Natural Product Research
Vol. 24, No. 10, 15 June 2010, 920–925

Taylor & Francis
Taylor & Francis Group

Flavonol glycosides with α-D-aldohexoses from *Rhododendron irroratum*

Ming Hua Yang, Jian Guang Luo, Xue Feng Huang and Ling Yi Kong*

Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, P.R. China

(*Received 18 January 2008; final version received 22 August 2008*)

Two new flavonol glycosides which contain rare α-D-galactose or α-D-glucose were obtained from the flowers of *Rhododendron irroratum* Franch., namely myricetin 3-*O*-β-D-galactoside-3′-*O*-α-D-glucoside (**1**) and myricetin 3-*O*-β-D-galactoside-3′-*O*-α-D-galactoside (**2**). Their structures were determined by UV, IR, HR–ESI–MS, ESI–MS, 1D- and 2D-NMR techniques.

Keywords: *Rhododendron irroratum*; flavonol glycosides; α-D-galactose; α-D-glucose

1. Introduction

Rhododendron irroratum Franch. is a species of rhododendron. The rhododendron plants mainly contain flavonoids, and many species are generally used in Chinese folk medicine for the treatment of coughs and inflammation. Some diterpenoids, flavonols and their flavonol glycosides have been reported from several species of the rhododendron, such as *R. molle*, *R. anthopogonoides* and *R. fortunei* (Dai, Chen, & Yu, 2004; Yang, Li, & Bian, 2003; Zhong, Hu, Wei, & Weng, 2005). However, the chemical constituents of *R. irroratum*, which is widely distributed in the Yunnan Province of China, have been less well studied. In our present study of the chemical constituents of its flowers, two unusual flavonol glycosides with α-D-aldohexoses were isolated from the EtOAc-soluble fraction. This article describes the isolation and structural elucidation of these two new flavonol glycosides (**1** and **2**, Figure 1).

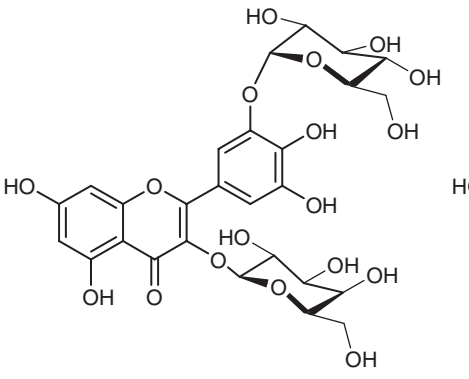
2. Results and discussion

Compound **1** was obtained as an amorphous yellow powder, and gave a positive result to HCl/Mg and the Molish test, which indicated that **1** was a flavonol glycoside. In the HR–ESI–MS, the quasi-molecular ion peak showed at m/z 665.1332 $[M+Na]^+$, corresponding to the molecular formula $C_{27}H_{30}O_{18}$ (Calcd for $C_{27}H_{30}O_{18}Na$, m/z 665.1324). Its UV showed absorption bands at 254 and 360 nm, which are the characteristic bands of flavonol. The IR spectrum exhibited absorption bands of hydroxyls (3421 cm^{-1}), an α,β-unsaturated ketone (1656 cm^{-1}) and aromatic groups (1608 and 1499 cm^{-1}). In ^1H NMR spectrum, two series of *meta* coupled doublets at δ 7.70

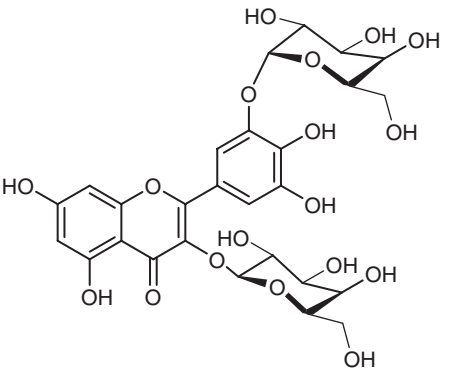
*Corresponding author. Email: lykong@jlonline.com

ISSN 1478–6419 print/ISSN 1029–2349 online

DOI: 10.1080/14786410802425811
http://www.informaworld.com



(**1**)



(**2**)

