Chemistry of Natural Compounds, Vol. 35, No. 6, 1999

FLAVONOIDS OF *Caragana alaica*

A. V. Butayarov,[1] E. Kh. Batirov,[2] M. M. Tadzhibaev,[1] and M. P. Yuldashev[3]

UDC 547.972

A new isoflavonglycoside is isolated from the terrestrial part of Caragana alaica *A. Pojark in addition to the known flavonoids 3-O-α-L-rhamnopyrano- and 3-O-β-D-glucopyranosides of isorhamnetin, quercetin, and vistin. The structure 3′-hydroxy-6,4′-dimethoxy-7-O-β-D-glucopyranosylisoflavone is established on the basis of chemical and spectral data.*

Plants of the Fabaceae family are rich sources of biologically active phenolic compounds [1, 2]. We continued our research in this area by studying flavonoids of the terrestrial part of *Caragana alaica* A. Pojark (alaic caragana) [3]. At present 12 flavonoids have been isolated from the five studied plant species of the *Caragana* Lam. genus. These include quercetin, isorhamnetin, myricetin, and their glycosides [1]. Flavonoids of alaic caragana have not previously been studied.

Six pure flavonoids were isolated by column chromatography from various fractions of the methanol extract of the terrestrial part of plants collected during the fruit-bearing phase on slopes of the Pamir-Alai range (Republic of Tadzhikistan). Five of these were identified by acid hydrolysis and UV, PMR, and mass spectra as the known flavonol glycosides isorhamnetin-3-O-α-*L*-rhamnopyranoside (**1**), quercetin-3-O-α-*L*-rhamnopyranoside (**2**) [4], isorhamnetin-3-O-β-*D*-glucopyranoside (**3**), quercetin-3-O-β-*D*-glucopyranoside (**4**) [5], and the isoflavone glycoside afrormosin-7-O-β-*D*-glucopyranoside (vistin) (**5**) [6].

A compound of composition $C_{23}H_{24}O_{11}$ (**6**) has a UV spectrum (λ_{max} 257, 262, 287, 317 nm) typical of an isoflavone derivatives [7]. Its PMR spectrum contains signals of protons on two methoxy groups, H-2, H-5, H-8, a 3′,4′-disubstituted ring *B*, an anomeric proton, and other protons of a single carbohydrate unit. Therefore, compound **6** is a 6,7,3′,4′-tetrasubstituted isoflavone glycoside. Acid hydrolysis of glycoside **6** produced the aglycone **7** of composition $C_{17}H_{14}O_6$ and *D*-glucose. The mass spectrum of aglycone **7** gives peaks for $(a + 1)^+$ with *m/z* 167 and *b* with *m/z* 148 is that formed via retrodiene decomposition of the molecular ion with *m/z* 314. This is consistent with the presence of one methoxy group and one hydroxyl in rings *A* and *B* [8].

Comparison of the UV spectra of compounds **6** and **7** in the presence of NaOAc indicates that, in contrast with the spectrum of glycoside **6**, the short-wavelength band in the spectrum of the aglycone **7** undergoes a bathochromic shift by 8 nm. This is consistent with a free 7-OH group in the molecule of aglycone **7** [7].

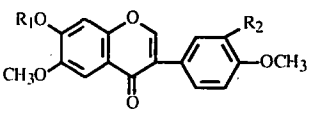
The placement of the carbohydrate residue was determined by methylating glycoside **6** with dimethylsulfate in the presence of K_2CO_3. Acid hydrolysis of the resulting methylation product produced substance **8**, which was identical in physicochemical and spectral properties to cladrastin (7-hydroxy-6,3′,4′-trimethoxyisoflavone) [9]. Therefore, the carbohydrate residue in glycoside **6** is bonded to the 7-OH. One of the methoxy groups occupies the 6-position of ring *A*.

Biogenetic considerations (the presence of a methoxy group in the 4′-position of vistin) suggest that the second methoxy group in glycoside **6** is probably located on C-4′. This is confirmed by comparing the PMR spectra of glycoside **6** and pratensein (5,7,3′-trihydroxy-4′-methoxyisoflavone) taken in Py-D_5 [10]. The good agreement of the proton chemical shifts of ring *B* in glycoside **6** (7.70 ppm, H-2′; 6.93 ppm, H-5′; 7.24 ppm, H-6′) with those of pratensein (7.63 ppm, H-2′; 6.93 ppm, H-5′; 7.20 ppm, H-6′) confirms that the hydroxyls in ring *B* of both flavonoids are situated in the same positions.

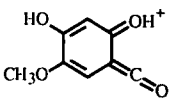
The spin—spin coupling constant of the signal for the anomeric proton in the PMR spectrum of glycoside **6** is J = 6.5 Hz. This indicates that the monosaccharide ring has the C1-conformation. Therefore, the glycoside center of *D*-glucose has the β-configuration [11].

