Acetylated and Non-acetylated Flavonol Triglycosides from *Galega officinalis*

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Three flavonol triglycosides kaempferol 3-[2^{Gal}-(4-acetylrhamnosyl)robinobioside], kaempferol 3-(2^{Gal}-rhamnosylrobinobioside) and quercetin 3-(2^{G}-rhamnosylrutinoside) have been isolated from a methanolic extract of *Galega officinalis* aerial parts. They are reported for the first time in the genus *Galega*; moreover, the acetylated triglycoside is a new natural product.

Key words *Galega officinalis*; Fabaceae; acylated flavonol triglycoside; kaempferol 3-[2^{Gal}-(4-acetylrhamnosyl)robinobioside]

Goat's rue (*Galega officinalis* L.) has been used in traditional medicine for treatment of diabetes mellitus.[1] The plant has been previously investigated and the rare norterpenoid glucoside dearabinosyl pneumonanthoside was isolated.[2] We carried out a chemical investigation of *G. officinalis* and isolated a new acetylated flavonol triglycoside along with two known flavonol triglycosides. This paper describes the isolation and structural elucidation of these components.

The methanolic extract of the aerial parts[2] was suspended in H_2O and partitioned by CH_2Cl_2 and EtOAc successively. Compounds **1**—**3** were present in the EtOAc soluble part. Compounds **2** and **3** were identified as mauritianin[3] and quercetin 3-(2^{G}-rhamnosylrutinoside),[4,5] respectively, by comparing their ^{1}H- and ^{13}C-NMR spectral data with reported values. Compound **1** is new, while **2** and **3** were isolated for the first time from this plant.

Among the three isolated triglycosides, component **1** was the least polar on silica gel and BAW-cellulose TLC systems (see Experimental). Its UV spectra in the usual shift reagents indicated a kaempferol 3-conjugated structure[6] as for **2**. Furthermore, the positive FAB mass spectrum exhibited a signal at *m/z* 783 $(M+H)^+$ consistent with a molecular formula $C_{35}H_{42}O_{20}$ for the glycoside. Other significant peaks visible at *m/z* 637 $[(M+H)-146]^+$, *m/z* 595 $[(M+H)-146-42]^+$, *m/z* 449 $[(M+H)-146-42-146]^+$ and finally *m/z* 287 $[(M+H)-146-42-146-162]^+$ indicated the successive loss of two branched rhamnose units of which one was acetylated as was as an inner hexose, the base signal at *m/z* 287 corresponding to kaempferol. The two branched α-L-rhamnosyl moieties were confirmed by the ^{1}H broad doublets at δ 4.52 (J=1.4 Hz) and δ 5.23 ppm (J=1.3 Hz) for anomeric protons and the doublets (J=6.2 Hz) at δ 1.17 and 0.88 ppm for the methyls, as well as the ^{13}C-NMR peaks at δ 101.9 and 102.6 ppm for both anomeric carbons and δ 18.0 and 17.4 for CH_3 groups. The inner hexose exhibited ^{1}H- and ^{13}C-NMR values characteristic of β-D-galactose at δ 5.52, d (J=7.7 Hz) and δ 101.2 for the anomeric CH as well as those at δ 3.77, br d (J=3.3 Hz) and δ 70.7 for CH-4 as recorded for component **2** (Tables 1 and 2). Finally, a substituted robinobiose structure was evidenced in this compound as for **2** by all the ^{1}H- and ^{13}C-NMR shifts and multiplicities relative to the osidic part, thus locating the second rhamnosyl unit at C-2 of the galactose as indicated by δ 3.92 and δ 77.9 (Tables 1 and 2). However, the supplementary rhamnosyl group was different from that of **2** by an acetyl function located at C-4, and was

