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FLAVONOID GLYCOSIDES FROM *Astragalus galegiformis* LEAVES

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New flavonoid oligosides, the structures of which were established by chemical transformations and UV, IR, PMR, and ^{13}C NMR spectra, were isolated from Astragalus galegiformis *leaves.*

Key words: *Astragalus galegiformis,* flavonol, quercetin, isorhamnetin, flagaloside.

We reported earlier on the isolation from *Astragalus galegiformis* L. (Fabaceae L.) flowers of flavonoid glycosides that were derivatives of isorhamnetin, astragalegoside and isoastragalegoside, which possess diuretic and hyponitrogenous activity [1, 2].

Herein we examine the structure of flavonoid glycosides isolated from leaves of *A. galegiformis*. They are difficult to separate by chromatography (R_f 0.34 ± 0.05) and isolate in pure states. This may be due to either H-bonds between the compounds or gums in the *Astragalus* species.

We were able to isolate six compounds by using classical separation methods for flavonoids and their modifications. Two compounds turned out to be known flavonoids that were previously isolated from other *Astragalus* species, astragalin (**1**) and kaempferol (**2**) [3].

The other compounds were new. We called them flagalosides (**2**-**5**). They all gave a positive qualitative reaction characteristic of flavonoid glycosides [4]. Their IR spectra contained absorption bands for hydroxyls (3200-3400 cm^{-1}), γ-pyrone carbonyl (1665-1660 cm^{-1}), and aromatic rings (1620, 1575 cm^{-1}) [5].

UV spectra of **3** and **4** in ethanol with added ionizing and complexing reagents produced absorption bands that revealed the absence of substituents on C_7, C_5, C_4, and $C_{3'}$; for **2** and **5**, the presence of substituents on C_7 and C_3, and C_3 and $C_{3'}$, respectively.

Acid hydrolysis of the glycosides produced the aglycons quercetin (from **2**-**4**) and isorhamnetin (from **5**). The yields of the aglycons indicated that **2** was a tetraoside; **3**, a pentaoside; **4**, a trioside; and **5**, a bioside. The carbohydrate part of **2** contained D-glucose, D-galactose, L-arabinose, and L-rhamnose bonded to C_3 and C_7 of quercetin; of **3**, D-glucose, D-galactose, L-arabinose, D-xylose, and L-rhamnose also bonded to C_3 of quercetin.

In view of the fact that **2** and **3** were isolated in minor amounts, it was not possible to establish their complete chemical structures.

The PMR spectrum of **4** recorded in CD_3OD (Table 1) contained a strong singlet at 12.8 ppm, which is characteristic of a 5-OH group. The presence of this hydroxyl was confirmed by the UV spectrum recorded in ethanol with added $AlCl_3$, in which the absorption band at 401 nm underwent a bathochromic shift of 46 nm [6]. The size of bathochromic shifts produced by adding AcONa + H_3BO_3 and MeONa is indicative of a dihydroxy group in the 3′,4′-positions and a free 4′-OH group [6]. Signals of aromatic protons are observable at weak field in the PMR spectra at 7.65, 6.90, and 6.92 (ring B) and 6.45 and 5.25 ppm (ring A). The chemical shifts and SSCC of protons in ring B indicated that they were located on $C_{2'}$, $C_{5'}$, and $C_{6'}$ (Table 1).

The ^{13}C NMR spectra led to an analogous conclusion, where signals for secondary C atoms at C-5, C-7, C-4′, and C-3′ were clearly observed. The chemical shift of C-3 was indicative of its glycosylation compared with C-3 of the aglycon. This C atom appeared at 135.6 ppm (136.73 ppm [7]).

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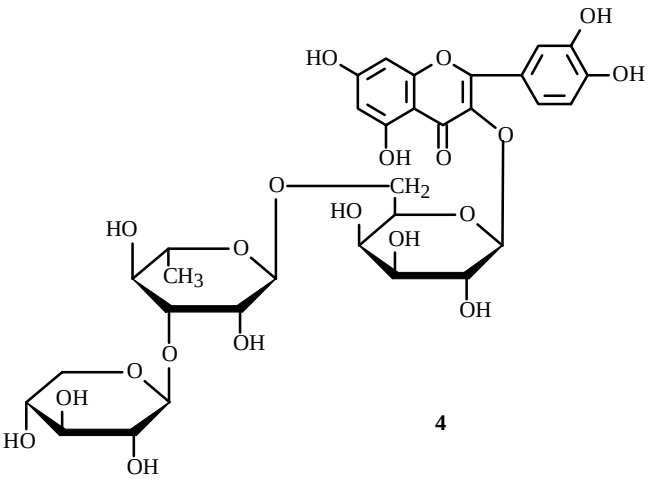
TABLE 1. PMR and ^{13}C NMR Spectra of Flavonoid Glycosides from *Astragalus galegiformis* Leaves

