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# Quercetin and Kaempferol Glycosides from *Ficaria verna* Flowers and Their Structure Studied by 2D NMR Spectroscopy

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From the flowers of *Ficaria verna* Huds. (Ranunculaceae), two flavonol triglycosides were isolated and their structures were elucidated by spectroscopic analysis (UV, NMR, MS) as 3-O-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-O-(β-D-glucopyranosyl) – quercetin (**1**) and 3-O-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-O-(β-D-glucopyranosyl) – kaempferol (**2**). In addition, the structure of **1** was determined using homo- and heteronuclear 2D NMR techniques.

**Key words**: *Ficaria verna*, flavonoids, triglycosides, 2D NMR

In the flora of Poland, the genus *Ficaria* (Ranunculaceae) is represented only by two species, *F. verna* Huds. (syn.: *Ranunculus ficaria* ssp. *bulbifer* Lawalrée, *Ficaria ranunculoides* ROTH) and F. *nudicaulis* Kerner (syn.: *Ranunculus ficaria* L. ssp. *calthifolius* (RCHB.) ARCANG., *Ficaria calthifolia* (RCHB.) [1,2]. In previous papers [3–4], we have reported the isolation and structural elucidation of flavonoid compounds from the flowers and leaves of *F. verna*, commonly known as pilewort. In this report, we describe the isolation and structure elucidation of next two flavonoids from flowers of *F. verna*.

## RESULTS AND DISCUSSION

Compounds **1–2** (Figure 1) were isolated by repeated column chromatography on a polyamide, cellulose and Sephadex LH-20 from butanolic extract of *F. verna* flowers.

Compound **1** was obtained as yellow needles. The complete acid hydrolysis gave, besides quercetin, glucose and rhamnose (coTLC). The UV spectrum showed absorption bands and reagents shifts of **1** to be a 3,7-disubstituted quercetin derivative. The UV analysis of **1** proved the presence of the lack of 3-hydroxyl group in the compound (the bathochromic shift (47 nm) of Band I (in MeOH) to Band I (in the presence of $AlCl_3$/HCl)). The absence of free 7-hydroxyl group in **1** was observed in lack of shift of Band II in the presence of NaOAc. The $^1H$ and $^{13}C$ NMR spectra of **1** display characteristic signals for quercetin moiety and three sugar residues.

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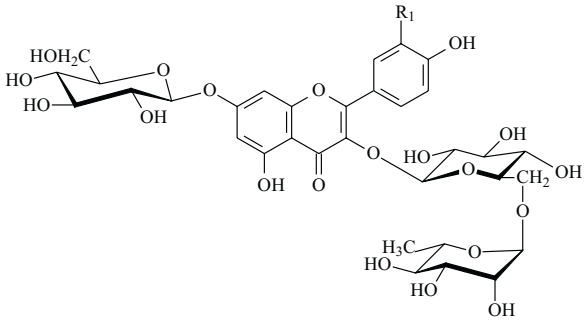

| | $R_1$ |
|---|---|
| Compound **1** | OH |
| Compound **2** | H |

**Figure 1.** Structure of isolated flavonoid compounds (**1–2**).

**Table 1.** $^{1}$H- and $^{13}$C-NMR spectral data of compounds **1–2** in DMSO-$d_6$, multiplicities and coupling constants ($J$, Hz), δ ppm.