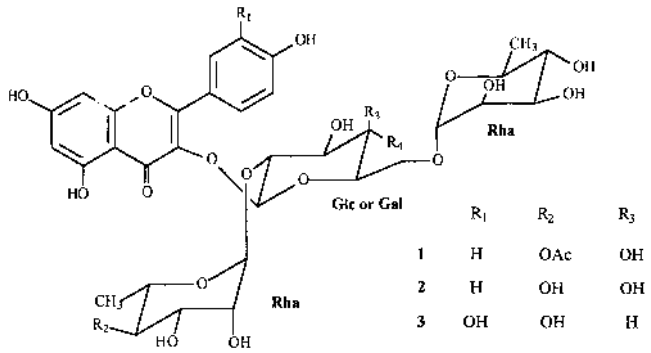

Table 1. ^{1}H-NMR Data of Compounds **1**—**3** (400 MHz, CD_3OD/TMS)[a]

No.	1	2	3
Kaempferol or quercetin			
6	6.20 d (2.0)	6.18 d (1.6)	6.18 d (2.0)
8	6.39 d (2.0)	6.38 d (1.6)	6.37 d (2.0)
2′	8.05 d (8.9)	8.06 d (8.8)	7.59 br s
3′	6.89 d (8.9)	6.89 d (8.8)	—
5′	6.89 d (8.9)	6.89 d (8.8)	6.87 d (8.1)
6′	8.05 d (8.9)	8.06 d (8.8)	7.60 dd (8.1, 2.2)
Robinobiose or rutinose[b]			
1	5.52 d (7.7)	5.60 d (7.8)	5.59 d (7.7)
2	3.92 dd (9.5, 7.7)	3.93 dd (9.5, 7.8)	3.64 dd (9.0, 7.7)
3	3.70 dd (9.5, 3.2)	3.70 dd (9.5, 3.4)	3.54 br t (9.0, 8.7)
4	3.77 br d (3.3)	3.77 br d (3.3)	3.27 br t (9.5, 8.9)
5	3.63 br t (6.1)	3.63 br t (6.3)	3.32 m
6_A	3.45 dd (10.3, 6.7)	3.44 dd (10.2, 6.6)	3.40 br d (11.6)
6_B	3.71 dd (10.2, 5.4)	3.72 dd (10.3, 5.7)	3.82 br d (11.6)
1	4.52 br d (1.4)	4.52 br d (1.2)	4.50 br d (1.3)
2	3.57 dd (3.4, 1.6)	3.56 dd (3.3, 1.6)	3.58 dd (3.3, 1.6)
3	3.50 dd (9.3, 3.4)	3.50 dd (9.3, 3.3)	3.49 dd (9.5, 3.4)
4	3.27 t (9.5)	3.26 t (9.5)	3.23 t (9.5)
5	3.52 dq (9.5, 6.2)	3.52 dq (9.5, 6.2)	3.41 dq (9.5, 6.2)
6	1.17 d (6.2)	1.17 d (6.2)	1.08 d (6.2)
Rhamnose			
1	5.23 br d (1.3)	5.21 br d (1.0)	5.22 br d (1.2)
2	4.04 dd (3.3, 1.6)	4.00 br dd (3.0, 1.4)	4.00 dd (3.3, 1.6)
3	4.00 dd (9.8, 3.3)	3.80 dd (9.8, 3.3)	3.80 dd (9.8, 3.4)
4	4.89 t (9.8)	3.34 br t (9.5)	3.35 br t (9.8)
5	4.26 dq (9.8, 6.2)	4.06 dq (9.6, 6.2)	4.08 dq (9.6, 6.2)
6	0.88 d (6.2)	0.98 d (6.2)	1.00 d (6.2)
Acetyl			
CH_3	2.00 s	—	—

a) δ ppm (J Hz). *b*) Robinobiose: Gal $^6\rightarrow^1$ Rha; rutinose: Glc $^6\rightarrow^1$ Rha.

