C-Glycosylflavones with Acetyl Substitution from *Rumex acetosa* L.

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Six flavone *C*-glycosides: 2″,6″-di-*O*-acetylisoorientin (1), 2″,6″-di-*O*-acetylorientin (2), 2″-*O*-acetylisoorientin (3), 2″-*O*-acetylorientin (4), isoorientin (5) and orientin (6) were identified from the aerial parts of *Rumex acetosa* L. (Polygonaceae), in addition to two flavonol *O*-glycosides: avicularin (7) and hyperin (8). Of these, 1—3 were new compounds and 4—7 were isolated for the first time from this species. The structures were established by ultraviolet, fast atom bombardment mass and nuclear magnetic resonance spectral data.

Keywords *Rumex acetosa*; Polygonaceae; acetyl *C*-glycosylflavone; orientin; isoorientin; 2″-*O*-acetylorientin; 2″-*O*-acetylisoorientin; 2″,6″-di-*O*-acetylorientin; 2″,6″-di-*O*-acetylisoorientin; *O*-glycosylflavonol

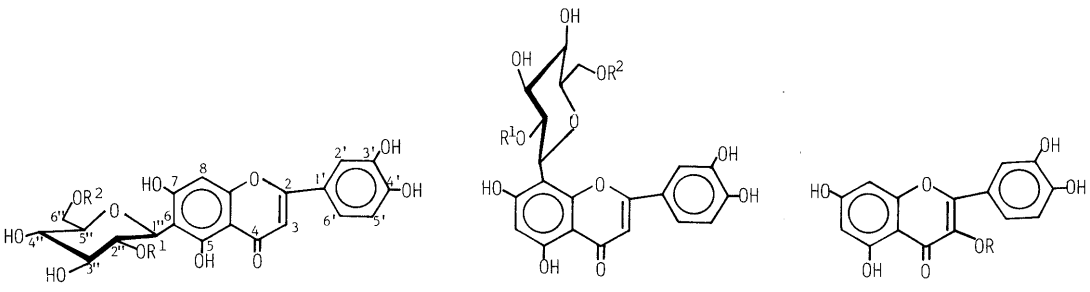
We earlier reported the isolation of anthraquinones and their glycosides from the dried roots of dioecious *Rumex acetosa* L.[1] In our detailed survey of other polar fractions than those containing anthraquinone glycosides to determine the criteria of dioecious discrimination, six flavone *C*-glycosides: 2″,6″-di-*O*-acetylisoorientin (**1**), 2″,6″-di-*O*-acetylorientin (**2**), 2″-*O*-acetylisoorientin (**3**), 2″-*O*-acetylorientin (**4**), isoorientin (**5**) and orientin (**6**), and two flavonol *O*-glycosides: avicularin (**7**) and hyperin (**8**), were obtained from the aerial parts of this plant. To date, **8**, vitexin, rutin, kaempferol, quercetin and myricetin have been identified as flavonoidal constituents in these aerial parts.[2] In this paper, we report the isolation of these additional compounds (**1**—**8**), **1**—**3** being new compounds and **4**—**7** isolated for the first time from this species.

All eight compounds were obtained from the ethyl acetate fraction of the methanolic extract of the fresh aerial parts by repeated chromatography as described in Experimental.

Identifications of **4**[3] and **5**[4,5] were performed by the comparison of physicochemical data with those reported, and **6**—**8** by direct comparison with authentic samples.[6]