| Position C/H | DEPT | **1** | | | **2** |
|---|---|---|---|---|---|
| | | δ H[a] | δ C[b] | HMBC correlations **1** | δ H[a] |
| 2 | C | | 157.18 | H-2′ | |
| 3 | C | | 133.53 | | |
| 4 | C | | 177.53 | | |
| 5 | C | | 160.89 | H-6 | |
| 6 | CH | 6.43 (d, 2.0) | 99.30 | H-8 | 6.43 (d, 2.1) |
| 7 | C | | 162.83 | H-1′′′′, H-6, H-8 | |
| 8 | CH | 6.71 (d, 2.0) | 94.50 | H-6 | 6.73 (d, 2.1) |
| 9 | C | | 156.01 | H-8 | |
| 10 | C | | 105.60 | H-6, H-8 | |
| 1′ | C | | 120.92 | H-2′, H-5′ | |
| 2′ | CH | 7.53 (d, 1.4) | 115.21 | H-6′ | 7.99 (d, 8.9) |
| 3′ | C | | 144.81 | H-2′, H-5′ | 6.84 (d, 8.9) |
| 4′ | C | | 148.74 | H-6′ | |
| 5′ | CH | 6.82 (d, 7.6) | 116.41 | | 6.84 (d, 8.9) |
| 6′ | CH | 7.54 (dd, 1.4, 7.4) | 121.68 | H-2′, H-5′ | 7.99 (d, 8.9) |
| β-glc | | | | | |
| 1′′ | CH | 5.36 (d, 6.9) | 101.04 | | 5.30 (d, 7.0) |
| 2′′ | CH | | 74.05 | | |
| 3′′ | CH | | 76.37 | | |
| 4′′ | CH | | 69.99 | | |
| 5′′ | CH | | 76.37 | | |
| 6′′ | $CH_2$ | | 66.99 | H-1′′ | |
| α-rhm | | | | | |
| 1′′′ | CH | 4.37 (s) | 100.73 | | 4.36 (s) |
| 2′′′ | CH | | 70.35 | H-1′′ | |
| 3′′′ | CH | | 70.53 | | |
| 4′′′ | CH | | 71.81 | | |
| 5′′′ | CH | | 68.24 | | |
| 6′′′ | $CH_3$ | 0.98 (d, 6.1) | 17.76 | | 0.98 (d, 6.1) |
| β-glc | | | | | |
| 1′′′′ | CH | 5.06 (d, 7.4) | 99.83 | | 5.05 (d, 7.3) |
| 2′′′′ | CH | | 73.12 | | |
| 3′′′′ | CH | | 76.37 | | |
| 4′′′′ | CH | | 69.54 | | |
| 5′′′′ | CH | | 77.15 | | |
| 6′′′′ | $CH_2$ | | 60.62 | | |

[a]measured at 500.13 MHz, [b]measured at 125.77 MHz.

The $^1$H NMR spectrum of **1** (see Table 1) indicated a triglycoside moiety from the appearance of three, well-separated anomeric proton signals at δ 5.30 (1H, d, *J* = 7.0 Hz), 5.05 (1H, d, *J* = 7.3 Hz) and 4.36 (1H, s). Consideration of the coupling constants suggested the presence of two β-glucopyranoses and one α-rhamnopyranose. The complete assignments of the NMR data and the composition of the sugar radical of **1** were achieved by 2D NMR techniques. The HMBC experiment clarified all intramolecular connections between carbons and protons, revealing **1** to be a disubstituted quercetin derivative (Fig. 2).

