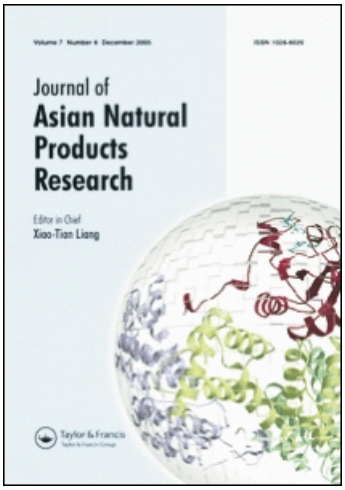

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Flavonoids from *Pyrrosia petiolosa* (Christ) Ching

N. Wang[a]; J. -H. Wang[a]; X. Li[a]; J. -H. Ling[a]; N. Li[a]
[a] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China

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Note

Flavonoids from *Pyrrosia petiolosa* (Christ) Ching

N. WANG, J.-H. WANG, X. LI*, J.-H. LING and N. LI

School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang110016, China

A new kaempferol glycoside, kaempferol-3-*O*-β-D-glucopyranoside-7-*O*-α-L-arabinofuranoside (**1**), was isolated from the EtOH extract of *Pyrrosia petiolosa* together with six known flavonoids already reported from the same plant. Structural elucidation was performed by means of physico-chemical methods including MS, and 1D and 2D NMR spectroscopy.

Keywords: *Pyrrosia petiolosa*; genus *Pyrrosia*; Flavonoids

1. Introduction

The genus *Pyrrosia* comprises over 110 species and more than 50 have been found in China. *Pyrrosia petiolosa* is one of the three main sources (*P. petiolosa, P. lingua* and *P. sheareri*) of the traditional Chinese medicine "ShiWei" and has been traditionally used for the treatment of nephritis (inflammation of the kidney) and asthma. Previous phytochemical research work on this plant showed the presence of triterpenoids, flavonoids and phenolic acids [1–3]. We had reported that *P. petiolosa* is very rich in phenolic acids [3] which showed significant anti-inflammatory and anti-oxidant activities. That prompted us to further investigate the flavonoids in this species in more detail. Extensive chromatography of the EtOH extract of *P. petiolosa* had led to the isolation of a new flavonoid and six known flavonoids already reported from this plant [2]. This paper deals with the isolation and structural elucidation of the new compound (**1**).

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder from methanol, mp 175–177°C, $[\alpha]_D^{20}$ − 132.9 (*c* 0.35, MeOH); the molecular formula, $C_{26}H_{28}O_{15}$, was deduced from HRFAB-MS (*m/z* 581.1511 $[M + H]^+$). Acid hydrolysis of **1** gave kaempferol, glucose and

*Corresponding author. E-mail: proflixian@163.com

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Table 1. ^{1}H NMR and ^{13}C NMR data for compound **1** in DMSO-d_6.[a]

Aglycone moiety				*Sugar moiety*			
No.	δ_H	δ_{OH}	δ_C	*No.*	δ_H	δ_{OH}	δ_C
2			156.9	1″	5.49 d (7.4)		100.9
3			133.6	2″	3.20 m	5.39 brs	74.4
4			177.8	3″	3.22 m	5.10 brs	76.6
5		12.62 s	161.0	4″	3.09 brs	4.99 brs	70.0
6	6.43 d (3.5)		99.5	5″	3.09 brs		77.7
7			162.4	6″	3.33/3.58 m	4.31 t (5.2)	61.0
8	6.77 d (3.5)		94.7	1″	5.61 s		106.5
9			156.1	2″	4.14 brs	5.74 brs	82.1
10			105.6	3″	3.83 brs	5.39 brs	76.6
1′			120.9	4″	3.90 m		85.7
2′, 6′	8.09 d (8.7)		131.1	5″	3.48/3.58 m	4.86 brs	61.2
3′, 5′	6.90 d (8.7)		115.3				
4′		10.25 brs	160.3				

[a] The ^{1}H NMR and ^{13}C NMR spectra were measured at 600 and 150 MHz, respectively, and the *J* values (parentheses) are in hertz.