C atom	Compound			
	4		**5**	
	δ_C	δ_H	δ_C	δ_H
2	158.10		158.20	
3	135.60		134.25	
4	178.00		179.10	
5	162.10		163.10	
6	99.20	6.26, d, 2.22	99.70	6.20, d, 2.2
7	165.00		165.80	
8	94.30	6.45, d, 2.08	94.61	6.45, d, 2.02
9	157.60		158.30	
10	105.10		105.95	
1′	122.10		123.45	
2′	117.20	7.92, d, 2.10	114.52	7.95, d, 2.20
3′	145.00		150.55	
4′	149.50		146.89	
5′	115.90	6.90, d, 8.0	116.05	6.93, d, 8.43
6′	122.10	7.65	123.38	7.64, dd, 2.2; 8.43
OCH_3			56.92	3.81, s
1″	105.70, d, 8.30	5.09, d, 8.30	104.06	4.80, d, 7.50
2″	72.70	3.86, dd, 8.3; 9.4	83.27	4.36, d, 7.30
3″	75.00	3.60, dd, 9.40; 3.8	75.65	3.41, t, 9.0
4″	69.90	3.83	70.36	3.78, td, 9.0; 5.0
5″	75.20	3.71	66.35	3.65, dd, 10.0; 9.0
6″	67.60	3.54; 3.75		3.90, dd, 10.0; 5.0
1‴	101.60	4.56, d, 2.1		
2‴	71.40	3.78, dd, 3.8; 2.1		
3‴	82.30	3.57, dd, 9.6; 3.8		
4‴	72.50	3.47, dd, 9.60		
5‴	69.10	3.60		
6‴	17.60	1.19		
1⁗	106.30	4.36, d, 7.30	106.10	4.71, d, 7.0
2⁗	75.00	3.25, dd, 9.0; 7.3	75.02	3.20
3⁗	77.20	3.33, t, 9.0	77.20	3.32
4⁗	70.70	3.49, td, 9.5	70.70	3.45
5⁗	66.60	3.14, dd, 9.0; 9.0 3.82, dd, 10.5; 5.0	66.70	3.14, 3.80

The glycoside was readily hydrolyzed by aqueous H_2SO_4 to afford an aglycon with mp 303-305°C that was identified as quercetin [8]. The aglycon yield was 38%, from which the trioside nature of this flavonoid was deduced. This conclusion was confirmed by the PMR spectrum, in which signals of three anomeric protons were observed at 5.09 (d, J = 8.3 Hz), 4.56 (d, J = 2.1 Hz), and 4.36 ppm (d, J = 7.3 Hz). The methyl of L-rhamnose appeared at 1.19 ppm.

PC of the hydrolysate using System 4 detected D-galactose, L-rhamnose, and D-xylose. The bonding between sugar units was determined using ^{13}C NMR spectra (Table 1) and chemical transformations of **1**. Stepwise hydrolysis of **4** formed D-xylose and a less polar glycoside, enzymatic decomposition of which produced robinobiose [9] and quercetin. It was established that D-galactose was directly bonded to the aglycon through a 3→1 bond. L-Rhamnose was bonded to it through a 6→1 bond. The terminal unit was D-xylose bonded to L-rhamnose through a 3→1 bond with the pyranose form of the rings [10, 11]. Alkaline hydrolysis of flagaloside C did not change the structure of the glycoside.

Judging from the results, glycoside **4** was characterized as quercetin-3-*O*-*β*-D-galactopyranosyl-(6→1)-*O*-*α*-L-rhamnopyranosyl-(3→1)-*O*-*β*-D-xylopyranoside (flagaloside C).



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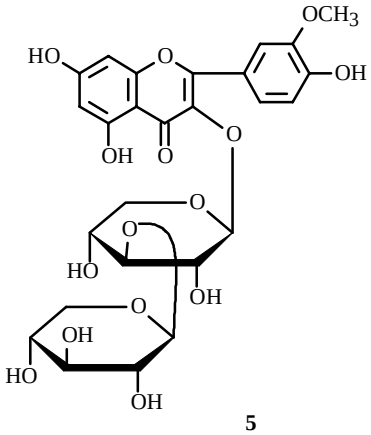

5

UV spectra of glycoside **5** (356, 255 nm) were characteristic of flavonol derivatives. Its IR spectrum had absorption bands due to hydroxyls (3560-3200 cm^{-1}), methoxyl (2980), γ-pyrone carbonyl (1650), aromatic rings (1635, 1587), and carbohydrate C–O vibrations (1100, 1078, 1050).

