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NEW FLAVONOID GLYCOSIDE FROM *Thalictrum minus*

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The new flavonoid diglycoside thamiflaside was isolated from the aerial part of Thalictrum minus. *The chemical structure of this glycoside was determined as apigenin 7-*O*-α-L-2‴-methoxyrhamnopyranosyl-(1→6)-β-D-glucopyranoside based on PMR and* ^{13}*C NMR (DEPT, HSQC, HMBC) physicochemical methods.*

Keywords: flavonoid glycoside, *Thalictrum minus*, meadow rue, glucose, rhamnose, NMR spectra.

Plants of the genus *Thalictrum* are widely used in folk medicine owing to their rich composition that includes biologically active compounds such as alkaloids [1–6], flavonoids [7], triterpene glycosides and saponins [8–11], vitamins [12], steroids, and fatty [13] and organic acids [14].

Triterpene saponins [10] with antitumor and contraceptive activity [8, 9] were isolated from *T. minus* L. and *T. foetidum* L. growing in Irkutsk Region, Russia.

Little information is available on the chemical composition and structure of flavonoids from plants of this genus. Alkaloids [5] and cycloartane glycosides [11] were isolated for the first time from plants of this genus during research conducted at the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan.

T. minus plants were collected in Surxondaryo Region, Uzbekistan (Kyzylkum, Peter I ridge). The ground aerial part of the air-dried plant (6 kg) was exhaustively extracted with EtOH at room temperature. The obtained total extracted substances were successively extracted with hexane and $CHCl_3$ to remove nonpolar constituents and then with BuOH.

The BuOH extract was separated by column chromatography over silica gel. Compound **1** was isolated by elution with $CHCl_3$–EtOH–H_2O (70:23:1.5).

Compound **1** was a light-yellow powder of formula $C_{28}H_{32}O_{14}$, *m*/*z* 593.300 $[M + 1]^+$. The chemical structure of **1** was established using PMR and ^{13}C NMR spectral data and two-dimensional HSQC and HMBC experiments.

The PMR spectrum of **1** (Table 1) showed resonances characteristic of glycosylated flavones. The region of aromatic protons exhibited two 1H doublets at δ 6.40 and 6.77 ppm with spin–spin coupling constant (SSCC) J = 2 Hz and *meta* splitting that were attributed to H-6 and H-8. Resonances of two 2H doublets at δ 7.08 and 7.98 with SSCC J = 9 Hz and *ortho* splitting were indicative of a substituent in the 4′-position of ring *B*. The H-3 resonance appeared as a singlet at δ 6.88 ppm and confirmed that the isolated compound was a flavone. A 1H singlet at δ 12.86 ppm corresponded to a chelated OH on C-5. The weak-field ^{13}C NMR spectrum also had resonances for aromatic C atoms of the aglycon part of the molecule. The presence of these resonances indicated that apigenin was the aglycon [15].

The PMR spectrum of **1** contained additional resonances for 10 protons as multiplets at δ 3.10–3.62 ppm that were characteristic of carbohydrates. The anomeric protons resonated at δ 5.01 and 4.50 ppm as clear doublets with SSCC J = 7.22 and 1.6 Hz. The strong-field spectrum exhibited a doublet at δ 1.03 ppm that unambiguously indicated the presence of a methyl, meaning that one of the monosaccharides in the structure of the new compound was rhamnose. A characteristic methyl C resonance at strong field of 17.88 ppm in the ^{13}C NMR spectrum was also indicative of this.

Acid hydrolysis of the new flavonoid glycoside **1** produced apigenin. D-Glucose and L-rhamnose were identified by paper chromatography in the presence of authentic samples in the carbohydrate part of the acid-hydrolysis products.

^{13}C NMR and HSQC spectra of **1** revealed the presence of 28 resonances for eight quaternary, including one carbonyl, 17 methine, one methylene, and two methyl C atoms.

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TABLE 1. ^{1}H and ^{13}C NMR and HMBC Data of Thamiflaside (**1**) (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_H	δ_C	HMBC	C atom	δ_H	δ_C	HMBC
	Aglycon				*β*-D-Glc*p* residue		
2	–	163.96		1″	5.01 (d, J = 7.2)	99.96	7
3	6.88 (s)	103.83	2, 4	2″	3.56 (m)	75.70	4″
4	–	182.06		3″	3.20 (m)	73.12	2″
5	–	161.18		4″	3.10 (m)	69.63	
6	6.40 (d, J = 2.0)	99.68	5, 7	5″	3.25 (m)	76.30	
7	–	162.98		6″	3.37 (m); 3.81 (m)	66.15	
8	6.77 (d, J = 2.0)	94.82	7, 9		*α*-L-2‴-OCH_3-Rha*p* residue		
9	–	157.0		1‴	4.50 (d, J = 1.6)	100.58	2‴
10	–	105.49		2‴	3.40 (m)	70.79	1‴
1′	–	122.68		3‴	3.62 (m)	70.41	
2′, 6′	7.98 (2H, d, J = 9.0)	128.48	2, 4′	4‴	3.10 (m)	72.11	3‴, 6‴
4′	–	162.45		5‴	3.35 (m)	68.39	
3′, 5′	7.08 (2H, d, J = 9.0)	114.73	1′	6‴	1.03 (d, J = 6.2)	17.88	
OH	12.86 (s)			OCH_3	3.80 (s)	55.60	1‴

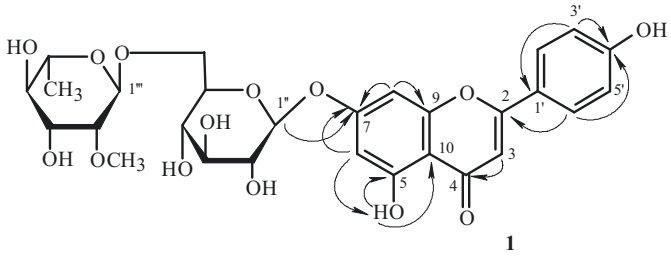

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Fig. 1. Chemical structure of thamiflaside (**1**) and key HMBC cross-peaks.

