Violarvensin, a New Flavone Di-C-glycoside from *Viola arvensis*

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A new flavonoid di-C-glycoside, violarvensin (**1**), was isolated from the aerial parts of *Viola arvensis*, together with the known derivative violanthin (**2**). The structure of **1** was established as apigenin-6-C-β-D-glucopyranosyl-8-C-β-D-6-deoxygulopyranoside by spectral analysis.

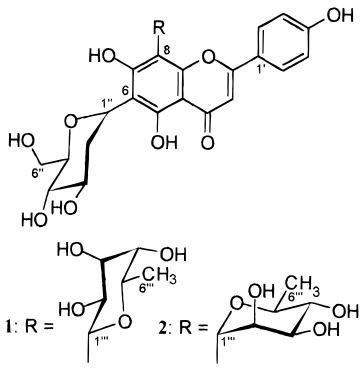
The wild pansy *Viola arvensis* Murray (Violaceae), closely related to *Viola tricolor* L., is a very common species found in open and cultivated land in Europe.[1] It was described by early authors as a variety or subspecies of *V. tricolor*.[2] In traditional medicine, the herb has been used as an expectorant, a diuretic, and an antiinflamatory for bronchitis, rheumatism, skin eruptions, and eczema. Its properties are ascribed to the presence of mucilage, salicylic derivatives, and flavonoids.[3] The main flavonoid in *V. arvensis* was reported to be rutin.[4] This compound[5] and also several flavone C-glycosides—violanthin,[6] vitexin, saponaretin, orientin, isoorientin, and vicenin-2,[7]—were found in *V. tricolor*.

As part of an on-going study of French medicinal plants, we report here the isolation of a new apigenin di-C-glycoside, together with the known flavonoid, violanthin, from *V. arvensis*. The structure of the new compound (**1**), which was named violarvensin, was determined from spectroscopic evidence.

The MeOH extract of the aerial parts of *V. arvensis* was chromatographed on a polyamide column, and the flavonoid-containing fractions were further purified on a Sephadex LH-20 column to afford compounds **1** and **2**. Compound **2** was identified as violanthin (apigenin-6-C-β-D-glucopyranosyl-8-C-α-L-6-rhamnopyranoside) by comparison of its ^{13}C-NMR spectra with literature data.[8]

Like that of **2**, the UV spectrum of **1** showed characteristic flavone absorptions at 272 and 336 nm, and **1** was resistant to acidic hydrolysis (HCl 2 N, 100 °C, 2 h), suggesting a C-glycoside structure.

The FABMS of **1** showed an ion peak at *m/z* 579 [M + H]$^+$ in the high mass region, corresponding to a molecular weight of 578. The molecular formula $C_{27}H_{31}O_{14}$ was deduced from the pseudomolecular ion at *m/z* 579.1714 on the HRFABMS.



The ^{1}H-NMR spectrum of compound **1** confirmed the presence of a flavone skeleton characterized by two doublet signals at δ 6.89 and 8.09 assigned to H-3′, H-5′ and H-2′, H-6′, respectively, and a singlet at δ 6.77 (H-3) (Table 1). In addition, the absence of signals for H-6 and H-8, the presence of two anomeric sugar protons at δ 4.70 and 5.56, and the ^{13}C chemical shift of anomeric carbons (δ 73.6 and 63.6) indicated a 6,8-di-C-glycosyl apigenin structure.

In the HMBC spectrum of **1**, correlations were observed between H-1″ and C-5, C-6, and C-7 and between H-1‴ and C-7, C-8, and C-9; thus, the two sugar moieties were attached to C-6 and C-8, respectively, as in **2**. The sugars were identified from COSY, TOCSY, HMQC, and HMBC spectra in conjunction with the magnitude of the ^{1}H−^{1}H vicinal coupling constants. As for **2**, the NMR data obtained for **1** showed that glucose was bound to C-6. For the second hexose residue, the anomeric proton at δ 5.56 was coupled to a proton at δ

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Table 1. NMR Data (DMSO-d_6) for **1** and **2**