Compound **1**: Yellow needles, mp 180—182 °C, and $[\alpha]_D^{22}$ +4.8° (MeOH); gave positive color reaction in the Mg–HCl test. The ultraviolet (UV) spectrum having maxima at 350, 270, 258 and 243 sh nm, and the bathochromic shifts with typical test reagents ($AlCl_3$, $AlCl_3$+HCl, NaOMe and NaOAc) agreed very well with those of **5**[5] (see Experimental). In addition, the molecular ion $[(M+H)^+]$ at *m/z* 533 (448, isoorientin +84, Ac_2) in the fast atom bombardment mass spectrum (FAB-MS) and the acetyl signals in the nuclear magnetic resonance (NMR) spectra (*vide infra*) suggested **1** as a diacetate of **5**. Then, hydrolysis of **1** with 2 N HCl–MeOH was attempted and **5** was yielded according to the expectation. The determination of acetylated positions in **1** was performed by analysis of the NMR spectra as follows. In the ^{1}H-NMR spectrum of **1** (Table I), the signals of H-2″ (δ 5.56 ppm) and H-6″ (δ 4.43 and 3.97 ppm) were shifted to downfield compared with those of **5**. Thus, two acetyl signals at δ 1.77 and 2.02 ppm were assigned to 2″- and 6″-*O*-acetyl groups, respectively. The assignment of 2″-*O*-acetyl to δ 1.77 ppm was based on the upfield shift due to shielding by A-ring of the flavone nucleus. In the aglycone moiety, two singlets due to one proton each at δ 6.47 and 6.70 ppm were attributed respectively to H-8 and H-3 of **5** type flavone,[4a] and the signals at δ 7.42, 6.91 and 7.43 ppm were respectively assigned to H-2′, H-5′ and H-6′ on B-ring from their coupling constants. The anomeric proton was observed at δ 4.81 ppm as doublet with J=10 Hz to indicate β-configuration for glycosidic linkage. In the ^{13}C-NMR spectrum of **1** (Table II), the signals due to the aglycone moiety agreed with those of **5**, and two acetyl signals were observed as two carbonyl signals (δ 168.9 and 170.3 ppm) and two methyl signals (δ 20.5 and 20.6 ppm). Complete assignments of the carbon signals in the sugar moiety by the selective proton decoupling experiments revealed that the carbon signals of C-2″ and C-6″ were shifted to downfield and the signals of their α-position carbons (C-1″, C-3″ and C-5″) were shifted to upfield compared with those of **5**. These findings indicate that compound **1** is 2″,6″-di-*O*-acetylisoorientin.

Compound **2**: Yellow prisms, mp 175—176 °C, and $[\alpha]_D^{21}$ +36.2° (MeOH); was determined to be 2″,6″-di-*O*-acetylorientin as follows. A positive coloration was



1: R^1=Ac, R^2=Ac
3: R^1=Ac, R^2=H
5: R^1=H, R^2=H

2: R^1=Ac, R^2=Ac
4: R^1=Ac, R^2=H
6: R^1=H, R^2=H

7: R=α-arabinofuranosyl
8: R=β-galactopyranosyl

Chart 1

exhibited in the Mg–HCl test. The UV spectrum with maxima at 348, 290 sh, 268 and 257 nm, and the bathochromic shifts with typical test reagents agreed very well with those of **6**[5] (see Experimental). FAB-MS of **2** gave an $[(M+H)^+]$ ion peak at m/z 533 (448, orientin +84, Ac_2). Further, since the NMR data of **2** were similar to those of **1** in the sugar moiety, **2** was suggested to be a diacetate of **6**. As expected, hydrolysis of **2** with 2 N HCl–MeOH gave **6**. In the ^{1}H-NMR spectrum of **2** (Table I), the signals of H-2″ (δ 5.37 ppm) and H-6″ (δ 4.47 and 4.12 ppm) were shifted to downfield compared with those of **6** (δ3.2—3.9 ppm), and two acetyl signals were observed at δ 1.72 and 1.93 ppm. The signal further upfield (δ 1.72 ppm) was assigned to 2″-*O*-acetyl groups due to shielding by the A rings of the flavone nucleus and the other acetyl signal (δ 1.93 ppm) to 6″-*O*-acetyl group.[7] In the aglycone moiety, two singlets due to one proton each at δ 6.27 and 6.70 ppm were attributed respectively to H-6 and H-3 of **6** type flavone,[4a] and the signals at δ 7.50, 6.95 and 7.55 ppm were respectively assigned to H-2′, H-5′ and H-6′ on B-ring from their coupling constants. The anomeric proton was observed at δ 4.92 ppm as doublet with J = 10 Hz