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Table 2. ^{13}C-NMR Data of Compounds **1**—**3** (100 MHz, CD_3OD/TMS, δ ppm)

No.	**1**	**2**	**3**
Kaempferol or quercetin			
2	158.9	158.7	159.0
3	134.6	134.5	134.5
4	179.4	179.5	179.3
5	163.2	163.2	163.2
6	99.8	99.8	99.8
7	165.7	165.7	165.7
8	94.8	94.7	94.7
9	158.5	158.5	158.5
10	105.9	105.9	106.0
1′	123.0	123.1	123.6
2′	132.2	132.3	117.5
3′	116.2	116.2	146.0
4′	161.4	161.3	149.6
5′	116.2	116.2	116.1
6′	132.2	132.3	123.5
Robinobiose or rutinose			
1	101.2	100.9	100.5
2	77.9	77.6	80.1
3	75.7	75.8	79.0
4	70.7	70.8	71.9
5	75.3	75.4	77.1
6	67.3	67.2	68.3
1	101.9	101.9	102.3
2	72.1	72.1	72.2
3	72.3	72.3	72.3
4	73.9	73.9	73.9
5	69.7	69.7	69.8
6	18.0	18.0	17.9
Rhamnose			
1	102.6	102.7	102.7
2	72.5	72.5	72.5
3	70.4	72.4	72.4
4	75.8	74.1	74.1
5	67.7	69.9	70.0
6	17.4	17.6	17.6
Acetyl			
CO	172.9	—	—
CH_3	21.1	—	—

responsible for the large ^{1}H and ^{13}C deshielding of CH-4 at δ 4.89 and δ 75.8 in comparison with δ 3.34 and δ 74.1 for **2**. This group also induced downfield shifts to vicinal protons H-3 (δ 4.00, $\Delta\delta$ +0.20 ppm) and H-5 (δ 4.26, $\Delta\delta$ +0.20 ppm) by decreasing, as expected, the electron density caused by the conjugated 4-oxygen with the carbonyl. Inversely, upfield shifts were recorded for the corresponding carbons C-3 (δ 70.4, $\Delta\delta$ −2.0 ppm) and C-5 (δ 67.7, $\Delta\delta$ −2.2 ppm) following both the anisotropic effect involving the carbonyl and steric hindrance of the substituent.[7] These results which were completely confirmed by both 2D-NMR including ^{1}H–^{1}H and ^{1}H–^{13}C COSY correlation spectroscopy experiments as well as by acid hydrolysis resulted in assignation of the new structure kaempferol 3-*O*-[4-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnosylpyranosyl-(1→6)]-β-D-galactopyranoside or kaempferol 3-[2^{Gal}-(4-acetylrhamnosyl)robinobioside] to compound **1**.

Experimental

General CC was achieved on polyamide SC-6 (Macherey-Nagel) and Sephadex LH20 (Pharmacia). Chromatographic mobilities were recorded in three systems: system 1 (Silica gel F_{254}, EtOAc–H_2O–HCO_2H–HOAc, 20 : 2 : 1 : 1), system 2 (Cellulose F_{254}, *n*-BuOH–HOAc–H_2O, 4 : 1 : 5, upper phase), system 3 (Cellulose F_{254}, H_2O–HOAc, 9 : 1). Prep. HPLC was performed on a Merck model (Prep Septech) with Lichrospher 100 DIOL (10 μm, 250×10 mm). UV spectra were recorded on a Jasco V-560 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured in CD_3OD at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR on a Bruker DPX Avance spectrometer using tetramethylsilane as internal standard. The complete proton and carbon assignments were based on 1D (^{1}H standard, ^{13}C *J* mod and ^{13}C distortionless enhancement by polarization transfer (DEPT)), 2D (^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{13}C heteronuclear multiple quantum coherence (HMQC) and ^{1}H–^{13}C heteronuclear multiple bond correlation (HMBC)) NMR experiments. Fast atom bombardment (FAB) mass spectra were obtained on a Nermag Sidar V 3.1 spectrometer (70 eV) in the positive ion mode using glycerol matrix. Acid hydrolysis was performed at 110 °C for 2 h with 9 mg for **1**, 5 mg for **2** and **3** in 4 ml of 2 N HCl. After neutralization with NaOH, the aqueous residue was extracted twice with Et_2O. Aglycones were detected in the organic layer whilst sugars were identified in the aqueous phase. Glucose, galactose and rhamnose were visualized by TLC (20 cm) on silica gel (EtOAc–H_2O–MeOH–HOAc 13 : 3 : 3 : 4) after spraying *p*-anisidine phthalate reagent.