1) Andizhan State Medical Institute; 2) Namangan Engineering-Pedagogical Institute, Namangan, fax (36922) 6 22 93; 3) Academician S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (371) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 734-737, November-December, 1999. Original article submitted November 22, 1999.

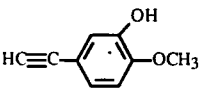
 0009-3130/99/3506-0628$22.00



5: R_1=β-*D*-Glcp, R_2=H
6: R_1=β-*D*-Glcp, R_2=OH
7: R_1=H, R_2=OH
8: R_1=H, R_2=OCH_3



$(a+1)^+$, *m/z* 167



b, *m/z* 148

Thus, glycoside **6** has the structure 3′-hydroxy-6,4′-dimethoxy-7-O-β-*D*-glucopyranosylisoflavone and is a new natural compound. Flavonoids **1-5** from alaic caragana are isolated for the first time.

EXPERIMENTAL

General. The solvent systems $CHCl_3$—CH_3OH [97:3 (1), 95:5 (2), 92:8 (3), and 9:1 (4)] and butan-1-ol—pyridine-water [6:4:3 (5)] were used. Silufol UV-254 plates were used for TLC. Silica gel (KSK, 100-160 μm) was used for column chromatography.

Compounds on TLC plates were visualized using ammonia, iodine, and ethanolic NaOH (1%). Monosaccharides were detected using paper chromatography (Filtrak N11) with acidic anilinium phthalate spray and subsequent heating for 3-5 min at 90-100°C. Melting points were measured on a Boetius apparatus with an RNMK 0.5 viewer.

PMR spectra were recorded on a Tesla BS-567A spectrometer in Py-D_5. Mass spectra were measured on an MS 25RF (Kratos) instrument with a DS-90 data processing system. UV spectra were recorded on a Perkin—Elmer Lambda 16 spectrometer.

Isolation and Separation of Flavonoids. Dried and ground terrestrial parts (0.6 kg) of the fruit-bearing plant (20 August 1990) were extracted six times with hot methanol. The combined extracts were concentrated under vacuum to 0.8 l and diluted with water to 1.6 l. The aqueous-alcohol extract was sequentially shaken with petroleum ether (4 × 0.5 l), $CHCl_3$ (6 × 0.5 l), ethylacetate (6 × 0.5 l), and *n*-butanol (4 × 0.5 l). Evaporation of the solvents gave fractions of 8.9 g (petroleum-ether), 5.2 g ($CHCl_3$), 4.6 g (ethylacetate), and 22.0 g (butanol). The ethylacetate fraction was chromatographed on a silica-gel (260 g) column (120 × 3 cm) with elution by $CHCl_3$ and systems 1, 2, and 3. Fractions of 200-ml volume were collected. Elution of the column by system 1 gave fractions from which vistin (**5**, 0.32 g) and isoflavonglycoside **6** (0.093 g) were isolated. Elution of the column by system 2 gave glycoside **1** (0.115 g) and glycoside **3** (0.076 g).

The butanol fraction (22.0 g) was chromatographed on a silica-gel (450 g) column (140 × 5 cm) with elution by systems 1, 2, and 3. Fractions of 500- ml volume were collected. Elution of the column by system 3 gave fractions from which **1** (0.14 g), **2** (0.225 g), **3** (0.337 g), and **4** (0.482 g) were isolated.

Isorhamnetin-3-O-α-*L*-rhamnopyranoside (1). $C_{22}H_{22}O_{12}$, mp 156-158°C, λ_{max} (ethanol) 256, 266, 360 nm. Acid hydrolysis of glycoside **1** gave isorhamnetin and *L*-rhamnose.

Quercetin-3-O-α-*L*-rhamnopyranoside (2). $C_{21}H_{20}O_{11}$, mp 187-189°C, λ_{max} (ethanol) 255, 266 (sh), 356 nm. Acid hydrolysis of compound **2** gave quercetin and *L*-rhamnose.

Isorhamnetin-3-O-β-*D*-glucopyranoside (3). $C_{22}H_{22}O_{12}$, mp 164-166°C, λ_{max} (ethanol) 257, 267, 359 nm. Acid hydrolysis of glycoside **3** gave isorhamnetin and *D*-glucose.

Quercetin-3-O-β-*D*-glucopyranoside (4). $C_{21}H_{20}O_{12}$, mp 237-238°C, λ_{max} (ethanol) 255, 265 (sh), 362 nm. Acid hydrolysis of this compound gave quercetin and *D*-glucose.

