# FLAVONOIDS FROM THE AERIAL PART OF *Vicia subvillosa*

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*The new flavone glucoside viscioside, luteolin-4′*-O-β-*D-galactopyranoside, in addition to the known flavonoids apigenin, luteolin, quercetin, cinaroside, luteolin-4′*-O-β-*D-glucoside, and isoquercitrin were isolated from the aerial part of* Vicia subvillosa.

**Key words:** *Vicia subvillosa*, apigenin, luteolin, quercetin, cinaroside, luteolin-4′-*O*-*β*-D-glucoside, isoquercitrin, luteolin-4′-*O*-*β*-D-galactopyranoside.

Plants of the genus *Vicia* (Fabaceae) contain flavonoids, anthocyans, carotinoids, and vitamins. Some of them are used in folk medicine for ascites, paralyses, epilepsy, flu, and cholic [1, 2].

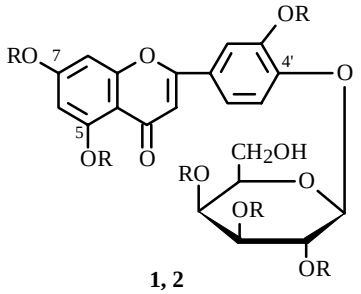
*Vicia subvillosa* (Ledeb.) Trautv. is a perennial indigenous to steppes and sometimes alkaline areas and foothills of Central Asia [3].

Herein we report results of a study of flavonoids from the aerial part of *V. subvillosa* collected during flowering (April 1997) near the Alimtau mountains (Chimkentsk Oblast, Republic of Kazakhstan). Column chromatography of various fractions of the ethanol extract isolated a new flavone glycoside (**1**) of composition $C_{21}H_{20}O_{11}$, which we called viscioside, in addition to the known flavonoids apigenin [4, 5], luteolin [4, 6], quercetin [7, 8], cinaroside [4, 7], luteolin-4′-*O*-*β*-D-glucoside [9], and isoquercitrin [7, 8].

The UV spectrum of **1** was characteristic of flavone derivatives [5, 10].

The IR specrum of **1** had absorption bands for hydroxyls, γ-pyrone carbonyls, aromatic C=C bonds, and glycoside C–O vibrations.

The PMR spectrum of **1** in DMSO-$d_6$ exhibited signals for five aromatic protons, H-3, an anomeric proton (H-1″), and phenolic hydroxyls on C-5 and C-7.




**1, 2**

**1**: R = H; **2**: R = $COCH_3$

The chromatic mobility and IR and PMR spectra indicated that **1** was a glycoside. Acid hydrolysis of **1** to produce luteolin (5,7,3′,4′-tetrahydroxyflavone) [6, 7] and D-galactose confirmed this.

Acetylation of **1** by acetic anhydride in pyridine produced the heptaacetate derivative **2** of formula $C_{35}H_{34}O_{18}$ ($[M]^+$ 742).

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A bathochromic shift of UV band I of **1** upon addition of sodium acetate and a proton signal at 10.85 ppm in the PMR spectrum indicated that **1** had a free hydroxyl in the 7-position [5, 10].

The site of attachment of the carbohydrate to the aglycon was found by comparing the UV spectra of **1** and luteolin that were recorded with ionizing and complexing additives [2, 5, 10].

The bathochromic shift of band I in the spectrum of **1** upon addition of $AlCl_3$ and $AlCl_3$/HCl (+46 nm) indicated that ring B did not have an ortho dihydroxy group. Band I in the UV spectrum of **1** underwent a bathochromic shift by 55 nm with a decreased intensity for the absorption maximum if $CH_3ONa$ was added. This indicated that the luteolin 4′-OH group was glycosylated in **1** [5, 10].

The signal for the anomeric proton of D-galactose in the PMR spectrum of **1** appeared at 4.90 ppm as a doublet with SSCC 7.0 Hz. This indicated that the glycosidic D-galactose had the *β*-configuration [5]. Therefore, **1** had the structure luteolin-4′-*O*-*β*-D-galactopyranoside.

Although luteolin 4′-*O*-arabinoside, 4′-*O*-glucoside, and 4′-*O*-glucuronide have been reported, the 4′-*O*-*β*-D-galactopyranoside has not [11].

Viscioside (**1**) is a new natural compound. Apigenin, luteolin, quercetin, cinaroside, luteolin-4′-*O*-*β*-D-glucoside, and isoquercitrin were isolated for the first time from *V. subvillosa*.

## EXPERIMENTAL

The solvent systems $CHCl_3$:$CH_3OH$ (19:1, 1; 9:1, 2; 85:15, 3) and *n*-butanol:pyridine:water (6:4:3, 4) were used. TLC used Silufol UV-254 plates. Column chromatography was performed over KSK silica gel of particle size 100/160 μm. Paper chromatography (PC) used Filtrak No. 12 chromatography paper.