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

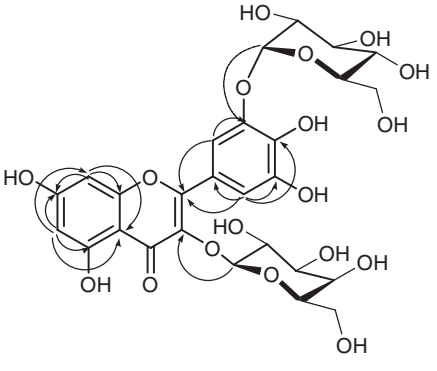
(1H, $J = 2.1$ Hz) and δ 7.33 (1H, $J = 2.1$ Hz), δ 6.42 (1H, $J = 2.0$ Hz) and δ 6.21 (1H, $J = 2.0$ Hz) could be assigned to H-2′ and H-6′, H-6 and H-8, respectively. The ^{1}H and ^{13}C NMR data (Table 1) of **1** assigned from HSQC, HMBC experiments (Figure 2) indicated that the aglycone was myricetin (Arya, Babu, Ilyas, & Nasim, 1992; Zhong et al., 1997), which was further confirmed by thin layer chromatography (TLC) comparison of the sample obtained by acid hydrolysis with an authentic sample.

In addition, two doublets for one proton at δ 5.39 ($J = 7.7$ Hz) and another at δ 5.32 ($J = 3.7$ Hz) were observed as anomeric protons of two sugars. A comparison of the ^{1}H and ^{13}C NMR data of the sugar moieties with methylated sugars (Agrawal, 1992) (Table 2) indicated the existence of one D-glucoside and another D-galactoside, which were further confirmed by comparing with standard sugar on TLC after the acid hydrolysis. The coupling constants of both of the anomeric protons at δ 5.39 ($J = 7.7$ Hz) and δ 5.32 ($J = 3.7$ Hz), which correlated with carbons at δ 101.8, 99.9 in the HSQC, indicate the presence of two sugar units in β and α form, respectively. It is rare to find flavonol glycosides with α-D-glucopyranose. The positions of connectivity of the sugars were determined by HMBC experiment; the correlations between the proton H-1″ (δ 5.39) and C-3 (δ 133.6) indicated the β-D-galactose was bound to C-3. Similarly, the α-D-glucose was linked at C-3′ by the correlations between H-1‴ (δ 5.32) and C-3′ (δ 145.3) (Figure 2), and this was also confirmed by the downshifts of C-2′ and C-6′ from δ 107.2 (Shen, Chang, & Ho, 1993) to δ 111.1 and δ 109.9. Thus, the structure of compound **1** was established as myricetin 3-*O*-β-D-galactoside-3′-*O*-α-D-glucoside.

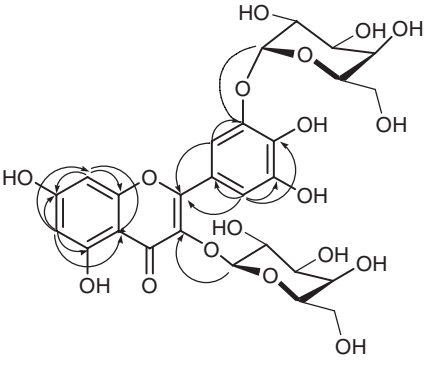
Compound **2** was an amorphous yellow powder, and gave a positive result to HCl/Mg and the Molish test. It had the same molecular formula ($C_{27}H_{30}O_{18}$) as **1** from HR–ESI–MS. Its UV and IR also showed the characteristic absorption bands of a flavonol. The ^{1}H and ^{13}C NMR spectral data (Table 1) indicated that **2** possessed the same aglycon as **1**, but differed in the sugar chains. The sugar moiety was identified as two D-galactoses by comparing the ^{1}H and ^{13}C NMR data with those of methylated sugars (Agrawal, 1992) (Table 3) and standard sugar on TLC after the acid hydrolysis. The coupling constants of both of the anomeric protons ($J = 7.7$ Hz and 3.9 Hz) indicated the presence of two D-galactosides in β and α form, respectively. Thus one was α-D-galactoside and the other was β-D-galactoside. Although α-D-galactosides have

Table 1. The ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** in DMSO-d_6.