Afrormosin-7-O-β-*D*-glucopyranoside (5). $C_{23}H_{24}O_{10}$, mp 214-215°C, λ_{max} (ethanol) 264, 323 (sh) nm. PMR spectrum (δ, ppm, Py-D_5): 3.57 and 3.60 (s, 3H each, 2 × OCH_3), 4.05-4.60 (carbohydrate protons), 5.75 (1H, d, 6.5 Hz, H-1″), 6.95 (2H, d, 9.0 Hz, H-2′, -6′), 7.52 (1H, s, H-8), 7.67 (2H, d, 9.0 Hz, H-3′, -5′), 7.73 (1H, s, H-5), 8.01 (1H, s, H-2).

Acid hydrolysis of **5** (5% HCl, 3 h on a water bath) gave *D*-glucose and afrormosin [$C_{17}H_{14}O_5$, mp 232-235°C, λ_{max} (ethanol) 259, 322 nm].

Mass spectrum, *m/z* (%): 298 [M^+] (100), 283 (10), 268 (4), 255 (5.5), 240 (3), 175 (2.5), 166 (20), 149 (8.5), 133 (4),

132 (11).

3′-Hydroxy-6,4′-dimethoxy-7-O-β-*D*-glucopyranosylisoflavone (6). $C_{23}H_{24}O_{11}$, mp 196-198°C (ethanol), λ_{max} (ethanol) 257, 262, 287 (sh), 317 nm; +NaOAC 263, 319 nm.

PMR spectrum (δ, ppm, Py-D_5): 3.59 and 3.65 (s, 3H each, 2 × OCH_3), 4.05-4.60 (glucose protons), 5.76 (1H, d, 6.5 Hz, H-1″), 6.93 (1H, d, 9.0 Hz, H-5′), 7.24 (1H, dd, 9.0 and 2.0 Hz, H-6′), 7.50 (1H, s, H-8), 7.70 (1H, d, 2.0 Hz, H-2′), 7.75 (1H, s, H-5), 8.05 (1H, s, H-2).

Acid Hydrolysis. Glycoside **6** (30 mg) was heated with HCl (5%, 10 ml) for 3 h on a water bath. The solid was filtered off and recrystallized from CH_3OH. Yield 12 mg of aglycone $C_{17}H_{14}O_6$, mp 210-212°C, λ_{max} (ethanol) 256, 259, 318 nm; +NaOAc 267, 321 nm. Mass spectrum, *m/z* (%): 314 [M^+] (100), 313 (25), 299 [M - CH_3]$^+$ (24), 271 (16), 243 (11), 228 (4), 205 (10), 167 (11), 151 (5.5), 148 (5), 133 (12), 105 (23).

Cladrastin (8) from Glycoside 6. Glycoside **6** (36 mg) in anhydrous acetone (5 ml) was treated with dimethylsulfate (0.4 ml) and anhydrous K_2CO_3 (50 mg). The mixture was boiled on a water bath for 6 h. The solid was filtered off. The filtrate was evaporated. The solid was dissolved in ethanol (5 ml), treated with conc. HCl (0.5 ml), and heated on a water bath for 3 h. The solvent was removed. The solid was treated with water (15 ml) and extracted with $CHCl_3$. The $CHCl_3$ extract was dried, filtered, and evaporated. The solid was recrystallized from CH_3OH. Yield 13 mg of compound **8**, $C_{18}H_{16}O_6$, mp 205-206°C, λ_{max} (ethanol) 263, 318 nm, +NaOAc, 270, 320 nm. Mass spectrum, *m/z* (%): 328 [M^+] (100), 313 (19), 310 (7), 283 (6), 167 (13), 148 (8).

REFERENCES

1. *Plant Resources of the USSR. Flowering Plants, Their Chemical Composition and Use. Hydrangeaceae-Halorigaceae Family* [in Russian], Nauka, Leningrad (1987), p. 125.
2. A. S. Ammosov and V. I. Litvinenko, *Rastit. Resur.*, **31**, No. 3, 116, 136 (1995).
3. *Flora of the USSR* [in Russian], USSR Academy of Sciences Press, Moscow and Leningrad (1945), Vol. 11, p. 349.
4. G. G. Zapesochnaya, *Khim. Prir. Soedin.*, 695 (1982).
5. Sh. V. Abdullaev, A. Sattikulov, E. Kh. Batirov, et al., *Khim. Prir. Soedin.*, 104 (1983).
6. T. Konoshima, E. Okamoto, M. Kozuka, et al., *J. Natur. Prod.*, **51**, 1270 (1988).
7. K. R. Markham, *Techniques of Flavonoid Identification*, Academic Press, London (1982), p. 113.
8. *Biochemical Applications of Mass Spectrometry*, New York (1980), Vol. 1, p. 1859.
9. M. Shamma and L. D. Stiver, *Tetrahedron*, **25**, 3887 (1969).
10. S. S. Yusupova, E. Kh. Batirov, F. Kiyamitdinova, and V. M. Malikov, *Khim. Prir. Soedin.*, 639 (1986).
11. C. Altona and C. A. G. Haasnoot, *Org. Magn. Reson.*, **13**, 417 (1980).