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6,8-Di-*C*-methyl-flavonoids with neuroprotective activities from *Rhododendron fortunei*

Yongji Lai [a,b], Hong Zeng [a], Meijun He [c], Huiqin Qian [a], Zhaodi Wu [a], Zengwei Luo [a], Yongbo Xue [a], Guangmin Yao [a,*], Yonghui Zhang [a,*]

[a] Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China
[b] Department of Pharmacy, Central Hospital of Wuhan, Wuhan 430014, People's Republic of China
[c] Institute of Chinese Herbal Medicines, Hubei Academy of Agricultural Sciences, Enshi 445500, People's Republic of China

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ABSTRACT

Six 6,8-di-*C*-methyl-flavonoids, (2*R*,3*R*)-6,8-di-*C*-methyl-5,7,4′-trihydroxyflavanonol 7-*O*-β-D-gluco-pyranoside (**1**), (2*R*,3*R*)-6,8-di-*C*-methyl-5,7,4′-trihydroxyflavanonol 7-*O*-β-D-xylopyranosyl(1 → 6)-β-D-glucopyranoside (**2**), 6,8-di-*C*-methylkaempferol 7-*O*-β-D-glucopyranoside (**3**), (2*R*)-farrerol (**4a**), (2*R*/2*S*)-farrerol 7-*O*-β-D-glucopyranoside (**5**), and (2*R*/2*S*)-farrerol 7-*O*-β-D-xylopyranosyl(1 → 6)-β-D-glucopyranoside (**6**), and four known analogues, farrerol (**4b**), (2*R*,3*R*)-6,8-di-*C*-methyldihydrokae-mpferol (**7**), 6,8-di-*C*-methylkaempferol 7-*O*-β-D-glucopyranoside (**8**), and 6,8-di-*C*-methylkaempferol (**9**), were isolated from the twigs and leaves of *Rhododendron fortunei*. The structures of compounds **1**–**9** were determined by spectroscopic analyses (HRESIMS, 1D and 2D NMR, and CD) and chemical methods. Compounds **1**–**9** were evaluated for their neuroprotective effects on the human neuroblastoma SH-SY5Y cells apoptosis induced by hydrogen peroxide (H_2O_2) and amyloid β peptide (Aβ), respectively. Compounds **1**–**3** and **5**–**9** exhibited significant neuroprotective effects against H_2O_2-induced SH-SY5Y cell apoptosis, and compound **8** exhibited the strongest activity with a improvement of cell viability by about 30% at the concentration of 10 μM. Compounds **1**–**9** showed significant neuroprotective effects against Aβ-induced SH-SY5Y cell apoptosis.

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease, and is the leading cause of dementia [1]. Oxidative stress-induced cell damage and the deposition of β-amyloid (Aβ) peptide into senile plaques in the extracellular space are the manifest pathological process of AD [2,3]. Natural products from plants, marine organisms, and bacteria have been reported to be an important source of Alzheimer's drug leads [4,5]. Flavonoids are widely distributed in plants, and are reported to possess neuroprotective effects by inhibiting the formation of reactive oxygen species (ROS), as well as by preventing Aβ aggregation and Aβ-induced toxicity [6,7]. Although flavonoids are abundant in nature, *C*-methyl-flavonoids having methylations on carbon are rare [8]. Literature survey revealed that *C*-methyl-flavonoids were mainly reported from plants of Caesalpiniaceae [9], Didieraceae [10,11], Dryopteridaceae [12], Ericaceae [13,14], Fabaceae [15], Myrtaceae [16–18], Onocleaceae [19], Pinaceae [8,20–23], and Ranunculaceae [24], and demonstrated anticancer [8], antifungal [15], antiviral [17], antiinflammatory [9], antibacterial [9], antioxidant [18], antimicrobial [24], neuroprotective [7], peroxynitrite scavenging [20], and aldose reductase inhibitory [19] activities.

Rhododendron fortunei Lindil. (Ericaceae), an evergreen shrub or small tree, is native to China, and widely distributes in Anhui, Fujian, Guangdong, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangxi, Shaanxi, Sichuan, Yunnan, and Zhejiang provinces of China [25]. The leaves and flowers of *R. fortunei* are used as a folk medicine in China to treat skin ulcers, menstrual disorder, leucorrhea, hemorrhage of digestive tract, and cough [26]. Literature survey revealed limited phytochemical studies on *R. fortunei*. Preliminary phytochemical studies of our term on the twigs and leaves of *R. fortunei* led to the isolation of twelve known compounds, five flavonoids including a *C*-methyl-flavonoids, (2*R*, 3*R*)-6,8-di-*C*-methyldi-hydrokaempferol (**7**, in the reference the structure was determined as (2*R*, 3*S*)-6,8-di-*C*-methyldihydrokaempferol by mistake), a diterpenoid, and six phenylpropanoids [27]. Farrerol (**4b**), a well-known 6,8-di-*C*-methyl-flavonoid in the genus of *Rhododendron*, has been reported to possess the neuroprotective activity [7]. In order to discovery more 6,8-di-*C*-methyl-flavonoids with neuropective activity from *R. fortunei*, the chemical constituents of this plants were further investigated. In the follow-up study, six 6,8-di-*C*-methyl-flavonoids (**1**–