	Compound **1**			Compound **2**	
No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
2	155.9		2	155.8	
3	133.6		3	133.7	
4	177.5		4	177.5	
5	161.2		5	161.2	
6	98.6	6.21 (1H, d, 2.0)	6	98.6	6.20 (1H, d, 2.1)
7	164.2		7	164.2	
8	93.5	6.42 (1H, d, 2.0)	8	93.5	6.41 (1H, d, 2.1)
9	156.3		9	156.3	
10	103.9		10	103.9	
1′	120.1		1′	120.1	
2′	111.1	7.33 (1H, d, 2.1)	2′	111.5	7.38 (1H, d, 2.1)
3′	145.3		3′	145.3	
4′	138.6		4′	138.8	
5′	145.4		5′	145.3	
6′	109.9	7.70 (1H, d, 2.1)	6′	110.0	7.59 (1H, d, 2.1)
β-Gal			β-Gal		
1″	101.8	5.39 (1H, d, 7.7)	1″	101.9	5.34 (1H, d, 7.7)
2″	70.9	3.70	2″	71.1	3.62
3″	73.1	3.38	3″	73.2	3.37
4″	68.0	3.65	4″	67.9	3.64
5″	75.8	3.32	5″	75.8	3.33
6″a	60.0	3.45	6″a	60.0	3.45
6″b		3.28	6″b		3.29
α-Glu			α-Gal		
1‴	99.9	5.32 (1H, d, 3.7)	1‴	100.5	5.32 (1H, d, 3.9)
2‴	71.8	3.37	2‴	68.4	3.76
3‴	73.7	3.56	3‴	69.4	3.80
4‴	69.7	3.25	4‴	68.6	3.86
5‴	72.9	3.73	5‴	72.3	3.83
6‴	60.4	3.69	6‴	60.3	3.50
6‴		3.55	6‴		3.58



(**1**)



(**2**)

Figure 2. Selected HMBC correlations of compounds **1** and **2**.

Table 2. The ^{1}H and ^{13}C NMR data of glucose in compound **1** and methyl α/β-D-glucoside.

	^{1}H NMR (*J* in Hz)			^{13}C NMR		
No.	β-D	α-D	**1**	β-D	α-D	**1**
1	4.27 (d 6~8)	4.70 (d 1~4)	5.32 (d 3.7)	104.0	100.0	99.9
2	3.15	3.46	3.37	74.1	72.2	71.8
3	3.38	3.56	3.56	76.8	74.1	73.7
4	3.27	3.29	3.25	70.6	70.6	69.7
5	3.36	3.54	3.73	76.8	72.5	72.9
6a	3.62	3.66	3.55	61.8	61.6	60.4
6b	3.82	3.77	3.69			

Table 3. The ^{1}H and ^{13}C NMR data of galactose in compound **2** and methyl α/β-D-galactoside.

	^{1}H NMR (*J* in Hz)			^{13}C NMR		
No.	β-D	α-D	**2**	β-D	α-D	**2**
1	4.20 (d 6~8)	4.73 (d 1~4)	5.32 (d 3.9)	104.5	100.1	100.5
2	3.39	3.72	3.76	71.7	69.2	68.4
3	3.53	3.68	3.80	73.8	70.5	69.4
4	3.81	3.86	3.86	69.7	70.2	68.6
5	3.57	3.78	3.83	76.0	71.6	72.3
6a	3.64	3.61	3.50	62.0	62.2	60.3
6b	3.69	3.67	3.58			

been reported in triterpenoid saponins (Jia, Koike, & Nikaido, 1999), this is the first example of the α-D-galactopyranosyl chain in a flavonol glycoside. Similar to **1**, the linkage positions for the sugar units were established by HMBC correlations between $\delta_{H\text{-}1''}$ 5.34 with $\delta_{C\text{-}3}$ 133.7 and $\delta_{H\text{-}1'''}$ 5.32 with $\delta_{C\text{-}3'}$ 145.3, respectively. Based on the above information, the structure of compound **2** was elucidated as myricetin 3-*O*-β-D-galactoside-3′-*O*-α-D-galactoside.

3. Experimental

3.1. *General*

IR spectra were measured on a Bruker Tensor-27 spectrophotometer and UV on a Shimadzu UV-2501 PC spectrophotometer. ESI–MS and HR–ESI–MS experiments were performed on an Agilent 1100 Series LC/MSD trap mass spectrometer and an Agilent TOF MSD 1946D mass spectrometer. NMR spectra were recorded on a Bruker DRX-500 instrument. Absorbents for column chromatography were silica gel (200–300 mesh, Qingdao Marine Chemistry Ltd) and Sephadex LH-20 (20–100 μ, Pharmacia). The silica gel GF254 for TLC was from Qingdao Marine Chemistry Ltd. Preparative HPLC was performed using an Agilent 1100 Series instrument with a Shim-Park RP-C_{18} column (200 × 20 mm i.d.) and an MWD detector with UV at 260 nm.

