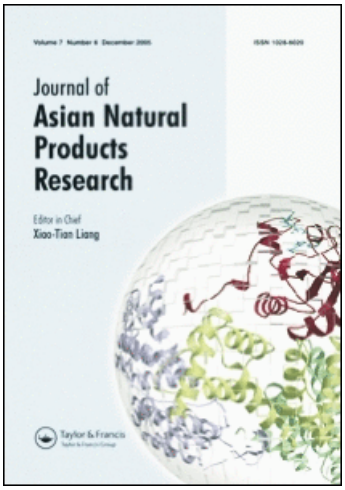

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Note

A new flavonoside from *Leonurus heterophyllus*

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In a chemical investigation on the flavone composition of *Leonurus heterophyllus* a new flavonoside has been isolated. By means of physico-chemical evidences and spectral analysis its structure has been established as quercetin-3-*O*-[3-(4-hydroxy-3,5-dimethoxybenzyl)-α-L-rhamnopyranosyl]-(1→6)-β-D-galactopyranoside (**1**).

Keywords: *Leonurus heterophyllus*; Flavonoside; Heteronoside

1. Introduction

The whole plant of *Leonurus heterophyllus* (Labiatae), called 'Yi Mu Cao' in China, is a well-known herb in Chinese medicine for the treatment of gynaecological problems, including irregular menstruation, gravida vaginal bleeding parodynia, menalgia and puerperal congestion [1]. During our systematic phytochemical studies on *Leonurus heterophyllus* we have isolated six known compounds, syringic acid, megastigmane, leonurine, tiliroside, ajugoside, 2,6-dimethyl-2*E*,7-octadiene-1,6-diol, and a new flavonoside named heternoside (**1**). We report here the isolation and structural elucidation of the new flavonoside.

2. Results and discussion

Heteronoside (**1**) was isolated from the ethyl acetate part of a 95% ethanol extract of the aerial parts of *Leonurus heterophyllus* by silica-gel column chromatography.

Compound **1** was obtained as a yellow amorphous power, mp 201–203°C. It displayed pseudomolecular ion peaks at *m/z* 1603.9 $[2M + Na]^+$, 813.2 $[M + Na]^+$, 791.0 $[M + H]^+$ in

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ESI-MS, and 813.1823 $[M + Na]^+$ in its HRMS spectrum. This information, combined with NMR data, allowed its molecular formula to be assigned as $C_{36}H_{38}O_{20}$. The FTIR spectrum displays diagnostic absorption bands for hydroxyl (3405 cm^{-1}), carbonyl (1654 cm^{-1}) and aromatic moieties (1608, 1513 cm^{-1}), and the UV spectrum shows maximum absorptions at λ_{max}^{MeOH}(nm) : 210, 266 and 359 due to the flavonol chromophore. The ^{13}C NMR spectrum of **1** contains 36 signals, including 15 aromatic carbon signals at δ 158.1 (C-2), 135.4 (C-3), 178.9 (C-4), 162.8 (C-5), 100.1 (C-6), 157.8 (C-7), 94.9 (C-8), 166.1 (C-9), 105.9 (C-10), 121.2 (C-1′), 118.8 (C-2′), 146.8 (C-3′), 150.9 (C-4′), 116.6 (C-5′), 122.4 (C-6′), along with its ^1H NMR data at δ 6.73 (1H, s, H-6), 6.72 (1H, s, H-8), 8.02 (1H, dd, $J = 8.4$,2.1 Hz, H-6′), 7.34 (1H, d, $J = 8.4$ Hz, H-5′), 8.52 (1H, d, $J = 2.1$ Hz, H-2′), 13.1(1H, s, HO-5). In the HMBC spectrum, long-range cross-peaks appear between proton signals at δ 8.02 (dd, $J = 8.4$, 2.1 Hz, H-6′), 8.52 (d, $J = 2.1$ Hz, H-2′) and carbon signal at δ 158.1 (C-2), and also between proton signals at δ 6.73 (s, H-6), 6.72 (s, H-8) and the carbon signal at δ 157.8 (C-7). Proton signals at δ 7.34 (d, $J = 8.4$ Hz, H-5′) and 8.52 (d, $J = 2.1$ Hz, H-2′) both correlate with the carbon signals at δ 146.8 (C-3′) and 150.9 (C-4′). In the $^1H—^1H$ COSY spectrum, proton signals at δ 7.34 (d, $J = 8.4$ Hz, H-5′) and 8.52 (d, $J = 2.1$ Hz, H-2′) correlate with the proton signals at δ 8.02 (dd, $J = 8.4$, 2.1 Hz, H-6′). All these indicate that **1** contains a quercetin moiety. In addition, in the ^{13}C NMR spectrum, four other olefinic carbons resonate at δ 122.8 (C-1″), 108.9 (C-2″, 6″) 148.8 (C-3″, 5″), 142.8 (C-4″), a carbonyl carbon signal at δ 167.2 (C-7″) and two methoxy carbon signals at δ 56.6 (C-8″, 9″). The ^1H NMR spectrum exhibits two proton signals at δ 7.72 (2H, s, H-2″, 6″) and 3.72 (6H, s, H-8″, 9″). In the HMBC spectrum, proton signals at δ 7.72 (H-2″, 6″) and 3.72 (H-8″, 9″) correlate with carbon signals at δ 142.8 (C-4″) and