arabinose. This was further proved by the fragments in the ESI-MS spectrum. In the positive ion ESI-MS spectrum, ion peaks at *m/z* 1183.2, 603.2, 581.1, 471.1, 419.1 and 287.1 were ascribed to $[2M + Na]^+$, $[M + Na]^+$, $[M + H]^+$, $[M + Na - ara]^+$, $[M + H - glc]^+$ and $[M + H - glc - ara]^+$, respectively, while ion peaks at *m/z* 615.0, 579.1, 447.0 and 285.0 found in the negative ion ESI-MS spectrum were due to $[M + Cl]^-$, $[M - H]^-$, $[M - H - ara]^-$ and $[M - H - ara - glc]^-$, respectively. The ^{1}H NMR spectrum of **1** displayed the characteristic signals of the kaempferol nucleus: two doublets at δ 6.77 and 6.43 (each 1H, d, $J = 3.5$ Hz) were assigned to the H-8 and H-6 protons respectively, and a pair of AA′BB′ aromatic system protons at δ 6.90 and 8.09 (each 2H, d, $J = 8.7$ Hz) were assignable to H-3′, 5′ and H-2′, 6′, respectively. The doublet of the glucose anomeric proton appeared at δ 5.49 and a diaxial coupling constant $J_{1,2} = 7.4$ Hz indicated the β configuration of glucopyranosyl unit. The presence of the anomeric proton singlet at δ 5.61 indicated the α configuration of the arabinofuranosyl residue. The proton signals of H-6 and H-8 shifted downfield significantly by 0.24 and 0.33 chemical shifts, respectively, in comparison with those of kaempferol, suggesting that C-7 was substituted by sugar moiety. Comparing with kaempferol, downfield shifts of C-2 (146.6 to 156.9), C-4 (175.9 to 177.8), C-6 (98.2 to 99.5) and C-8 (93.4 to 94.7), and upfield shifts of C-3 (135.6 to 133.6) and C-7 (163.8 to 162.4) were also observed in the ^{13}C NMR spectrum. The foregoing evidence showed that **1** was 3,7-diglycosyl kaempferol. The proposed structure was confirmed by HMBC experiments: the anomeric protons of glucosyl and arabinosyl correlated with the carbons C-3 (δ 133.6) and C-7 (δ 162.4), respectively. Thus, **1** was concluded to be kaempferol 3-*O*-β-D-glucopyranoside-7-*O*-α-L-arabinofuranoside. The assignments of the chemical shifts of **1** were deduced unequivocally by HMBC, HSQC and ^{1}H-^{1}H COSY experiments (see Table 1). Some significant HMBC and ^{1}H-^{1}H COSY correlations are shown in Figure 1.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanaco MP-S3 micro-melting point apparatus and were uncorrected. The optical rotation was measured on a Perkin-Elmer 241 MC polarimeter.

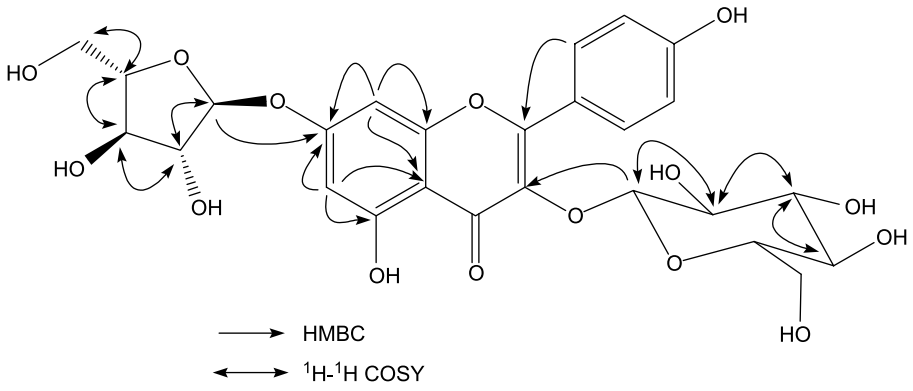

Figure 1. Significant HMBC and ^{1}H-^{1}H COSY correlations of **1**.

NMR spectra were recorded on Bruker AV-600 and ARX-300 NMR spectrometers using TMS as internal standard. HRFAB-MS spectrum recording was performed on a Qstar LCQ mass spectrometer and ESI-MS data were obtained with a Finnigan LCQ mass spectrometer.

3.2 Plant material

The whole plants of *Pyrrosia petiolosa* were collected in July 2002 from Bening City, Liaoning Province, China, and identified by Professor Qishi Sun, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China. A voucher specimen (200207–02) is deposited at the Department of Natural Medicines, Shenyang Pharmaceutical University, China.