Table I. ^{1}H-NMR Spectral Data for **1**—**6**

	1	2	3	4	5	6
Aglycone moiety						
H-3	6.70 (s)	6.70 (s)	6.68 (s)	6.66 (s)	6.70 (s)	6.69 (s)
H-6	—	6.27 (s)	—	6.24 (s)	—	6.31 (s)
H-8	6.47 (s)	—	6.45 (s)	—	6.55 (s)	—
H-2′	7.42 (d, J=2)	7.50 (s)	7.40 (d, J=2)	7.53 (d, J=2)	7.42 (s)	7.50 (d, J=2)
H-5′	6.91 (d, J=8)	6.95 (d, J=8)	6.89 (d, J=8)	6.89 (d, J=8)	6.94 (d, J=8)	6.91 (d, J=8)
H-6′	7.43 (dd, J=8, 2)	7.55 (d, J=8)	7.41 (dd, J=8, 2)	7.59 (dd, J=8, 2)	7.44 (d, J=8)	7.55 (dd, J=8, 2)
OH (5)	13.65 (s)	13.22 (s)	13.60 (s)	13.19 (s)	13.60 (s)	13.18 (s)
Sugar moiety						
H-1″	4.81 (d, J=10)	4.92 (d, J=10)	4.77 (d, J=10)	4.84 (d, J=10)	4.62 (d, J=9)	4.71 (d, J=10)
H-2″	5.56 (t, J=10)	5.37 (t, J=10)	5.51 (t, J=10)	5.28 (t, J=10)	4.08 (t, J=9)	3.2—3.9[a]
H-3″	3.2—3.6[a]	3.2—3.9[a]	3.2—3.8[a]	3.2—3.9[a]	3.2—3.9[a]	3.2—3.9[a]
H-4″	3.2—3.6[a]	3.2—3.9[a]	3.2—3.8[a]	3.2—3.9[a]	3.2—3.9[a]	3.2—3.9[a]
H-5″	3.2—3.6[a]	3.2—3.9[a]	3.2—3.8[a]	3.2—3.9[a]	3.2—3.9[a]	3.2—3.9[a]
H-6″	4.43 (d, J=11)	4.47 (d, J=12)	3.2—3.8[a]	3.2—3.9[a]	3.2—3.9[a]	3.2—3.9[a]
	3.97 (dd, J=11, 6)	4.12 (dd, J=12, 5)				
Ac (2″)	1.77 (s)	1.72 (s)	1.75 (s)	1.69 (s)	—	—
Ac (6″)	2.02 (s)	1.93 (s)	—	—	—	—

Measured in DMSO-d_6 (added trace amounts of D_2O) at 270 MHz with TMS as the internal standard, δ = ppm, J = Hz. The following abbreviations are used: s, singlet; d, doublet; dd, double doublet; t, triplet. *a*) Overlapped with other proton signals of sugar moiety.