* Corresponding authors.
E-mail addresses: gyap@hust.edu.cn (G. Yao), zhangyh@mails.tjmu.edu.cn (Y. Zhang).

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3, **4a**, **5**–**6**) and four known analogues (**4b**, **7**–**9**) (Fig. 1) were obtained. Here, we described the isolation and structural determination of the ten 6,8-di-*C*-methyl-flavonoids, and their neuroprotective effects on the human neuroblastoma SH-SY5Y cells apoptosis induced by hydrogen peroxide (H_2O_2) and amyloid-β (Aβ) peptide, respectively.

2. Results and discussion

The dried twigs and leaves of *R. fortunei* were extracted by 95% EtOH. The extract was concentrated under reduced pressure to remove the EtOH, and successively extracted by petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH to yield four extraction fractions. Repeated chromatographic purification of the EtOAc and *n*-BuOH fractions by silica gel, RP C-18, Sephadex LH-20, and HPLC resulted in the isolation of ten *C*-methyl-flavonoids, of which six are new compounds. Comparing their NMR data with reported values, we assigned the sructures of the four known *C*-methyl-flavonoids to be farrerol (**4b**) [22], (2*R*,3*R*)-6,8-di-*C*-methyldi-hydrokaempferol (**7**) [21], 6,8-di-*C*-methylkaempferol 7-*O*-β-D-glucopyranoside (**8**) [23], and 6,8-di-*C*-methyl-kaempferol (**9**) [18].

Compound **1** was obtained as a yellow amorphous solid. The molecular formula of **1** was determined to be $C_{23}H_{26}O_{11}$ by the quasi-molecular ion $[M + Na]^+$ at *m/z* 501.1354 (calcd for $C_{23}H_{26}O_{11}Na$, 501.1373) in the HRESIMS and the ^{13}C NMR data. The IR spectrum of **1** showed the presence of hydroxy (3381 cm^{-1}) and conjugated carbonyl (1630 cm^{-1}) groups. In addition, the strong absorption at 285 and 368 nm in the UV spectrum inferred **1** to be a flavonoid. The ^1H NMR spectrum of **1** (Table 1) exhibited resonances for a typical AA′BB′ coupling system, two oxymethines, a glucopyranose at 4.74 (d, J = 7.6 Hz, H-1″), 3.77 (dd, J = 5.2, 11.8 Hz, H-6″a), 3.66 (dd, J = 2.2, 11.8 Hz, H-6″b), 3.53 (t, J = 8.3 Hz, H-2″), 3.44 (t, J = 8.8 Hz, H-3″), 3.39 (t, J = 8.8 Hz, H-4″), and 3.18 (m, H-5″), and two methyl singlets. The ^{13}C NMR (Table 2) and DEPT spectra displayed resonances for a ketone carbonyl, eight substituted aromatic carbons, four aromatic methines, a glucopyranose unit (δ_C 105.5, C-1″; 78.3, C-5″; 78.0, C-3″; 75.9, C-2″; 71.6, C-4″; 62.8, C-6″), two oxymethines, and two methyls. The above NMR data suggested that compound **1** is a glycosylated derivatives of the known compound **7** [21]. The HSQC, ^1H–^1H COSY, and HMBC data were used to establish the planar structure of compound **1** (Fig. 2). The HMBC correlations of 6-CH_3 (δ_H 2.15) with C-5 (δ_C 159.7), C-6 (δ_C 113.5), and C-7 (δ_C 163.2) and 8-CH_3 (δ_H 2.08) with C-7, C-8 (δ_C 112.3), C-9 (δ_C 159.1) indicated that these two methyl groups were located at C-6 and C-8. Moreover, the glucosidation position was deduced to be at C-7 based on the HMBC correlation of the anomeric proton H-1″ (δ_H 4.74) and C-7. To determine the absolute configuration of the glucopyranose, **1** was hydrolyzed by 2 M CF_3CO_2H, and the trimethylsilylthiazolidine derivatives of the hydrolysate were prepared. The absolute configuration of the glucose in **1** was determined to be D by comparing the GC retention time of the trimethylsilylthiazolidine derivatives of the hydrolysate with those of standard D- and L-glucose.