3.3 Extraction and isolation

The shade-dried whole plant of *Pyrrosia petiolosa* (9 kg) was extracted with 90% aqueous EtOH (90 l × 3) under reflux and the extract was concentrated *in vacuo* to give a gummy residue, which was then suspended in water and extracted with petroleum ether, $CHCl_3$, EtOAc and n-BuOH, successively. The EtOAc extract (55 g) was subjected to column chromatography on silica gel (300 g) and eluted with $CHCl_3$/MeOH to afford 409 fractions (each 500 ml). Fractions E41–E52 (400 mg, 100:1) were further chromatographed over silica gel with petroleum ether-EtOAc to give kaempferol (**2**, 58 mg). Fractions E170–E183 (2.0 g, 100:5) were applied to silica gel (16 g) chromatography with $CHCl_3$/acetone (6:4) as the eluent to afford kaempferol-3-*O*-β-D-glucopyranoside (**3**, 46 mg). (±)-Eriodictyol-7-*O*-β-D-glucuronide methyl ester (**4**, 65 mg) and (±)-eriodictyol-7-*O*-β-D-glucuronide ethyl ester (**5**, 26 mg) were obtained from frs. E184–E209 (4.7 g, 100:5) by polyamide (200–400 mesh, 20 g) column chromatography eluted with $CHCl_3$/MeOH. The n-BuOH extract (200 g) was subjected to column chromatography on silica gel (1000 g) with a $CHCl_3$/EtOH gradient system to give 153 fractions (each 500 ml). Fractions B76–B91 (18.5 g, 100:30) were further separated by polyamide (200–400 mesh, 80 g) and developed with $CHCl_3$/MeOH to give **1** (33 mg, 100:20) and gossypetin-7-*O*-β-D-glucopyranoside (**6**, 67 mg, 100:50). Chromatography of frs. B110-B125 (34 g) on silica gel (250 g) with EtOAc/MeOH, followed by

polyamide column chromatography, led to the isolation of gossypetin 7-*O*-(6-α-L-arabinofuranosyl)-β-D-glucopyranoside (**7**, 11 mg).

3.4 Kaempferol 3-O-β-D-glucopyranoside-7-O-α-L-arabinofuranoside (1)

Yellow amorphous powder (MeOH), mp 175–177°C; $[\alpha]_D^{20}$ − 132.9 (*c* 0.35, MeOH); HRFAB-MS, *m/z* 581.1511 $[M + H]^+$ (calcd for $C_{26}H_{28}O_{15}$, 581.1507); Positive ion ESI-MS, *m/z* 1183.2 $[2M + Na]^+$, 603.2, $[M + Na]^+$, 581.1 $[M + H]^+$, 471.2 $[M + Na - ara]^+$, 419.1 $[M + H - glc]^+$, 287.1 $[M + H - glc - ara]^+$; Negative ion ESI-MS *m/z* 615.0 $[M + Cl]^-$, 579.1 $[M - H]^-$, 447.0 [M − H-ara]$^-$, 285.0 [M − H-ara-glc]$^-$; NMR data: see Table 1.

3.5 Acid hydrolysis of 1

A solution of **1** (5 mg) was refluxed with 5% HCl (5 ml) in methanol for 1 h. Excess acid was precipitated with Ag_2O. After evaporation of the methanol, the reaction mixture was extracted with EtOAc, and the resulting aglycone was identified as kaempferol by TLC and comparison of its NMR data with those of compound **2**. The sugars in the aqueous solution were examined with authentic samples by PC with n-BuOH/HOAc/H_2O (4:1:5, upper layer) and PhOH/H_2O (4:1) (detection with aniline phthalate at 105°C), and TLC with $CHCl_3$/MeOH/H_2O (7:3:1, lower layer) (detection with 10% H_2SO_4 at 105°C).

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References

[1] J. Li, J.S. Yang, Y.Y. Tong. *Chin. Trad. Herbal Drugs*, **23**, 348 (1992).
[2] C. Yang, J.G. Shi, S.Y. Mao, Y.C. Yang. *J. Asian Nat. Prod. Res.*, **5**, 143 (2003).
[3] N. Wang, J.H. Wang, J. Cheng, X. Li. *J. Shenyang Pharm. Univ.*, **20**, 425 (2003).