Table II. ^{13}C-NMR Spectral Data for **1**—**6**

	1	2	3	4	5	6
Aglycone moiety						
C-2	163.7	163.9	163.7	164.0	163.6	164.0
C-3	102.8	101.9	102.8	102.2	102.8	102.5
C-4	181.7	181.8	181.7	181.8	181.8	181.9
C-4a	102.9	102.4	102.9	102.3	103.3	104.0
C-5	160.0	160.9	160.6	160.7	160.2	160.0
C-6	106.5	97.8	106.8	97.7	108.5	97.9
C-7	162.4	162.4	163.1	162.2	163.0	162.2
C-8	93.2	103.8	93.3	103.8	93.6	104.4
C-8a	156.4	156.4	156.3	156.3	156.2	156.0
C-1′	121.3	121.9	121.3	121.8	121.4	122.0
C-2′	113.3	113.9	113.2	113.9	113.1	113.8
C-3′	145.6	145.9	145.6	145.7	145.4	145.5
C-4′	149.6	149.7	149.7	149.6	149.4	149.3
C-5′	116.0	115.6	115.9	115.6	116.0	115.6
C-6′	119.0	118.9	118.9	119.3	119.0	119.4
Sugar moiety						
C-1″	70.2 (−2.7)[a]	70.9 (−2.4)[b]	70.4 (−2.5)[a]	70.8 (−2.5)[b]	72.9	73.3
C-2″	71.7 (+1.4)	72.1 (+1.5)	72.0 (+1.7)	72.3 (+1.7)	70.3	70.6
C-3″	75.7 (−2.9)	75.4 (−3.0)	76.0 (−2.6)	75.7 (−2.7)	78.6	78.4
C-4″	70.1	70.3	70.4	70.5	70.0	70.5
C-5″	78.0 (−3.3)	78.5 (−3.6)	81.6	82.1	81.3	81.7
C-6″	64.1 (+2.9)	63.9 (+2.5)	61.2	61.2	61.2	61.4
2″-OCOMe	168.9	169.1	168.8	169.0	—	—
2″-OCOMe	20.5	20.3	20.5	20.3	—	—
6″-OCOMe	170.3	170.4	—	—	—	—
6″-OCOMe	20.6	20.5	—	—	—	—

Measured in DMSO-d_6 at 67.8 MHz with TMS as the internal standard. Assignments of C-1″, C-2″ and C-4″ were made with the aid of selective proton decoupling experiments. Values in parentheses indicate $\Delta\delta$ from *a*) **5** and *b*) **6**.

to indicate β-configuration for glycosidic linkage. In the ^{13}C-NMR spectrum of **2** (Table II), the chemical shifts of carbon signals in the aglycone moiety and sugar moiety were consistent with those of **6** and **1**, respectively, indicating two acetyl groups at 2″ and 6″ positions of glucose moiety in **6**.

Compound **3**: Yellow needles, mp 209—210 °C, and $[\alpha]_D^{22}$ +11.4° (MeOH); gave positive Mg–HCl test. The UV spectrum showing maxima at 351, 270, 258 and 243 sh nm, and the bathochromic shifts with typical test reagents agreed very well with those of **5**[5] (see Experimental). FAB-MS of **3** showed an $[(M+H)^+]$ ion peak at *m/z* 491 (448, isoorientin +42, Ac). In the ^{1}H-NMR spectrum of **3** (Table I), an acetyl signal was observed at δ 1.75 ppm, and the downfield shift was observed by a triplet of H-2″ at δ 5.51 ppm in comparison with **5**. The doublet with $J=10$ Hz at δ 4.77 ppm was assigned as an anomeric proton to indicate β-configuration for glycosidic linkage. The observed chemical shifts of protons in aglycone moiety were very similar to those of **1** and **5**. Thus, the signals at δ 6.68, 6.45, 7.40, 6.89 and 7.41 ppm were assigned to H-3, H-8, H-2′, H-5′ and H-6′, respectively. In the ^{13}C-NMR of **3** (Table II), the carbon signals of the sugar moiety were observed at the similar chemical shifts to those of **4**. There, the signal of C-2″ was shifted to downfield, and the signals of C-1″ and C-3″ were shifted to upfield compared with those of **5**, because of an acetyl on C-2″. The signals of the aglycone moiety were observed at the similar chemical shifts to those of **5**. The structure of **3** was thus deduced as 2″-*O*-acetylisoorientin.

Redaelli and co-workers reported 2″- and 6″-*O*-acetyl-7-*O*-glucopyranosylapigenin, whose acetylation shifts of C-2″ and C-6″ are +3.0 and +2.4 ppm, respectively, in the ^{13}C-NMR spectra.[9] As shown in Table II, the acetylation shifts of C-2″ and C-6″ of **1**—**4** were +1.4 to +1.7 ppm and +2.5 to +2.9 ppm, respectively. It is interesting that those shifts of acetylated C-2″ in *C*-glycosides (**1**—**4**) were smaller than that of *O*-glycoside.[10]

Experimental

All the melting points were taken on a Yanagimoto micromelting-point apparatus and are uncorrected. The UV spectra were recorded on a Hitachi U-3200 spectrophotometer and FAB-MS's were measured with a JEOL DX-300 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL GX-270 spectrometer, using tetramethylsilane (TMS) as the internal standard. The optical rotations were determined on a JASCO J-20A spectropolarimeter. Column chromatography was carried out with silica gel, polyamide (Wako gel C-200, Polyamide C-200; Wako Pure Chemical Industry, Ltd.) and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd.).