3.2. *Plant material*

Flowers of *R. irroratum* were collected from Yunnan province, China, in September 2005. The botanical identification was made by Prof. Chang-Qin Zhang of the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (no. 050405) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3. *Extraction and isolation*

Flowers of *R. irroratum* (5 kg) were dried in the shade for one week and crushed into pieces. The pieces were refluxed with 80% EtOH (3×20 L $\times$ 2 h), then concentrated *in vacuo*. The solution continued to be concentrated to afford a residue. The residue was partitioned between H_2O and petroleum ether (5×0.5 L), $CHCl_3$ (5×0.5 L), EtOAc (5×0.5 L), and *n*-BuOH (5×0.5 L), successively. The EtOAc fraction (95 g) was subjected to chromatography (silica, $CHCl_3$–MeOH, $100:1 \rightarrow 100:50$) to offer six fractions (fr. I–VI). Fr. IV (8 g) was further rechromotographed (silica, $CHCl_3$–MeOH, $100:10 \rightarrow 100:25$) and finally subjected to Sephadex LH-20 to yield a mixture of **1** and **2**. With the help of preparative HPLC using elution as MeOH/H_2O (50% : 50%), two peaks were collected to afford **1** (8 mg t_R 23.5 min) and **2** (10 mg t_R 20.1 min).

3.3.1. *Myricetin-3-O-β-D-galactoside-3′-O-α-D-glucoside (**1**)*

Amorphous yellow powder; UV (MeOH) λ_{max} nm (log ξ): 360 (4.1), 254 (4.0), 209 (4.6); IR (KBr) ν_{max} cm^{-1}: 3421, 2926, 1656, 1608, 1499; ESI–MS *m/z*: 641 $[M-H]^+$; HR–ESI–MS *m/z*: 665.1332 $[M+Na]^+$ (Calcd for $C_{27}H_{30}O_{18}Na$, 665.1324). For ^1H and ^{13}C NMR details, see Table 1.

3.3.2. *Myricetin-3-O-β-D-galactoside-3′-O-α-D-galactoside (**2**)*

Amorphous yellow powder; UV (MeOH) λ_{max} nm (log ξ): 363 (4.2), 254 (4.1), 209 (4.6); IR (KBr) ν_{max} cm^{-1}: 3417, 2825, 1655, 1606, 1500; ESI–MS *m/z*: 641 $[M-H]^+$; HR–ESI–MS *m/z*: 665.1338 $[M+Na]^+$ (Calcd for $C_{27}H_{30}O_{18}Na$, 665.1324). For ^1H and ^{13}C NMR details, see Table 1.

3.3.3. *Acid hydrolysis of **1** and **2***

A solution of **1** or **2** (each 4 mg) in 5 mL of 1 N H_2SO_4 : MeOH (1 : 1) was refluxed at 90°C for 2 h and diluted with H_2O to 8 mL. The solution was partitioned between H_2O and EtOAc (4×8 mL). The water layer was subjected to amberlite and evaporated. Residue was dissolved in MeOH and analytical TLC (*n*-BtOH/Me_2CO/H_2O, 4 : 5 : 1) was compared with standard glucose and galactose. The EtOAc layer was evaporated and compared with standard myricetin on TLC ($CHCl_3$/MeOH, 10 : 1).

References

Agrawal, P.K. (1992). NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. *Phytochemistry, 31*, 3307–3330.

Arya, R., Babu, V., Ilyas, M., & Nasim, K.T. (1992). Myricetin 3′-rhamnoside-3-galactoside from *Buchanania lanzan* (Anacardiaceae). *Phytochemistry, 31*, 2569–2570.

Dai, S.J., Chen, R.Y., & Yu, D.Q. (2004). Studies on the flavonoid compounds of *Rhododendron anthopogonoides*. *China Journal of Chinese Materia Medica, 29*, 44–47.

Jia, Z.H., Koike, K., & Nikaido, T. (1999). Saponarioside C, the first α-D-galactose containing triterpenoid saponin, and five related compounds from *Saponaria officinalis*. *Journal of Natural Products, 62*, 449–453.

Shen, C.C., Chang, Y.S., & Ho, L.K. (1993). Nuclear magnetic resonance studies of 5,7-dihydroxyflavonoids. *Phytochemistry, 34*, 843–845.

Yang, B.F., Li, J.M., & Bian, C.M. (2003). Qualitative analysis of flavonoids in *Rhododendron fortune*. *Journal of Taizhou University, 25*, 65.

Zhong, X.N., Otsuka, H., Ide, T., Hirata, E., Takushi, A., & Takeda, Y. (1997). Three flavonol glycosides from leaves of *Myrsine seguinii*. *Phytochemistry, 46*, 943–946.

Zhong, G.H., Hu, M.H., Wei, X.Y., & Weng, Q.F. (2005). Grayanane diterpenoids from the flowers of *Rhododendron molle* with cytotoxic activity against a *Spodoptera frugiperda* cell line. *Journal of Natural Products, 68*, 924–926.