Spots of flavonoids on TLC were developed using ammonia vapors and vanillin (1%) in conc. $H_2SO_4$. Monosaccharides were developed by spraying chromatograms with anilinium acid phthalate with subsequent heating at 90-100°C.

PMR spectra were recorded on a Tesla BS-567A instrument at working frequency 100 MHz (δ, ppm, 0 = HMDS). IR spectra were recorded on a Perkin—Elmer System 2000 FT—IR Fourier spectrophotometer in KBr disks; UV spectra, on a Perkin—Elmer Lambda 16 spectrophotometer. Melting points were determined on a Boetius stage with a PHMK 0.5 visual device.

**Extraction and Isolation of Flavonoids.** Air-dried ground aerial part of *V. subvillosa* (1.0 kg) was extracted eight times at room temperature with ethanol. The combined alcohol extract was condensed in vacuo to 0.7 L and diluted with water to 1.4 L. The aqueous alcohol extract was successively shaken with $CHCl_3$ (5 × 0.5 L), ethylacetate (8 × 0.5 L), and *n*-butanol (10 × 0.5 L). Solvents were distilled to produce chloroform (20.5 g), ethylacetate (14.0), and butanol (25.0) fractions.

The ethylacetate fraction (14.0 g) was chromatographed over a silica-gel (350 g) column (2.8 × 160 cm) using systems 1-3. Fractions of 400 mL were collected. Elution of the column by system 1 isolated apigenin (0.15 g), luteolin (0.24), and quercetin (0.22). Elution by system 2 afforded viscioside (0.10 g), cinaroside (0.19), and luteolin-4′-*O*-*β*-D-glucoside (0.16); by system 3, isoquercitrin (0.12).

**Apigenin** (5,7,4′-trihydroxyflavone), $C_{15}H_{10}O_5$, mp 347-348°C, $\lambda_{max}$ (ethanol): 270, 311 nm [4].

**Luteolin** (5,7,3′,4′-tetrahydroxyflavone), $C_{15}H_{10}O_6$, mp 328-330°C (dec.), $\lambda_{max}$: 260, 270, 356 nm [4, 6].

**Quercetin** (3,5,7,3′,4′-pentahydroxyflavone), $C_{15}H_{10}O_7$, mp 313-314°C, $\lambda_{max}$: 257, 268, 371 nm [7, 8].

**Cinaroside** (luteolin-7-*O*-*β*-D-glucopyranoside), $C_{21}H_{20}O_{11}$, mp 240-242°C (dec.), $\lambda_{max}$: 256, 268, 350 nm [4, 6].

**Luteolin-4′-*O*-*β*-D-glucopyranoside**, $C_{21}H_{20}O_{11}$, mp 187-190°C, $\lambda_{max}$: 271, 290, 342 nm [9].

**Isoquercitrin** (quercetin-3-*O*-*β*-D-glucopyranoside), $C_{21}H_{20}O_{12}$, mp 238-239°C, $\lambda_{max}$: 255, 266, 362 nm; +$CH_3COONa$: 274, 323, 374 nm [7, 8].

**Viscioside (1)** (luteolin-4′-*O*-*β*-D-galactopyranoside), $C_{21}H_{20}O_{11}$, mp 178-180°C, $\lambda_{max}$: 272, 292, 340 nm; +$CH_3ONa$: 273, 395; +$CH_3COONa$: 271, 370; +$AlCl_3$: 279, 351, 386; +$AlCl_3$/HCl: 280, 352, 385. IR spectrum ($\nu_{max}$, $cm^{-1}$): 3490-3310 (OH), 1660 (*γ*-pyrone C=O), 1575, 1520 (aromatic C=C), 1090, 1025 (glycoside C–O). PMR spectrum (DMSO-$d_6$, δ, ppm, J/Hz): 3.40-3.83 (sugar protons), 4.90 (1H, d, J = 7.0, H-1″), 6.21 (1H, d, J = 2.0, H-6), 6.52 (1H, d, J = 2.0, H-8), 6.85 (1H, s, H-3), 7.25 (1H, d, J = 8.5, H-5′), 7.50 (1H, d, J = 1.5, H-2′), 7.54 (1H, dd, J = 1.5, 8.5, H-6′), 10.85 (1H, s, 7-OH), 12.85 (1H, br.s, 5-OH).

**Acid Hydrolysis of 1.** A solution of **1** (25 mg) in HCl (5%, 20 mL) was boiled for 4 h. The resulting precipitate of the aglycon was filtered off and recrystallized from ethanol to afford luteolin (8 mg), mp 327-330°C. PC (system 4) of the hydrolysate identified D-galactose.

**Heptaacetate (2).** A solution of **1** (20 mg) in pyridine (1.5 mL) and acetic anhydride (4 mL) was held at room temperature for 4 h. The usual workup afforded the heptaacetate (24 mg), mp 118-120°C.

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