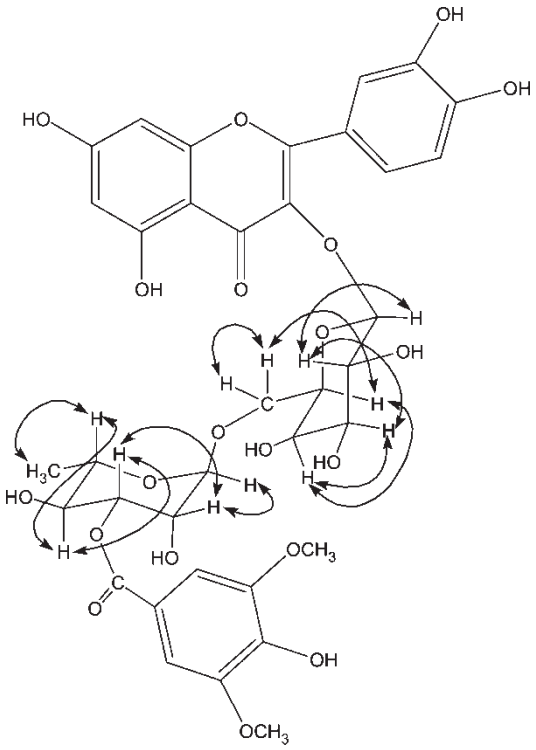

Figure 1. Key $^1H—^1H$ COSY correlations for **1**.

167.2 (C-7″), and 148.8 (C-3″, 5″), respectively. Consequently, it is concluded that **1** contains a syringic acid moiety.

Acid hydrolysis of **1** yielded galactose and rhamnose. ^{1}H and ^{13}C NMR, HMBC, HMQC and $^{1}H—^{1}H$ COSY spectra (figure 1) demonstrate that **1** has β-galactopyranosyl and α-rhamnopyranosyl moieties, assigned as shown in table 1; the D-galactopyranosyl and L-rhamnopyranosyl were confirmed by the comparison with reported data [2]. In the HMBC spectrum (figure 2), the anomeric proton signal at δ 6.01 (1H, d, $J = 7.8$ Hz, gal-1) has a long-range correlation with the carbon signal at δ 135.4 (C-3), indicating that the galactopyranosyl moiety is connected to C-3. Another anomeric proton signal at δ 5.28 (1H, brs, rham-1) is correlated with the carbon signal at δ 66.8 (gal-6), indicating the rhamnopyranosyl moiety is linked to gal C-6. Furthermore, another cross-peak occurs between a proton signal at δ 5.99 (m, rham-3) and a carbon signal at δ 167.2 (C-7″), suggesting that the syringic acid moiety is connected to rham C-3 (figure 1). Thus, the structure of **1** was established as quercetin-3-*O*-[3-(4-hydroxyl-3,5-dimethoxybenzyl)-α-L-rhamnopyranosyl]-(1 → 6)-β-D-galactopyranoside, named as heteronoside.

