Indzhikyan, *Zh. Obshch. Khim.*, 1989, **59**, 814 [*J. Gen. Chem. USSR*, 1989, **59** (Engl. Transl.)].
7. A. T. Babayan and A. G. Terzyan, *Dokl. Akad. Nauk Arm. SSR* [*Dokl. Acad. Sci. Arm. SSR*], 1948, **9**, 105 (in Russian).
8. J. Meisenheimer, J. Gasper, and M. Horing, *Ann.*, 1926, 449, 213.
9. A. T. Babayan and G. T. Martirosyan, *Dokl. Akad. Nauk Arm. SSR* [*Dokl. Acad. Sci. Arm. SSR*], 1960, **30**, 271 (in Russian).
10. A. T. Babayan and N. P. Gambaryan, *Izv. Akad. Nauk Arm. SSR, Ser. Fiz.-Mat. Tekhn. Nauk* [*Bull. Acad. Sci. Arm. SSR, Div. Phys.-Math. Sci.*], 1950, **3**, 563 (in Russian).

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Flavonoid glycosides from *Thalictrum squarrosum* St. ex Willd. and *Thalictrum minus* L.

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Two flavonoid allose diglycosides were found in the terrestrial part of *Thalictrum squarrosum* St. ex Willd. and *T. minus* L. (Ranunculaceae). 7,4′-Di-*O*-β-allopyranosylapigenin was isolated from *T. minus*. In *T. squarrosum*, its monoacetate was also found and characterized as 7-*O*-(6-*O*-acetyl-β-allopyranosyl)-4′-*O*-(β-allopyranosyl)apigenin. The sites of attachment of the carbohydrate residues were determined by HMBC; the location of the acetate group was identified by ROESY. Both substances were isolated from these plants for the first time.

Key words: *Thalictrum*, flavonoids, glycosides; ^{1}H and ^{13}C NMR spectroscopy, FAB MS.

The majority of plant species of the *Thalictrum* genus are widely used in folk and oriental medicine. As a continuation of the study of the chemical composition of *T. squarrosum* St. ex Willd.[1] and *T. minus* L.,[2] which grow in Siberia, we isolated two flavonoid glycosides. Previously, the flavonoids of *Th. squarrosum* St. ex Willd. have not been studied.

Results and Discussion

Both glycosides (**1** and **2**) were isolated from the butanol fractions of water-methanol extracts defatted preliminarily with chloroform.

Based on the chromatographic behavior, molecular mass (595.1625 $[M + H]^+$ for **1** and 637.1724 $[M + H]^+$ for **2**), and the data of IR and UV spectra, compounds **1** and **2** were identified as flavonoid diglycosides. Along with the carbonyl group of the γ-pyrone ring (1668 cm^{-1}), compound **2** contains an ester carbonyl group (1719 cm^{-1}).

Analysis of the ^{1}H and ^{13}C NMR and UV spectra (Table 1) of compounds **1** and **2** permitted the conclusion that 5,7,4′-trihydroxyflavone (apigenin) substituted at positions 7 and 4′ is the aglycone of both compounds.[3,4]

The data of FAB mass spectra (peaks of the ions $[M + Na - 162]^+$ with m/z 455, $[M + H - 162]^+$ with m/z 433, and $[M + H - 324]^+$ with m/z 271 for **1** and $[M + H - 42]^+$ with m/z 595, $[M + H - 162 - 42]^+$ with m/z 433, and $[M + H - 324 - 42]^+$ with m/z 271 for **2**) indicate that compounds **1** and **2** contain two hexose residues and, in addition, they confirm the presence of an acetate group in compound **2**.

The proton and carbon signals for the carbohydrate parts of both compounds were assigned using the COSY and HETCOR 2D procedures (see Table 1). The data obtained make it possible to conclude that in both compound **1** and compound **2**, apigenin is glycosylated by two identical hexose molecules, which are attached directly to the aglycone. The chemical shifts for the

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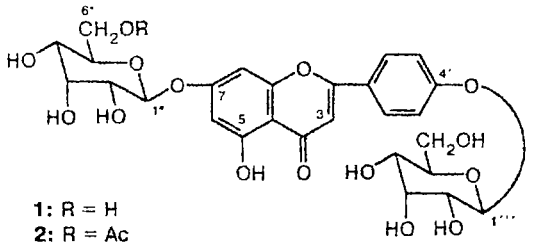
Table 1. NMR data for compounds **1** and **2**