Extraction and Isolation The aerial parts of *Rumex acetosa* L. were collected at Saitama prefecture in May 1989. The fresh leaves (7 kg) were extracted with MeOH (20 l × 3) at room temperature. The MeOH extract was concentrated *in vacuo* to give a dark green mass, which was then dissolved in H_2O. The H_2O solution was extracted with *n*-hexane, AcOEt and *n*-BuOH, successively. The AcOEt solution was concentrated to give a dark mass (20 g), which was then chromatographed on SiO_2 with $CHCl_3$–MeOH mixture (gradient up to 20%) to give fractions 1—20. Fractions 1—5 (0 to 0.5% MeOH–$CHCl_3$) (*ca.* 10 g) formed an oily dark green mass containing anthraquinones and other unidentified components. Fraction 6 (5% MeOH–$CHCl_3$) (0.5 g) and fraction 7 (5% MeOH–$CHCl_3$) (0.7 g) were each chromatographed on polyamide with a mixture of benzene, MeOH, 2-butanone and H_2O (55 : 20 : 22 : 3) (solvent A) to give **1** (70 mg) and **2** (50 mg), respectively. Fraction 10 (5% MeOH–$CHCl_3$) (0.6 g) was chromatographed on Sephadex LH-20 eluting with MeOH after chromatography on polyamide with solvent A to give **3** (55 mg), and **4** (77 mg). The latter (**4**) was obtained as pale yellow needles with mp 210—212 °C (MeCN–H_2O) and gave FAB-MS *m/z*: 491 $(M+H)^+$. UV and NMR data of **4** were consistent with those reported for 2″-*O*-acetylorientin.[3] Fractions 17—19 (20% MeOH–$CHCl_3$) (0.8 g) were chromatographed repeatedly on polyamide with solvent A and benzene–MeOH (1 : 1), respectively, to give **5** (65 mg) and **6** (68 mg).

The Former (**5**): Yellow needles, mp 235—237 °C, and FAB-MS *m/z*: 449 $(M+H)^+$; was identified as isoorientin by the comparison of spectral data with that reported in the literature.[4,5]

The Latter (**6**): Yellow needles, mp 275—277 °C, and FAB-MS *m/z*: 449 $(M+H)^+$; was identified as orientin by direct comparisons of UV, ^{1}H- and ^{13}C-NMR spectra with those of an authentic sample. Avicularin (quercetin 3-*O*-α-L-arabinofuranoside) (**7**) (200 mg), mp 117—118 °C, was obtained by polyamide chromatography (benzene–MeOH, 1 : 1) from fraction 8 (5% MeOH–$CHCl_3$) and hyperin (quercetin 3-*O*-β-D-galactopyranoside) (**8**) (60 mg), mp 240—242 °C, was obtained by the trituration with MeOH from fraction 12 (10% MeOH–$CHCl_3$). These flavonol *O*-glycosides were identified by direct comparisons with the authentic samples.[6] The other fractions are under study.

Acidic Hydrolysis The solution of **1** or **2** (each *ca.* 10 mg) in 2 N HCl–MeOH (2 : 1, 3 ml) was heated for 1 h in a steam bath, then cooled with ice-water to yield yellow precipitates, which gave pale yellow powders (*ca.* 60% and 50% from **1** and **2**, respectively) when washed with cold water. These products from **1** and **2** were respectively identical with **5** and **6**, by direct comparison using thin layer chromatography, high performance liquid chromatography and ^{1}H-NMR.