	1				**2**		
	δ_C	δ_H	multiplicity[a]		δ_C	δ_H	multiplicity[a]
apigenin				apigenin			
2	163.6			2	163.3		
3	102.2	6.77	s	3	102.7	6.81	s
4	182.2			4	182.2		
5	159.3			5	160.0		
6	108.4			6	109.1		
7	163.6			7	162.5		
8	104.4			8	102.7		
9	154.7			9	153.1		
10	102.4			10	103.0		
1′	121.4			1′	121.2		
2′	129.0	8.09	d (8.4)	2′	128.5	7.91	d (8.5)
3′	116.0	6.89	d (8.4)	3′	116.0	6.95	d (8.5)
4′	161.2			4′	161.3		
5′	116.0	6.89	d (8.4)	5′	116.0	6.95	d (8.5)
6′	129.0	8.09	d (8.4)	6′	128.5	7.91	d (8.5)
6-C-glucosyl				6-C-glucosyl			
1″	73.6	4.70	d (9.5)	1″	73.1	4.62	d (9.8)
2″	70.9	3.90	dd (9.5; 9.5)	2″	70.1	4.08	dd (9.8; 9.0)
3″	78.7	3.26	dd (9.5; 9.5)	3″	79.0	3.21	dd (9.0; 9.0)
4″	70.1	3.25[b]		4″	70.7	3.12	dd (9.0; 9.0)
5″	81.5	3.25[b]		5″	81.7	3.16	ddd (9.0; 4.0; 1.2)
6″	60.9	3.70	dd (11.2; < 1)	6″	61.5	3.70	dd (11.2; 1.2)
		3.52	dd (11.2; 4.5)			3.42	dd (11.2; 4.0)
8-C-6-deoxygulosyl				8-C-rhamnosyl			
1‴	63.6	5.56	d (10.0)	1‴	75.0	5.26	s
2‴	65.9	4.24	dd (10.0; 2.0)	2‴	72.3	3.90	d (2.6)
3‴	71.6	3.94	dd (2.0; 4.0)	3‴	74.2	3.60	dd (2.6; 9.0)
4‴	72.0	3.71[c]	d (4.0)	4‴	71.8	3.40	dd (9.0; 9.0)
5‴	74.8	4.04[c]	q (7.2)	5‴	77.3	3.45	dq (9.0; 6.0)
6‴	16.2	1.44	d (7.2)	6‴	18.2	1.28	d (6.0)

[a] Coupling constants J (in Hz) are in parentheses. [b] Signal patterns unclear due to overlapping. [c] J (4‴−5‴) # 0.

4.24 (dd, J = 10.0, 2.0 Hz), corresponding to H-2‴. In the COSY spectrum, a cross peak was observed between H-2‴ and a resonance at δ 3.94 (dd, J = 2.0, 4.0 Hz). On the basis of the large coupling constant (10.0 Hz) between H-1‴ and H-2‴, the two protons were established as axial and the configuration of the sugar as β. On the other hand, the configuration of H-3‴ was indicated to be equatorial. The correlations observed between H-3‴ and a resonance at δ 3.71 (d, J = 4.0 Hz), between the resonance at δ 3.71 and 4.04 (q, J = 7.2 Hz) and between the resonance at δ 4.04 and 1.44 (d, J = 7.2 Hz) permitted the assignments of H-4‴, H-5‴, and Me-6‴, respectively. Thus, a 6-deoxy-β-hexose is proposed.

Acetylation of **1** gave a decaacetate, the NMR spectrum of which showed large downfield shifts of the H-2‴, H-3‴, and H-4‴ protons of the 6-deoxy-β-hexose unit. The chemical shifts of H-3‴, H-4‴, and H-5‴ and the magnitude of all vicinal proton coupling constants are similar to those reported for 1,2,3,4-tetra-*O*-acetyl-6-deoxy-β-D-gulopyranoside.[9] Thus the second hexose in **1** was identified as 6-deoxy-β-D-gulopyranoside, and the structure of **1** was determined, in accordance with the molecular formula deduced from the HRFABMS, to be apigenin-6-C-β-D-glucopyranosyl-8-C-β-D-6-deoxygulopyranoside.

Although the overall ^{13}C-NMR data obtained for **2** are similar to those reported in the literature, the reassignment of three chemical shifts is proposed on the basis of COSY and HMQC experiments. The correct assignments for C-1‴, C-2‴, and C-5‴ are δ 75.0, 72.3, and 77.3, respectively. Literature values[8] were given as δ 77.3, 75.0, and 72.2, respectively.