Table 1. NMR data for heteronoside (**1**).

	Correlated proton			
Carbon No.	δ_C	δH	*HMBC*	$^{1}H—^{1}H$ *COSY*
2	158.1			
3	135.4			
4	178.9			
5	162.8			
6	100.1	6.73 (s)	C-7, C-8, C-10	
7	157.8			
8	94.9	6.72 (s)	C-6, C-7, C-10	
9	166.1			
10	105.9			
1′	121.2			
2′	118.8	8.52 (d, $J = 2.1$ Hz)	C-2, C-3′, C-4′ C-6′	6′
3′	146.8			
4′	150.9			
5′	116.6	7.34 (d, $J = 8.4$ Hz)	C-3″, C-4′	6′
6′	122.4	8.02 (dd, $J = 8.4$, 2.1 Hz)	C-2, C-2′	5′2′
1″	122.8			
2″,6″	108.9	7.72 (2H,s)	C-7″, C-4″	
3″,5″	148.8			
4″	142.8			
7″	167.2			
8″,9″	56.6	3.72 (6H,s)	C-3″, C-5″	
gal-1	105.0	6.01 (d, $J = 7.8$ Hz)	C-3	Gal-2
2	73.4	4.79 (m)	C-gal-3,4	Gal-1,3
3	75.3	4.24 (dd, $J = 9.6$, 3.0 Hz)		Gal-2,4
4	70.6	4.52 (br s)		Gal-3,5
5	75.2	4.13 (br t, $J = 6$ Hz)	C-gal-1,4,6	Gal-4,6
6	66.8	3.99 (m), 4.46 (m)	C-gal-5, C-rah-1	Gal-5,6
rha-1	102.0	5.28 (br s)	C-rha-3,5, C-gal-6	Rha-2
2	69.8	4.79 (br s)	C-rha-4	Rha-1,3
3	76.6	5.99 (m)	C-7″, C-rha-4	Rha-2,4
4	71.2	4.58 (m)	C-rha-3,5,6	Rha-3,5
5	70.3	4.41 (m)	C-rha-4	Rha-4,6
6	18.1	1.57 (d, $J = 6.0$ Hz)	C-rha-5	Rha-5

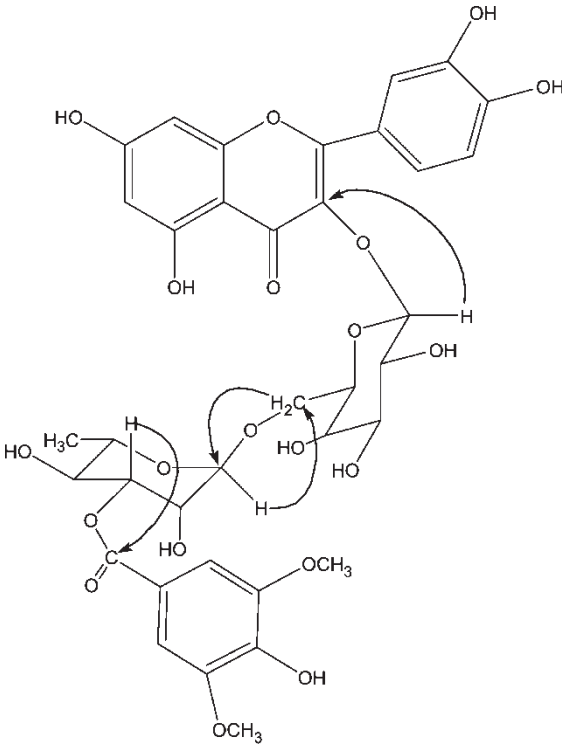

Figure 2. Key HMBC correlations for **1**.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yamaco MP-S3 Micro-hot stage and are uncorrected. The UV spectrum was recorded on a Shimadzu UV-2201 spectrometer and the IR spectrum was obtained with an IFS-55 spectrum instrument. ESI-MS was performed on a Finnigan LCQ mass spectrometer. HRMS was performed on a QSTAR LCQ mass spectrometer. NMR spectra were taken in pyridine-d_5 on a Bruker ARX-300 spectrometer. Silica gel for chromatography was produced by the Qingdao Ocean Chemical Group Co. of China.

