.80 (d, 15.6)		2.80 (d, 15.6)	
	5		171.8		169.6		169.6
	6		173.2		172.8		172.8
	3-OH			5.85 (brs)		5.85 (brs)	
S1	1′	4.86 (d, 7.2)	100.7	4.86 (d, 7.2)	100.7	4.86 (d, 7.8)	100.7
	2′	3.23 (m)	73.6	3.23 (m)	73.6	3.23 (m)	73.6
	3′	3.26 (m)	77.0	3.26 (m)	77.0	3.26 (m)	77.0
	4′	3.16 (t, 9.0)	70.1	3.16 (t,9.6)	70.1	3.16 (m)	70.1
	5′	3.35–3.31 (m)	77.4	3.34–3.31 (m)	77.4	3.34–3.31 (m)	77.4
	6′	3.69 (t, 9.0)	61.1	3.68 (m)	61.1	3.72–3.67 (m)	61.1
		3.51 (m)		3.52 (m)		3.47–3.44 (m)	
	2′-OH	5.33 (brs)		5.33 (brs)		5.34 (brs)	
	6′-OH	4.57 (brs)		4.57 (t like)		4.58 (brs)	
	1″		157.7		157.7		157.7
	2”/6”	7.01 (d, 8.4)	116.5	7.01 (d, 8.4)	116.5	7.01 (d, 8.4)	116.5
	3”/5”	7.28 (d, 9.0)	130.1	7.28 (d, 8.4)	130.1	7.27 (d, 8.4)	130.1
	4”		129.6		129.5		129.5
	7”	4.97 (d, 4.2)	65.8	4.98 (m)	65.9	4.97 (m)	65.9
S2/S3	1′	5.09 (d, 7.8)	100.3	5.09 (d, 7.8)	100.3	5.02 (d, 7.2)	100.5
	2′	3.49–3.44 (m)	71.7	3.50–3.45 (m)	71.7	3.45–3.39 (m)	73.8
	3′	5.06 (t, 9.6)	78.2	5.07 (t, 9.6)	78.2	3.60 (t, 9.0)	74.3
	4′	3.49–3.44 (m)	68.0	3.50–3.45 (m)	68.0	4.80 (t, 9.6)	71.6
	5′	3.49–3.44 (m)	77.1	3.50–3.45 (m)	77.1	3.72–3.67 (m)	74.9
	6′	3.69 (t, 9.0)	60.7	3.68 (m)	60.7	3.72–3.67 (m)	60.8
		3.51 (m)		3.52 (m)		3.47–3.44 (m)	
	2′-OH	5.64 (d, 5.4)		5.64 (d, 6.0)		5.62 (brs)	
	3′-OH					5.44 (brs)	
	4′-OH	5.35 (d, 5.4)		5.35 (d, 6.0)			
	6′-OH	4.67 (brs)		4.67 (brs)		4.77 (brs)	
	1”		157.4		157.4		157.5
	2”/6”	7.04 (d, 7.8)	116.6	7.05 (d, 8.4)	116.6	7.05 (d, 9.0)	116.6
	3”/5”	7.30 (d, 7.8)	129.8	7.28 (d, 8.4)	129.9	7.27 (d, 8.4)	129.9
	4”		129.8		129.6		129.6
	7”	5.00 (s)	66.3	4.97 (m)	66.6	4.96 (m)	66.5
S2/S3	1‴		134.6		134.6		134.4
	2‴/6‴	7.75 (m)	128.8	7.75 (m)	128.8	7.74 (m)	128.8
	3‴/5‴	7.44 (m)	129.4	7.44 (m)	129.4	7.44 (m)	129.4
	4‴	7.44 (m)	130.8	7.44 (m)	130.8	7.44 (m)	131.0
	7‴	7.68 (d, 16.2)	144.7	7.69 (d, 15.6)	144.7	7.68 (d, 15.6)	145.3
	8‴	6.70 (d, 16.2)	119.2	6.70 (d, 16.2)	119.2	6.67 (d, 16.2)	118.5
	9‴		166.2		166.2		165.9

suggested unit S1 and S2 was respectively located at C-1 and C-6. The HMBC correlation from H-3′ (δ_H 5.06) of unit S2 to C-9‴ (δ_C 166.2) confirmed the *trans*-cinnamoyl group linked to C-3′ of unit S2. All chemical shift assignments of compound **1** were established by analysis of the HMQC, HMBC and ^{1}H-^{1}H COSY spectra as shown in Table 1. The absolute configuration of the sugar was determined on the basis of GC–MS analysis of its chiral derivative, only D-glucose was detected by comparison of the retention time with that of authentic samples prepared in the same way (the t_R of D- and L-glucose was 22.47 and 24.78 min, respectively). On the basis of the above results, the structure of **1** was established and named parishin X.

Compound **2** was isolated as a light yellow powder. The positive ion at *m/z* 1149.3428 $[M+Na]^+$ (calcd. 1149.3422 $[M+Na]^+$) in HR-ESI-MS established the molecular formula of **2** as $C_{54}H_{62}O_{26}$. The IR spectrum indicated the presence of hydroxyl group (3432 cm^{-1}), ester (1730 cm^{-1}) and aromatic ring (1613, 1514 cm^{-1}). ^{1}H NMR spectral data (Table 1) showed signals assigned to a citrate moiety [δ_H 2.93, 2.80 (each 2H, d, J = 15.0 Hz, H-2, 4)], a *trans*-cinnamoyl group [δ_H 7.75 (2H, m), 7.69 (1H, d, J = 15.6 Hz), 7.44 (3H, m), 6.70 (1H, d, J = 16.2 Hz)], three *p*-hydroxybenzyl alcohol moieties [δ_H 7.28 (6H, d, J = 8.4 Hz), 7.05 (2H, d, J = 8.4 Hz), 7.01 (4H, d, J = 8.4 Hz), 5.00-4.94 (6H, m)] and three anomeric protons [δ_H 5.09 (1H, d, J = 7.8 Hz), 4.86 (2H, d, J = 7.2 Hz)], which were quite similar to those of compound **1** except for the presence of one more glucopyranosyloxybenzyl alcohol moiety. Moreover, the two methylene signals (δ_H 2.93, 2.80) of the citrate moiety in compound **2** were different to those of compound **1** (δ_H 2.90, 2.77 and δ_H 2.76, 2.62). This observation implied that one more glucopyranosyloxybenzyl alcohol moiety may be connected to C-5 in compound **2**. The HMBC correlations (Fig. 2) from H-7″ of unit S1 to C-1 and C-5 were further confirmed the above inference. The HMBC correlations between H-3′ of unit S2 and C-9‴ of unit S2, and between H-7″ of unit S2 and C-6 indicated that the *trans*-cinnamoyl group and unit S2 were placed at C-3′ of unit S2 and C-6, respectively. The absolute configuration of the sugar was determined to be D-glucose followed the same method for compound **1**. Consequently, the structure of **2** (Fig. 1) was unambiguously determined and named parishin Y.

Compound **3** was obtained as a light yellow powder. It possessed the same molecular formula of $C_{54}H_{62}O_{26}$ as compound **2**, as deduced from