Plant Material *G. officinalis* was harvested during flowering by Pharma et Plantes (Valanjou, France), and the aerial parts in powder form were deposited at Laboratoire de Pharmacognosie et de Phytochimie (Université de Limoges) along with a control certificate.

Extraction and Isolation *G. officinalis* powdered aerial parts (9 kg) were percolated at room temperature with successive solvents of increasing polarity: 57 l *n*-hexane (110 g), 200 l CH_2Cl_2 (120 g), 140 l EtOAc (52 g) and 100 l MeOH (500 g). A part of the MeOH extract (150 g) was suspended in 1.2 l of water and then divided into 5 portions and added with 150 ml of petrol ether, CH_2Cl_2, EtOAc, and *n*-BuOH. After concentration, residues were 2.3 g for the petrol ether part, 6.4 g for the CH_2Cl_2 part, 13.9 g for the EtOAc part, 61.3 g for the *n*-BuOH part and, finally, 59.4 g for the aqueous residue. The last mentioned EtOAc extract (13.9 g) was then fractionated on a Sephadex LH20 CC (800×45 mm, MeOH) to give nine fractions (A—I). Compounds **1**—**3** issued from fraction C were separated by a Lichroprep C18 MPLC (15—25 μm, 460×15 mm, MeOH gradient in H_2O) to give four fractions (I, II, III, IV). Fraction II (542 mg) eluted with H_2O–MeOH (1 : 1) was passed through two polyamide MPLC (230×15 mm, MeOH gradient in toluene) to afford compound **2** in the toluene–MeOH (4 : 1) mixture and compound **3** in toluene–MeOH (13 : 7) fractions. The final purification of **2** was carried out by centrifugal thin layer chromatography [Chromatotron, Silica gel 60 F_{254}, 1 mm thickness, *n*-hexane–EtOAc–MeOH (5 : 2 : 3)] affording 17 mg. Finally, compound **3** was subjected to a Sephadex LH20 CC (550×15 mm, MeOH) to give 17 mg . Fraction III (430 mg) eluted with H_2O–MeOH (2 : 3) was submitted to three successive polyamide MPLC (230×15 mm, MeOH gradient in toluene) and finally to a Lichrospher 100 DIOL HPLC [10 μm, 250×10 mm, *n*-hexane–isoPrOH–MeOH (12 : 3 : 10)] to yield 127 mg of pure **1**.

Kaempferol 3-[2^{Gal}-(4-acetylrhamnosyl)robinobioside] (**1**): A yellow amorphous powder, $C_{35}H_{42}O_{20}$. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. UV λ_{max}^{MeOH} nm: 266, 298 sh, 351; (+NaOH): 274, 329, 394; (+$AlCl_3$): 275, 306, 353, 401; (+$AlCl_3$+HCl): 275, 305, 351, 398; (+NaOAc): 270, 303, 356; (+NaOAc+H_3BO_3): 267, 301 sh, 350. Positive FAB-MS (glycerol): *m/z* 783 $(M+H)^+$; 637 $(M+H-Rha)^+$; 595 $(M+H-AcRha)^+$; 449 $(M+H-Rha-AcRha)^+$; 287 $(M+H-Rha-AcRha-Gal)^+$. Chromatographic mobilities: *Rf* 0.20 (system 1), *Rf* 0.44 (system 2), *Rf* 0.88 (system 3).

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