2″,6″-Di-*O*-acetylisoorientin: Luteolin 6-*C*-(2″,6″-Di-*O*-acetyl)-β-D-glucopyranoside (1) Recrystallization (MeCN–H_2O) gave yellow needles, mp 180—182 °C, and $[\alpha]_D^{22}$ +4.8° (*c*=0.42, MeOH). UV λ_{max}^{MeOH} nm (log ε): 243 sh (4.21), 258 (4.25), 270 (4.26), 350 (4.34); $\lambda_{max}^{MeOH+AlCl_3}$ nm: 277, 303 sh, 335, 419; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 264 sh, 277, 297, 362, 389; $\lambda_{max}^{MeOH+NaOMe}$ nm: 270, 279 sh, 337 sh, 400; $\lambda_{max}^{MeOH+NaOAc}$ nm: 269, 396. FAB-MS *m/z*: 533 $(M+H)^+$. The ^{1}H- and ^{13}C-NMR data are shown in Tables I and II, respectively. *Anal.* Calcd for $C_{25}H_{24}O_{13}\cdot 1/2H_2O$: C, 55.46; H, 4.65. Found: C, 55.23; H, 4.86.

2″,6″-Di-*O*-acetylorientin: Luteolin 8-*C*-(2″,6″-Di-*O*-acetyl)-β-D-glucopyranoside (2) Recrystallization (MeCN–H_2O) gave yellow prisms, mp 175—176 °C, $[\alpha]_D^{21}$ +36° (*c*=0.69, MeOH). UV λ_{max}^{MeOH} nm (log ε): 257 (4.22), 268 (4.20), 290 sh (3.94), 348 (4.27); $\lambda_{max}^{MeOH+AlCl_3}$ nm: 275, 303 sh, 333, 422; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 265 sh, 276, 298, 361, 386; $\lambda_{max}^{MeOH+NaOMe}$ nm: 269, 279 sh, 329 sh, 400; $\lambda_{max}^{MeOH+NaOAc}$ nm: 268, 393. FAB-MS *m/z*: 533 $(M+H)^+$. The ^{1}H- and ^{13}C-NMR data are shown in Tables I and II, respectively. *Anal.* Calcd for $C_{25}H_{24}O_{13}\cdot H_2O$: C, 54.54; H, 4.76. Found: C, 54.75; H, 4.77.

2″-*O*-Acetylisoorientin: Luteolin 6-*C*-(2″-*O*-Acetyl)-β-D-glucopyranoside (3) Recrystallization (MeOH–H_2O) gave yellow needles, mp 209—210 °C, $[\alpha]_D^{22}$ +11.4° (*c*=0.35, MeOH). UV λ_{max}^{MeOH} nm (log ε): 243 sh (4.20), 258 (4.24), 270 (4.25), 351 (4.33); $\lambda_{max}^{MeOH+AlCl_3}$ nm: 276, 303 sh, 335, 426; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 264 sh, 276, 297, 366, 388; $\lambda_{max}^{MeOH+NaOMe}$ nm: 269, 279 sh, 335 sh, 400; $\lambda_{max}^{MeOH+NaOAc}$ nm: 268, 396. FAB-MS *m/z*: 491 $(M+H)^+$. The ^{1}H- and ^{13}C-NMR data are shown in Tables I and II, respectively. *Anal.* Calcd for $C_{23}H_{22}O_{12}\cdot 2H_2O$: C, 52.47; H, 4.79. Found: C, 52.42; H, 4.59.

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7) In the ^{1}H-NMR spectrum of vitexin peracetate (5,7,4′,2″,3″,4″,6″-hepta-*O*-acetylglucopyranosylapigenin), the acetyl signal at the furthest upfield (δ 1.74 ppm) and the acetyl signal at the next furthest upfield (δ 1.91 ppm) were assigned to 2″- and 6″-*O*-acetyl groups

based on the shielding by A and B rings, respectively, of the flavone nucleus.[8)]

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