Atom	δ ^{13}C		δ ^{1}H (*J*/Hz)		HMBC,
	1	**2**	**1**	**2**	**2**
C(2)	163.61	163.62	—	—	
C(3)—H	105.40	105.48	6.96 (s)	6.85 (s)	2, 6, 8, 1′
C(4)	182.04	182.05	—	—	
C(5)	157.02	157.00	—	—	8
C(6)—H	99.58	99.59	6.40 (d, *J* = 2.0)	6.43 (d, *J* = 2.1)	3, 7, 8, 9
C(7)	163.30	163.01	—	—	6, 8, H anom
C(8)—H	94.80	94.77	6.84 (d, *J* = 2.0)	6.80 (d, *J* = 2.1)	3, 5, 6, 7
C(9)	161.06	161.03	—	—	6
C(10)	104.02	104.02	—	—	
C(1′)	123.71	123.66	—	—	3, 3′, 5′
C(2′)—H	128.34	128.31	8.08 (br.d, *J* = 8.5)	8.02 (br.d, *J* = 8.8)	4′, 6′
C(3′)—H	116.55	116.50	7.17 (br.d, *J* = 9.0)	7.20 (br.d, *J* = 9.1)	3, 4′, 5′
C(4′)	160.66	160.65	—	—	2′, 3′, 5′, 6′, H anom
C(5′)—H	116.55	116.50	7.17 (br.d, *J* = 9.0)	7.20 (br.d, *J* = 9.1)	3, 3′, 4′
C(6′)—H	128.34	128.31	8.08 (br.d, *J* = 8.5)	8.02 (br.d, *J* = 8.8)	2′, 4′
C(1″)*—H	98.37	98.21	5.26 (d, *J* = 7.5)	5.27 (d, *J* = 7.7)	7, 2″, 3″
C(1‴)*—H	98.21	98.15	5.24 (d, *J* = 7.5)	5.26 (d, *J* = 7.7)	4′, 2‴, 3‴
C(2″)—H	70.18	70.18	3.47 (m)	3.50 (m)	
C(2‴)—H	70.11	69.94	3.47 (m)	3.50 (m)	
C(3″)—H	71.40	71.43	3.94 (m)	4.01 (m)	
C(3‴)—H	71.40	71.32	3.94 (m)	4.01 (m)	
C(4″)—H	66.98	67.20	3.43 (m)	3.47 (m)	
C(4‴)—H	66.88	66.98	3.43 (m)	3.47 (m)	
C(5″)—H	74.82	71.60	3.80 (m)	3.78 (m)	
C(5‴)—H	74.81	74.81	3.80 (m)	4.06 (m)	
C(6″)—$H_{a,b}$	60.89	63.65	3.48 (m); 3.71 (m)	4.35 (dd, *J* = 11.5, 1.9); 4.11 (br.d)	
C(6‴)—$H_{a,b}$	60.89	60.89	3.48 (m); 3.71 (m)	3.72 (dd, *J* = 11.7, 1.9); 3.50 (m)	
$\underline{CH_3}CO$	—	170.20	—	2.0 (s)	6″

* The assignment of the signals for the corresponding pairs of atoms of the allosyl fragments in both carbohydrate chains is alternative.

carbohydrate C atoms observed in the ^{13}C NMR spectra coincide with the published data for β-allopyranose (compound **1**) and β-allopyranose acylated at the O(6) atom (compound **2**).[5] The magnitudes of the spin-spin coupling constants for anomeric protons (^{3}J = 7.5 and 7.7 Hz) confirm the β-configuration of allopyranose.



1: R = H
2: R = Ac

The sites of attachment of carbohydrates to the aglycone were confirmed by analysis of the ^{1}H—^{13}C HMBC spectrum of compound **2**. The presence of cross-peaks of the anomeric protons of carbohydrates with C(7) and C(4′) of apigenin points to glycosylation at these atoms of the aglycone. The presence of cross-peaks of the $\underline{CH_3}CO$ group and H(6) of one allose residue in this spectrum indicates that one primary alcoholic group in compound **2** is acetylated. This is confirmed by the paramagnetic shift of the signal for C(6Ī), equal to 2.76 ppm, and the diamagnetic shift, equal to 3.21 ppm, of the C(5Ī) signal of this allose residue; these shifts are adequately explained by a- and b-effects of acetylation. The position of the acetate group was determined in NOE experiments. The 2D ROESY spectrum implies coupling between the $\underline{CH_3}CO$ and the 5-OH groups (d 12.61), which is possible only when the O(6) atom of the allose residue bound to position 7 of aglycone is acetylated.

The incorporation of allose into flavonoid glycosides is fairly rarely encountered. Up to now, *Thalictrum thunbergii* has been the only plant from which such glycosides have been isolated.[6]

Experimental

Melting points were determined using a Koffler hot stage, and optical rotation was measured on a Polamat A polarimeter. IR spectra were recorded on an IFS25 Fourier Transform spectrometer for pellets with KBr, and UV spectra were measured on a Specord UV-VIS spectrophotometer in methanol. The ^{1}H and ^{13}C NMR spectra were run on a Varian VXR-500S instrument (499.843 MHz) equipped with a SUN 3/50

computer with a standard VNMR provision in DMSO at 26 °C. When recording 2D spectra, traces of acetic acid-d_4 were added. FAB mass spectra were recorded on a JEOL SX102A instrument with double focusing (thioglycerol as the matrix and xenon as the ionizing gas).