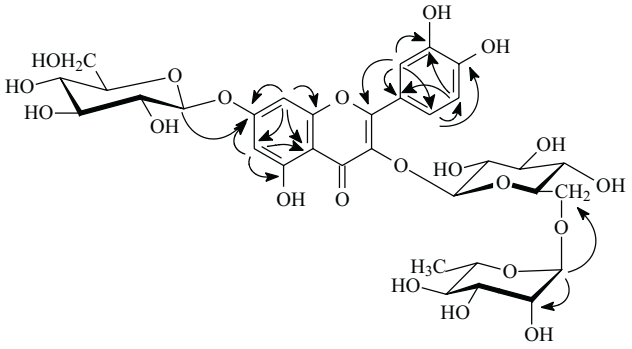


**Figure 2.** Heteronuclear multiple-bond correlations (HMBC) for **1**. Arrows point from proton to carbon.

The HMBC spectrum has shown long-range correlation between the anomeric proton (δ = 4.37) and the carbon of glucose (δ = 66.99). That fact indicated that α-L-rhamnose was attached to the C-6 of β-D-glucose moiety and this defined the disaccharide as a 3-O-rutinoside. In the HMQC spectrum one of the C-6 glucose protons is placed in upfield region. Observed in $^1$H-$^1$H TOCSY spectrum arrangement of hexoses protons shows that α-L-rhamnopyranose is connected to β-D-glucopyranose, which is substituted to C-3 position of aglycone. The positive and negative ion LSIMS mass spectra of **1** exhibited pseudomolecular ion, $(M+H)^+$ and $(M-H)^-$ at *m/z* 757.3 and 755.3, respectively, which were compatible with the molecular formula $C_{33}H_{40}O_{21}$. Therefore, the structure of **1** was determined as 3-O-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-O-(β-D-glucopyranosyl) – quercetin. Compound **2** was obtained as a colorless amorphous powder. The complete acid hydrolysis of **2** gave, besides kaempferol, glucose and rhamnose (coTLC). UV spectrum of **2** with diagnostic shift reagents indicated a flavonol substituted at positions C-3 and C-7, the same as **1** and showed absence of an *ortho*-dihydroxyl pattern at B ring. The $^1$H NMR spectrum suggested that **2** (see Table 1) is a trisaccharide of kaempferol on the basis of three signals in the sugar region at 5.30 (d, *J* = 7.0 Hz), 5.05 (d, *J* = 7.3 Hz) and 4.36 (s), corresponding to the anomeric protons of two β-glucoses and to the anomeric proton of the α-linked rhamnose, respectively. In addition, the structure of **2** was confirmed by LSIMS spectrum. The spectrum of **2** gave a molecular ion peak $(M+H)^+$ at *m/z* 757.3 (positive ion) and $(M-H)^-$ at *m/z* 755.3 (negative ion), indicating a molecular mass of 756.71. The pres-

ence of peaks at *m/z* 609.4 (M-rhm-H)$^-$, 593.4 (M-glc-H)$^-$, 447.3 (M-rut-H)$^-$, 285.3 (aglycone-H)$^-$ confirmed the presence of sugar units. Spectral analysis carried out for compound **2** and by comparison $^1$H NMR spectrum of sugar moiety of **2** and **1** suggests that compound **2** is 3-O-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-O-(β-D-glucopyranosyl) – kaempferol. The spectral properties of compounds **1** and **2** were verified by comparison of its spectral data with those described in the literature [5,6]. The presence of these compounds **1** and **2** have been reported in leaves of plants derived from Solanaceae and Liliaceae family [7–11] but their presence in *F. verna* has been described for the first time.

## EXPERIMENTAL

*General experimental procedures*. All melting points (m.p.) were determined on Büchi 535 melting point apparatus and are uncorrected. All solvents of analytical grade were purchased from POCH (Gliwice, Poland). TLC analysis was carried out using cellulose (TLCc) and Silica gel 60 $F_{254}$ (TLCg) plates (Merck, Darmstadt, Germany). Polyamide (Roth, Karlsruhe, Germany), cellulose CC11 (Whatman, England) and Sephadex LH-20 (Fluka, Buchs, Switzerland) were used for open column chromatography (CC). Chromatographic systems: TLCc: *n*-BuOH:HOAc:$H_2O$ (4:1:5 v/v/v) upper phase (system A), 15% HOAc (system B), HOAc:HCl conc.:$H_2O$ (30:3:10 v/v/v) (system C), *n*-BuOH:pyridine:$H_2O$ (6:4:3 v/v/v) (system D – for sugars); TLCg: EtOAc:HCOOH:$H_2O$ (18:1:1 v/v/v) (system E). Visualization of plates was performed using visible light, UV fluorescence and/or spraying with the following reagents: R1: 2% $AlCl_3$, R2: Naturstoffreagenz A (Roth, Karlsruhe, Germany), R3: aniline phthalate by heating at 110°C for 5–10 min – for sugars. Column chromatography (CC): $H_2O$:MeOH increasing gradient (system F), $H_2O$ – saturated *n*-BuOH (system G). Acid hydrolysis: the pure both compounds were treated with 5% HCl at 100°C for 4 hrs. UV spectra were recorded on a Specord 40 UV-VIS Spectrophotometer (Jena Analytik AG, Germany) according to Mabry *et al*. [12]. $^1$H-NMR and $^{13}$C-NMR were taken on a Bruker DRX 500 instrument (500.13 and 125.77 MHz, respectively). Chemical shifts values are given in δ-values (ppm) with tetramethylsilane (TMS) as internal standard. Mass spectra were obtained with Finnigan MAT 95 mass spectrometer.

*Plant material*. Flowers of *F. verna* Huds. (Ranunculaceae) were collected in March/April 1997 in the Białystok area. The plant material has been identified by Prof. J. Gudej, Faculty of Pharmacy, Medical Academy of Białystok and a voucher specimen (no. FV97004) has been deposited at the herbarium of the Department of Pharmacognosy, Medical Academy of Białystok, Poland.