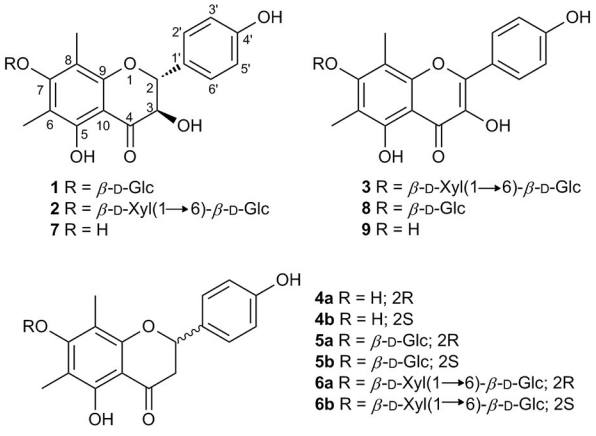

Fig. 1. Chemical structures of 6,8-di-*C*-methyl-flavonoids **1**–**9**.

Table 1
^1H NMR data (400 MHz) for compounds **1**–**4**, *J* in Hz.

Position	1[a]	2[b]	3[c]	4[a]
Aglycone				
2	4.98 d (11.8)	5.38 d (11.8)		5.29 dd (2.9, 12.8)
3α	4.56 d (11.8)	5.13 d (11.8)		2.71 dd (2.9, 17.1)
3β				3.05 dd (12.8, 17.1)
2′ 6′	7.37 d (8.5)	7.87 d (8.3)	8.10 d (8.8)	7.32 d (8.5)
3′ 5′	6.84 d (8.5)	7.30 d (8.3)	6.96 d (8.8)	6.83 d (8.5)
Me-6	2.15 s	2.68 s	2.19 s	2.00 s
Me-8	2.08 s	2.55 s	2.40 s	1.99 s
Glc				
1″	4.74 d (7.6)	5.46 d (6.8)	4.65 d (7.7)	
2″	3.53 t (8.3)	4.34 overlap	3.35 overlap	
3″	3.44 t (8.8)	4.33 overlap	3.26 m	
4″	3.39 t (8.8)	4.29 m	3.22 m	
5″	3.18 m	4.12 m	2.99 t (8.6)	
6″	3.66 dd (2.2, 11.8)	4.30 m	3.53 dd (4.0, 11.0)	
	3.77 dd (5.2, 11.8)	4.75 d (10.8)	3.85 d (11.0)	
Xyl				
1‴		4.86 d (7.4)	4.07 d (7.4)	
2‴		3.93 t (8.1)	2.88 d (8.1)	
3‴		4.08 m	3.24 m	
4‴		4.17 m	3.20 m	
5‴		4.33 overlap	2.95 t (11.2)	
		3.65 t (10.6)	3.63 dd (5.2, 11.2)	

[a] Measured in methanol-d_4.
[b] Measured in pyridine-d_6.
[c] Measured in DMSO-d_6.

Table 2
^1H NMR data (400 MHz) for compounds **1**–**4**, *J* in Hz.

Position	1[a]	2[b]	3[c]	4[a]
Aglycone				
2	84.9	84.9	147.7	80.1
3	74.2	74.3	136.1	44.1
4	200.8	201.5	176.5	198.4
5	159.7	159.9	155.0	160.3
6	113.5	113.0	113.1	104.8
7	163.2	163.1	158.5	164.2
8	112.3	112.1	109.8	104.1
9	159.1	158.8	151.0	159.3
10	105.0	105.2	106.2	103.2
1′	129.5	129.6	122.0	131.5
2′ 6′	130.4	130.8	129.7	128.8
3′ 5′	116.3	116.8	115.6	116.3
4′	159.3	160.1	159.5	158.8
Me-6	9.3	10.1	9.0	7.4
Me-8	9.8	10.5	9.2	8.1
Glc				
1″	105.5	106.2	104.5	
2″	75.9	76.2	74.0	
3″	78.0	78.8	76.2	
4″	71.6	71.9	69.7	
5″	78.3	78.2	76.5	
6″	62.8	70.3	68.5	
Xyl				
1‴		106.1	103.7	
2‴		75.3	73.2	
3‴		78.7	75.7	
4‴		71.6	69.5	
5‴		67.5	65.5	

[a] Measured in methanol-d_4.
[b] Measured in pyridine-d_6.
[c] Measured in DMSO-d_6.