3.2 Plant material

The material of *Leonurus heterophyllus* was collected from Changzhou city of Hebei Province, China and identified by Professor Qishi Sun (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University). A voucher specimen has been deposited at Shenyang Pharmaceutical University.

3.3 Extraction and isolation

Dried aerial parts of *Leonurus heterophyllus* (4.0 kg) were extracted with 95% EtOH (36 L × 3) under reflux. The extract was then evaporated under reduced pressure and

the residue extracted successively with light petroleum, $CHCl_3$ and EtOAc respectively. The EtOAc fraction (14 g) was subjected to column chromatography on silica gel (70 g), eluted with $CHCl_3$–MeOH with increasing polarity. Heteronoside (**1**) was obtained as a yellow amorphous solid from the eluent of $CHCl_3$–MeOH (8:1).

3.3.1 Heteronoside (1). A yellow amorphous powder, $C_{36}H_{38}O_{20}$; mp 201–203°C; $[\alpha]_D^{21} + 5.5$ (*c* 0.2, MeOH); IR(KBr) (cm^{-1}): 3405, 2924, 1654, 1608, 1513, 1457, 1347, 1304, 1212, 1114, 994, 814, 763, 653, 598; UV λ_{max}^{MeOH}(nm) : 359 ($\varepsilon, 2.02 \times 10^4$), 266 ($\varepsilon, 3.46 \times 10^4$) 210 ($\varepsilon, 6.40 \times 10^4$); HCl–Mg, $FeCl_3$–$K_3Fe(CN)_6$ and Molish reactions were positive; ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 6.20 (1H, s, H-6), 6.42 (1H, s, H-8), 7.69 (1H, br d, $J = 8.1$ Hz, H-6′), 6.85 (1H, d, $J = 8.1$ Hz, H-5′), 7.56 (1H, br s, H-2′), 12.61 (1H, s, HO-5), 7.29 (2H, s, H-2″,6″), 3.83 (6H, s, H-8″,9″), 5.38 (1H, d, $J = 7.2$ Hz, gal-1), 4.55 (1H, br s, rham-1), 4.61–5.20 (sugar-OH), 3.32–3.78 (m, sugar protons), 1.17 (3H, d, $J = 4.8$ Hz, rham-Me);^{13}C NMR (75.4 MHz, DMSO-d_6) δ (ppm): 177.5 (C-4), 165.5 (C-7″), 164.3 (C-7), 161.3 (C-5), 156.5 (C-2,9), 148.6 (C-4′), 147.5 (C-3″,5″), 144.9 (C-3′), 140.7 (C-4″), 133.7 (C-3), 122.0 (C-1′), 121.2 (C-6′),120.1 (C-1″), 116.2 (C-5′), 115.3 (C-2′), 107.5 (C-2″, 6″), 104.1 (C-10), 102.3 (gal-1), 100.1 (rham-1), 98.8 (C-6), 93.7 (C-8), 74.9 (rham-3), 73.4 (gal-5), 73.2 (gal-3), 71.3 (rham-4), 69.2 (gal-2), 68.6 (rham-2), 68.2 (rham-5), 68.0 (gal-4), 65.1 (gal-6), 56.3 (C-8″, 9″), 18.0 (rham-6); ^{1}H and ^{13}C NMR data in C_5D_5N see table 1; ESI-MS *m/z* 1603.9 $[2M + Na]^+$, 813.2 $[M + Na]^+$, 791.0 $[M + H]^+$ and HRMS *m/z* 813.1823 $[M + Na]^+$ (calcd. for $C_{36}H_{38}O_{20} + Na^+$, 813.1854).

Acknowledgements

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