This is the first report of a flavonoid glycoside containing 6-deoxy-D-gulose in its sugar moiety. This sugar, also known as antiarose, rarely occurs in the plant kingdom, being previously described only in some cardenolide glycosides from *Antiaris toxicaria*,[10] *Convallaria majalis*,[11] and *Cheiranthus allioni*,[12] and as a glycolipid constituent of *Ipomoea parasitica*.[13]

Experimental Section

General Experimental Procedures. Column chromatography was carried out on a polyamide column chromatograph (ICN) and Sephadex LH-20 (Pharmacia). Elution was monitored by TLC (cellulose F_{254} Merck) using 15% AcOH as solvent system and observation under UV at 365 nm after spraying with 2-aminoethyldiphenylborinate solution in MeOH. UV spectra were recorded on a Unicam model UV2 UV/vis spectrophotometer in MeOH. Optical rotation was measured on a JASCO model DIP 370 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AMX 360 spectrometer operating at 360.13 MHz (^{1}H) and 90.55 (^{13}C) using TMS as internal standard. Positive FABMS were obtained on a JEOL JMS SX-102 mass spectrometer.

Plant Material. The aerial parts of *V. arvensis* were collected near Orcines (Puy-de-Dôme, France) in September 1995. The plant was identified by Prof. J.-L. Lamaison, and a voucher specimen was deposited in the herbarium of the Faculty of Pharmacy, University of Auvergne.

Extraction and Isolation. The dried, powdered aerial parts with flowers (100 g) were extracted with

MeOH (2 × 500 mL). The concentrated extract (30 g) was dissolved in distilled H_2O (100 mL) and chromatographed on a polyamide column (500 g). Elution with H_2O gave 15 fractions of 250 mL (1−15) each. Podded fractions 12−14 (80 mg) were chromatographed on two successive columns on Sephadex LH-20 (50 g) with H_2O as elution solvent, giving 17 mg of **1**. Combined fractions 5−7 (110 mg) were purified analogously on Sephadex LH-20, giving 21 mg of **2**.

Violarvensin (1): yellow amorphous powder; R_f 0.45; $[\alpha]^{25}_D$ +14° (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 272 (4.09), 336 (4.37); positive FABMS *m/z* $[M + H]^+$ 579, $[M + Na]^+$ 601; HRFABMS $[M + H]^+$ 579.1714 (calcd for $C_{27}H_{31}O_{14}$, 579.1714); ^{1}H- and ^{13}C-NMR data, see Table 1.

Acetylation of 1. A solution of **1** (3 mg) in a mixture of Ac_2O (0.5 mL) and pyridine (0.5 mL) was allowed to stand at room temperature overnight and evaporated to dryness *in vacuo* to yield violarvensin decaacetate: ^{1}H NMR ($CDCl_3$) δ 7.98 (2H, H-2′, H-6′, d, J = 8.7 Hz), 7.24 (2H, H-3′, H-5′, d, J = 8.7 Hz), 6.50 (1H, H-3, s), 5.85 (1H, H-2‴, dd, J = 10.2, 2.9 Hz), 5.68 (1H, H-2″, dd, J = 9.7, 9.7 Hz), 5.44 (1H, H-3‴, dd, J = 2.9, 3.2 Hz), 5.31 (1H, H-3″, dd, J = 9.7, 9.7 Hz), 5.27 (1H, H-1‴, d, J = 10.2 Hz), 5.16 (1H, H-4″, dd, J = 9.7, 9.7 Hz), 4.89 (1H, H-1″, d, J = 9.7 Hz), 4.88 (1H, H-4‴, d, J = 3.2 Hz), 4.45 (1H, H-6″, dd, J = 12.7, 5.0 Hz), 4.28 (1H, H-5‴, q, J = 7.2 Hz), 3.96 (1H, H-6″, dd, J = 12.7, 1.5 Hz), 3.82 (1H, H-5″, ddd, J = 9.7, 5.0, 1.5 Hz), 1.51 (3H, H-6‴, d, J = 7.2 Hz), 2.48, 2.32, 2.22, 2.06, 2.05, 2.04, 2.01, 1.89, 1.84, and 1.76 (each 3H, s, −OAc × 10).

Violanthin (2): yellow amorphous powder; R_f 0.55; UV (MeOH) λ_{max} (log ϵ) 272 (4.16), 336 (4.45); ^{1}H- and ^{13}C-NMR data, see Table 1.

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