The glycoside was hydrolyzed by H_2SO_4 (2%) to form D-xylose and the aglycon (62%), indicating it was a biose. The aglycon melted at 289-300°C. Fusion with base gave phloroglucinol and vanillic acid [2]. The UV spectrum of the aglycon produced by adding CH_3COONa + H_3BO_3 was consistent with the absence of a C-3′,4′ dihydroxy group. Melting-point depression was not observed upon mixing with an authentic sample of isorhamnetin. Therefore, we characterized it as isorhamnetin [9].

Glycoside **5** was unaffected by base. This meant that the sugar was bonded to the aglycon at C-3. This conclusion was consistent with the PMR spectrum, in which signals for a 5-OH were observed at 12.05 ppm; five signals of aromatic H of rings A and B, at 7.65-6.25 ppm; and two signals of anomeric carbohydrate protons, at 4.80 and 4.71 ppm. The methoxyls of the aglycon were observed as a singlet at 3.18 ppm (Table 1).

The chemical shifts of the carbohydrate atoms (Table 1) indicated a 2→1 bond between the sugars and the β-configuration and pyranose form for the ring for xylose units bonded as a biose to C-3 of isorhamnetin [8, 10]. Thus, this glycoside was characterized as isorhamnetin-3-*O*-β-D-xylopyranosyl-(2→1)-*O*-β-D-xylopyranoside (flagaloside D).

EXPERIMENTAL

General Comments. Plants were collected near the resort Bakuriani (Georgia) during full flowering in 1999. Raw material was extracted with ethanol (80°). Chromatography was performed on Filtrak FN-11 paper using *n*-butanol:acetic acid:water (4:1:2, 1), acetic acid (15%, 2), ethylacetate:benzene:acetic acid (74.5:23.5:2, 3), and *n*-butanol: pyridine:benzene:water (5:3:1:3, 4). Column chromatography used polyamide [12]. Molecular weights were determined as before [13].

NMR spectra were recorded in CD_3OD on a Bruker DRX-600 instrument; UV spectra, on a Lomo SF-16 spectrophotometer; IR spectra, on a U-20 instrument as KBr disks.

Isolation of Flavonoids. Ground raw material (1 kg, air-dried) was extracted three times with ethanol (80°) in a 1:10 ratio. The extract was condensed to an aqueous residue and purified with $CHCl_3$. The purified aqueous extract was extracted exhaustively with ethylacetate. The ethylacetate was evaporated to afford a dry solid (3.8 g, fraction 1). After the ethylacetate was removed from the aqueous extract, it was fractionated over a polyamide column (d, 1.5; h, 50 cm) with elution successively by water and ethanol (45 and 90%). The alcohol (45%) fractions contained almost all flavonoids (7.2 g, fraction 2).

Fractions (3 g each) were separated over a polyamide column (d, 2.5; h, 90). Compounds of fraction 1 were eluted by $CHCl_3$ and $CHCl_3$:alcohol of increasing alcohol concentration. Compounds of fraction 2 were eluted by ethylacetate and ethylacetate:alcohol (1:4). This isolated compounds **1-6**.

Astragalin, mp 195-197°C, $[\alpha]_D$ -10° (*c* 0.1, ethanol); UV spectrum (EtOH, λ_{max}, nm): 375, 268; +$AlCl_3$: 410, 275; +CH_3ONa: 420, 290; +CH_3COONa: 385, 275.

Acid Hydrolysis of Astragalin. Glycoside (20 mg) and H_2SO_4 (2%) gave an aglycon with mp 270-272°C, $C_{15}H_{10}O_5$ that was identical to kaempferol [9] and D-glucose.

A mixed sample of **1** and astragalin did not show melting-point depression. It was identified as astragalin [9, 14].

Flagaloside A (2); pale white crystals; MW 921.5 [13]; soluble in aqueous alcohol; insoluble in water, alcohol, ethylacetate; mp 188-192°C; $[\alpha]_D$ -161.7° (*c* 0.271, ethanol:DMF, 1:10). UV spectrum (EtOH, λ_{max}, nm): 360, 260; +$AlCl_3$: 405, 270; $AlCl_3$ + HCl: 390, 265; +CH_3COONa: 369, 269; +CH_3COONa + H_3BO_3: 375, 265; IR spectrum (KBr, ν_{max}, cm^{-1}): 3400 (OH), 1650-1660 (γ-pyrone C=O).

Acid Hydrolysis of Flagaloside A. Glycoside (50 mg) was hydrolyzed by H_2SO_4 (5 mL, 2%) on a water bath. The resulting precipitate was separated, washed with water, dried, and recrystallized from aqueous ethanol to isolate the aglycon (15.2 mg), mp 303-305°C, which was identified as quercetin [14]. The filtrate was neutralized with AB-17 ion exchanger and evaporated. PC of the solid using system 4 detected D-glucose, D-galactose, L-arabinose, and L-rhamnose.