Preparative-scale chromatography was performed using a Servacel DEAE 23 SS anion-exchange cellulose; for column chromatography, silica gel Lachema L 40/100 and polyamide Woelm were used; and TLC was carried out with silica gel Lachema L 5/40 and polyamide Woelm TLC. The chromatographic plates were visualized by spraying with a 5% ethanolic solution of $AlCl_3$ followed by heating to 105 °C and examination in UV light.

Thalictrum squarrosum was gathered during blooming in Buryatiya. The dried and finely divided plant (2 kg) was subjected to exhaustive extraction with 80% aqueous methanol. The extract was concentrated, and the residue was treated successively with chloroform and butanol. The butanol fraction (70 g) was subjected to flash chromatography on silica gel using a chloroform—methanol—water mixture (70 : 23 : 1) as the eluent. This gave several fractions. On storage, a precipitate formed in some fractions; it was separated by centrifugation and washed with methanol to give 40 mg of compound **1** and 75 mg of **2**.

Thalictrum minus was gathered in the blooming phase near the Belaya river of the Cheremkhovskii district of the Irkutsk region. The finely divided terrestrial part (1.5 kg) was exhaustively extracted with 80% aqueous methanol. The extract was concentrated, and the aqueous residue was treated successively with chloroform and butanol. The concentrated butanol fraction was dissolved in the minimum volume of methanol and diluted with acetone (in 1 : 2 (v/v) ratio). The precipitate that formed was dissolved in water and filtered through Molselect G-25. Fractions free from carbohydrates were repeatedly chromatographed on silica gel in a chloroform—methanol—water mixture, 70 : 23 : 4. The resulting sum of flavonoids (1.3 g) was chromatographed on anion-exchange cellulose (OH^--form). Gradient elution was carried out with water—methanol mixtures with gradually increasing proportions of methanol (0→15%); elution with 12% methanol gave a fraction (30 mg) containing compound **1**. Individual glycoside **1** was isolated by chromatography on polyamide in the methanol—water (1 : 4) system repeated twice. This gave 7 mg of compound **1**.

7,4′-Di-*O*-(β-allopyranosyl)apigenin (1), m.p. 256—257 °C (methanol), $[\alpha]_{546}^{25}$ −95.5° (*c* 0.15; Py) (*cf.* Ref. 6: m.p. 248—251 °C, $[\alpha]_D$ −138.7° (*c* 1.06; Py)). MS, *m/z*: 595.1625 $[M + H]^+$ (calc. 595.1604), $C_{34}H_{27}O_{10}$. IR, ν/cm^{-1}: 3426 (OH), 1659 (C=O), 1610 (C=C), 1498, 836 (arom.). UV (MeOH), λ_{max}/nm: 271, 319; +$AlCl_3$ 280, 300, 339, 382; +NaOMe 297, 373. ^1H and ^{13}C NMR spectra are given in Table 1.

7-*O*-(6-*O*-Acetyl-β-allopyranosyl)-4′-*O*-(β-allopyranosyl)-apigenin (2), m.p. 246—248 °C (methanol), $[\alpha]_{546}^{25}$ −124° (*c* 2; Py) (*cf.* Ref. 6: m.p. 257—260 °C; $[\alpha]_D$ −102.4° (*c* 1.03; Py)). MS, *m/z*: 637.1724 $[M + H]^+$ (calc. 637.1710), $C_{36}H_{29}O_{11}$. IR, ν/cm^{-1}: 3538—3401 (OH), 1668 (C=O), 1613 (C=C), 1585, 829 (arom.), 1719 (C=O acetate). ^1H and ^{13}C NMR spectra are presented in Table 1.

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References

1. E. A. Khamidullina, A. S. Gromova, V. I. Lutskii, A. A. Semenov, and S. V. Zinchenko, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 1547 [*Russ. Chem. Bull.*, 1996, **45**, 1476 (Engl. Transl.)].
2. N. N. Trofimova, A. S. Gromova, V. I. Lutskii, A. A. Semenov, S. A. Avilov, A. I. Kalinovskii, D. Lee, and N. L. Owen, *Izv. Akad. Nauk, Ser. Khim.*, 1998, 1434 [*Russ. Chem. Bull.*, 1998, **47**, 1395 (Engl. Transl.)].
3. T. J. Mabry, K. R. Markham, and M. B. Thomas, in *The Systematic Identification of Flavonoids*, Springer-Verlag, Berlin—Heidelberg—New York, 1970, fig. 30.
4. V. M. Chari, M. Jordan, H. Wagner, and P. W. Thies, *Phytochemistry*, 1977, **16**, 1110.
5. V. M. Chari, R. J. Grayer-Barkmeijer, J. B. Harborne, and B.-G. Österdahl, *Phytochemistry*, 1981, **20**, 1977.
6. E. Shimizu, T. Tomimatsu, and T. Nohara, *Chem. Pharm. Bull.*, 1984, **32**, 5023.

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