A proton resonance at δ 3.80 ppm as a singlet was consistent with a methoxyl in **1**. A resonance at δ 55.60 ppm in the ^{13}C NMR spectrum that was characteristic of methoxyl confirmed this conclusion.

Resonances for nine C atoms that were characteristic of carbohydrates were found in the range δ 66–76 ppm. Resonances for two anomeric C atoms at δ 99.96 (C-1″) and 100.58 (C-1‴) were also observed in the ^{13}C NMR spectrum.

The PMR, ^{13}C NMR, HSQC, and HMBC spectral data revealed resonances for one D-glucose and one L-rhamnose and indicated that **1** was an apigenin dioside. The chemical shifts and SSCCs of the D-glucose and L-rhamnose protons were consistent with the pyranose form of *β*-D-glucopyranosyl and the *α*-L-rhamnosyl configuration in **1**.

The positions of the monosaccharides in **1** were established using HMBC experiments and C-atom chemical shifts. The HMBC spectrum of **1** exhibited cross-peaks H-1″/C-7; H-3/C-2, 4; 5-OH/C-5, 6, 10; H-2′ and H-6′/C-2, 4′; H-8/C-7, 9; H-6/C-5, 7; and OCH_3/C-1‴. These facts also confirmed that the glucose was bonded to apigenin C-7 with the methoxyl on C-2‴ of the rhamnopyranose residue.

The resonance for D-glucose C-6 in the ^{13}C NMR spectrum experienced a glycosylation effect and was observed at δ 66.14 ppm, which indicated that it was bonded to L-rhamnose at this atom. A nonglycosylated D-glucose C-6 resonated at δ 62–63 ppm.

The structure of the new compound, which we called thamiflaside, was established as apigenin 7-*O*-*α*-L-2‴-methoxyrhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**) (Fig. 1).

EXPERIMENTAL

General Comments. NMR spectra were recorded in DMSO-d_6 with TMS internal standard (0 ppm) for ^{1}H on a Varian Unity plus 400 spectrometer at operating frequency 400 MHz. ^{13}C NMR spectra used the DMSO-d_6 chemical shift (39.52 ppm vs. TMS) as the standard. Mass spectrum was recorded using GC-MS on an Agilent 1200 Infinity HPLC (Agilent Technologies, USA) with a 6420 Triple Quad LC/MS mass detector (electrospray ionization + ESI TIC Scan, Agilent

Technologies, USA). The purity of the compounds was determined by TLC on Silufol plates (Merck, Germany) with detection by I_2 vapor, NH_3, and vanillin solution (1%) in conc. H_2SO_4. Paper chromatography (PC) used Filtrak FN 11 paper and *n*-BuOH–Py–H_2O (6:4:3). Free monosaccharides on PC were detected by spraying with anilinium acid phthalate. Melting points were determined on a MEL-TEMP apparatus. Column chromatography used KSK silica gel (50–100 μm).

Extraction and separation of *T. minus* (6 kg) used EtOH (5 × 70 L) at room temperature. The obtained total extracted substances were successively extracted with hexane and $CHCl_3$ to remove nonpolar constituents and then with BuOH. After the extractant was evaporated, the whole total sum of extracted substances consisted of $CHCl_3$ (31.86 g) and BuOH extracts (153.67 g).

The BuOH extract was worked up as before [8] to afford a purified sum of extracted substances. The BuOH extract was chromatographed by TLC using various solvent systems to detect two flavonoids. Then, column chromatography over silica gel separated the BuOH extract using sequential elution by $CHCl_3$, $CHCl_3$–EtOH (20:1, 10:1), and $CHCl_3$–EtOH–H_2O (70:23:1.5) to afford a fraction containing **1** (1.5 g, 0.025% yield of air-dried raw material weight).

Thamiflaside (1). $C_{28}H_{32}O_{14}$, pale-yellow powder, mp 264–268°C (MeOH). IR spectrum (KBr, ν_{max}, cm^{-1}): 3466 (OH), 1659 (C=O), 1353, 906 (OCH_3). Table 1 lists the PMR (400 MHz) and ^{13}C NMR spectral data (100 MHz).

Acid Hydrolysis. Thamiflaside (**1**, 10 mg) was hydrolyzed by aqueous H_2SO_4 (25 mL, 5%) for 6 h on a boiling-water bath. The precipitate was filtered off and identified as apigenin. The carbohydrate part of the hydrolysate was neutralized with $BaCO_3$ and KU-2(H^+) cation exchanger. The residue was evaporated. D-Glucose and L-rhamnose were identified by PC using *n*-BuOH–Py–H_2O (6:4:3).

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