*Extraction and isolation*. Dried and powdered flowers of *F. verna* (0.5 kg) were extracted with petrol and $CHCl_3$ in a Soxhlet apparatus. Plant material purified in this way was successively extracted with MeOH. After solvent evaporation under reduced pressure, the MeOH extract was diluted with $H_2O$. This residue was left in the refrigerator overnight and filtered. The filtrate was partitioned between $Et_2O$, EtOAc and *n*-BuOH, successively. The *n*-BuOH-soluble fraction was concentrated and subjected to a polyamide column chromatography eluting with mixture $H_2O$-MeOH (increasing polarity), (system F). The fraction eluted with $H_2O$-MeOH (9.5:0.5 v/v) yielded mixture (45 mg), which was further chromatographed on a cellulose column chromatography using $H_2O$ – saturated *n*-BuOH (system G). Final purification was done by column chromatography on Sephadex LH-20 with MeOH as eluent to afford a pure (**1**) (35 mg, 0.07%) and (**2**) (9 mg, 0.018%).

*Identification of isolated flavonoids **1–2***. Compound (**1**): yellow needles; m.p. 214–215°C; $R_f$ TLCc: 0.03 – system A, R2; 0.65 – system B, R2; $R_f$ TLCg: 0.04 – system E; $R_f$ value of the aglycone (coTLCc, system C, R1) after acid hydrolysis of **1** it was identical with quercetin. $R_f$ values of sugars (coTLCc, system D, R3) after hydrolysis were identical with the standards, glucose and rhamnose; UV $\lambda_{max}$ (MeOH) nm: 268, 357; (NaOMe) 269, 299sh, 400; ($AlCl_3$) 274, 301sh, 346sh, 437; ($AlCl_3$/HCl) 270, 300sh, 362sh, 404; (NaOAc) 268, 294sh, 363, 422sh; (NaOAc/$H_3BO_3$) 261, 295sh, 382; $^1$H- and $^{13}$C-NMR, see Table 1; LSIMS *m/z*: 772.71 (Calcd. for $C_{33}H_{40}O_{21}$: 772.71); positive ion: 773.4 (M+H)$^+$, 611.3 (M-glc+H)$^+$, 627.4

$(M\text{-rhm}+H)^+$, 465.3 $(M\text{-rut}+H)^+$, 303.3 $(\text{aglycone}+H)^+$; negative ion: 771.4 $(M-H)^-$, 609.3 $(M\text{-glc-}H)^-$, 625.2 $(M\text{-rhm-}H)^-$, 463.3 $(M\text{-rut-}H)^-$, 301.3 $(\text{aglycone-}H)^-$.

Compound (**2**): pale-beige needles; m.p. 232–233°C; $R_f$ TLCc: 0.06 – system A, R2; 0.73 – system B, R2; $R_f$ TLCg: 0.07 – system E; $R_f$ value of the aglycone (coTLCc, system C, R1) after acid hydrolysis of **2** it was identical with kaempferol. $R_f$ values of sugars (coTLCc, system D, R3) after hydrolysis were identical with the standards, glucose and rhamnose; UV $\lambda_{max}$ (MeOH) nm: 266, 315sh, 350; (NaOMe) 244, 271, 303sh, 346sh, 392; ($AlCl_3$) 255sh, 274, 301, 353, 398; ($AlCl_3$/HCl) 275, 300, 350, 397; (NaOAc) 266, 320sh, 352, 416sh; (NaOAc/$H_3BO_3$) 266, 323sh, 350; $^1$H-NMR, see Table 1; LSIMS *m/z*: 756.71 (Calcd. for $C_{33}H_{39}O_{20}$: 756.71); positive ion: 757.3 $(M+H)^+$, 595.4 $(M\text{-glc}+H)^+$, 611.3 $(M\text{-rhm}+H)^+$, 449.3 $(M\text{-rut}+H)^+$, 288.4 $(\text{aglycone}+2H)^+$; negative ion: 755.3 $(M-H)^-$, 593.4 $(M\text{-glc-}H)^-$, 609.4 $(M\text{-rhm-}H)^-$, 447.3 $(M\text{-rut-}H)^-$, 285.3 $(\text{aglycone-}H)^-$.

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