Flagaloside B (3), pale white crystals, MW 1036.5 [13], mp 202-205°C; $[\alpha]_D$ +55.0° (*c* 0.68, ethanol:DMF, 1:10). UV spectrum (EtOH, λ_{max}, nm): 360, 260; +$Zr(NO_3)_3$: 400, 260; +$Zr(NO_3)_3$ + citric acid: 390, 255; +CH_3COONa: 375, 265; +CH_3COONa + H_3BO_3: 378, 265. IR spectrum (KBr, ν_{max}, cm^{-1}): 3200-3400 (OH), 1660-1600 (γ-pyrone C=O), 1550 (C=C).

Acid Hydrolysis of Flagaloside B. Glycoside (50 mg) was hydrolyzed as for **2** to afford the aglycon (13.2 mg), mp 301-303°C, which was identified as quercetin [8, 9]. PC of the carbohydrate part using system 4 identified D-glucose, D-galactose, D-xylose, L-arabinose, and L-rhamnose.

Flagaloside C (4); white crystals; mp 188-193°C; soluble in aqueous alcohol, water; insoluble in concentrated alcohol, acetone; MW 726.03 [13], $C_{32}H_{38}O_{19}$. UV spectrum (EtOH, λ_{max}, nm): 350, 270; +$AlCl_3$: 401, 350, 300sh, 267; +$AlCl_3$ + HCl: 400, 350, 295, 270; +CH_3COONa: 370, 360, 300sh, 270; +CH_3COONa + H_3BO_3: 370, 270; CH_3ONa: 400, 320, 270. IR spectrum (KBr, ν_{max}, cm^{-1}): 3200-3400 (OH), 1670 (γ-pyrone C=O), 1520, 1470 (C=C).

Acid Hydrolysis of Flagaloside C. Glycoside (20 mg) was hydrolyzed by H_2SO_4 (2%) as for **1** to afford the aglycon (7.52 mg), mp 300-302°C, which was identified as quercetin [9]. The carbohydrate part was neutralized with AB-17 anion exchanger. PC detected in it D-galactose, D-xylose, and L-rhamnose.

Enzymatic Hydrolysis of the Product of Partial Hydrolysis of 3 (I). Glycoside (75 mg) was hydrolyzed by aqueous H_2SO_4 (5%) on a water bath. The production of the intermediate product was monitored at 15-min intervals. The maximum amount of intermediate product had formed after 30 min. The hydrolysis was interrupted by diluting the reaction mixture with water and extracting with ethylacetate. The aqueous part was neutralized by AB-17 ion exchanger. PC detected in it D-xylose. The ethylacetate extract was washed with water until the rinsings were neutral and then evaporated. The solid was hydrolyzed by rhamnodiastase (37°C, 5 h). After the appropriate work up, PC of the hydrolysate using system 4 detected robinobiose by comparison with an authentic sample [9, 12].

Flagaloside D (5), white crystals, MW 564.5 [13], $C_{26}H_{28}O_{14}$, soluble in aqueous alcohol. UV spectrum (EtOH, λ_{max}, nm): 356, 255; +$AlCl_3$: 402, 268; +$AlCl_3$ + HCl: 396, 268; +CH_3COONa: 378, 325, 275; +CH_3COONa + H_3BO_3: 360, 255; +CH_3ONa: 415, 275. IR spectrum (KBr, ν_{max}, cm^{-1}): 3350 (OH), 2980 (OCH_3), 1650, 1620 (γ-pyrone C=O), 1580, 1410 (C=C).

Table 1 gives the PMR and ^{13}C NMR spectra.

Acid Hydrolysis of 5. Glycoside (10 mg) was hydrolyzed by H_2SO_4 (2%) for 2 h to afford the aglycon (65%), mp 299-303°C. UV spectrum (EtOH, λ_{max}, nm): 355, 308, 255; +$AlCl_3$: 405, 360sh, 267; +$AlCl_3$ + HCl: 400, 356, 265; +CH_3COONa: 377, 324, 274; +CH_3COONa + H_3BO_3: 358, 300sh, 258; +CH_3ONa: 422, 300sh, 268. IR spectrum (KBr, ν_{max}, cm^{-1}): 3400 (OH), 2980 (OCH_3), 1650, 1620 (γ-pyrone C=O), 1580, 1470 (C=C). An authentic sample of isorhamnetin and **5** gave a single spot on PC. It was characterized as isorhamnetin [8, 9].

Kaempferol (6), mp 270-272°C. UV spectrum (EtOH, λ_{max}, nm): 270, 350. IR spectrum (KBr, ν_{max}, cm^{-1}): 3200-3400 (OH), 1650 (γ-pyrone C=O).

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