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FLAVONOIDS OF *Juniperus zeravschanica*

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Isoquercitrin and the new flavonglycoside zeravschanoside, 5,6,8,3′,4′-pentahydroxy-7-O-β-D-glucopyranosylflavone, the structure of which was established from chemical transformations and spectral data, were isolated from Juniperus zeravschanica *Kom.*

Key words: *Juniperus zeravschanica*, isoquercitrin, 5,6,8,3′,4′-pentahydroxy-7-O-*β*-D-glucopyranosylflavone.

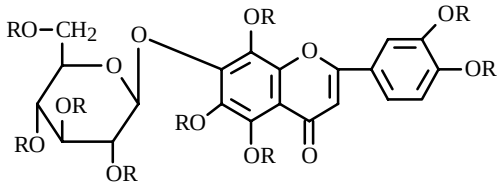
Many species of *Juniperus* L. (Cupressaceae) are rich sources of flavonoids [1] and are widely used in folk and official medicine [2].

Zeravschan juniper (*Juniperus zeravschanica* Kom.) is a dioecious evergreen tree 20 m in height that grows on rocky, gravelly, and thinly soiled slopes of mountains in Central Asia [3].

Compounds **1** and **2** were isolated from the alcohol extract of fruit collected in autumn on mountain slopes near Kumyshkan (Tashkent district).

The UV spectrum (λ_{max}, nm, 276, 343) of the new flavonoid **1** is characteristic of flavone derivatives [4]. The PMR of **1** contains signals for H-3, H-5′, H-6′, H-2′, an anomeric proton, and other protons of a carbohydrate. The chromatographic mobility and PMR spectrum are consistent with a glycoside. This is confirmed by formation of 5,6,7,8,3′,4′-hexahydroxyflavone and D-glucose monosaccharide upon acid hydrolysis of **1**. The aglycone of **1** gives a positive qualitative reaction with $SrCl_2$, which indicates the presence of *ortho*-dihydroxyls in the C-5 and C-6 positions [5], and a positive gossypetin test, which is consistent with hydroxyls on C-5 and C-8 [6].

Acetylation of **1** gave the nonaacetyl derivative **1a**, $C_{39}H_{38}O_{22}$, the mass spectrum of which exhibits a peak for the molecular ion with *m*/*z* 858 and strong peaks for fragments of tetraacetylhexose with *m*/*z* 331, 329, 271, and 169 [7] and the aglycone with *m*/*z* 318.



1: R = H;
1a: R = $COCH_3$

The absence of a bathochromic shift in the UV spectrum of **1** in the presence of NaOAc indicates that the 7-OH of the aglycone was glycosylated [4]. The anomeric proton of D-glucose resonates at 5.53 ppm as a doublet with SSCC 7.0 Hz. Therefore, there is a *β*-glycosidic bond between the carbohydrate and the aglycone [4].

Thus, **1** has the structure 5,6,8,3′,4′-pentahydroxy-7-O-*β*-D-glucopyranosylflavone.

Compound **2** is a flavonol glycoside according to spectral data. Acid hydrolysis produces quercetin (3,5,7,3′,4′-pentahydroxyflavone) [1, 8] and D-glucose. The PMR, UV, and mass spectra, chemical transformations, and comparison of the physicochemical properties with those in the literature identify **2** as isoquercitrin (quercetin-3-O-*β*-D-glucoside) [1, 8].

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EXPERIMENTAL

We used solvent systems $CHCl_3$—CH_3OH (9:1) (1) and *n*-butanol—pyridine—water (6:4:3) (2).

Preparative TLC used LSL 5/40 µm silica gel (Chemapol, Czech Rep.). TLC was performed on Silufol UV-254 plates. Compounds were visualized on TLC in UV light using ammonia vapor. Sugar was detected on PC (Filtrak No. 12) by spraying with acidic anilinium phthalate with heating for 3-5 min at 90-100°C.

Conditions for recording spectra have been published [9].

Extraction and Isolation of Flavonoids. Dried and ground fruit (0.2 kg) of zeravschan juniper that was collected in autumn 1996 on slopes near Kumyshkan (Tashkent) was extracted with ethanol (95%) in a Soxhlet apparatus for 4 h. The alcohol extract was condensed until dry. Then, recrystallization from methanol gave **1** (0.09 g). Preparative TLC of the mother liquor in system 1 gave **2** (0.07 g).

Zeravschanoside (1). $C_{21}H_{20}O_{13}$, mp 328-330°C (methanol). UV spectrum (EtOH, λ_{max}, nm): 276, 343; CH_3CO_2Na 275, 345. IR spectrum (KBr, ν_{max}, cm^{-1}): 3445 (OH), 1665 (C=O of γ-pyrone), 1600, 1560, 1510 (aromatic C=C), 1095, 1030 (C–O of glycosides).

PMR spectrum (δ, ppm, J/Hz, C_5D_5N): 3.50-4.50 (carbohydrate protons), 5.53 (1H, d, J = 7.0, H-1″), 6.86 (1H, s, H-3), 7.12 (1H, d, J = 9.0, H-5′), 7.63 (1H, dd, J = 9.0, J = 2.0, H-6′), 8.06 (1H, d, J = 2.0, H-2′).

Zeravschanoside Nonaacetate (1a). Glycoside **1** (15 mg) was dissolved in pyridine (1 mL) and acetic anhydride (2 mL), worked up as usual after 3 h, and recrystallized from ethanol to produce **1a** (14 mg), $C_{39}H_{38}O_{22}$, mp 248-250°C. Mass spectrum, *m*/*z*: M^+ 858, 331, 329, 318, 271, 169, 109, etc.

Acid Hydrolysis of 1. Glycoside **1** (18 mg) was hydrolyzed by aqueous-methanolic HCl (20 mL, 5%) for 4 h on a boiling-water bath. Then, the methanol was evaporated in vacua. The precipitated aglycone was filtered off and recrystallized from methanol. Yield of 5,6,7,8,3′,4′-hexahydroxyflavone, 8 mg, mp >360°C, $C_{15}H_{10}O_8$ (M^+ 318). D-Glucose was observed in the hydrolysate by PC using system 2.

Isoquercitrin 2. $C_{21}H_{20}O_{12}$, mp 238-239°C. UV spectrum (EtOH, λ_{max}, nm): 255, 265*, 362. IR spectrum (KBr, ν_{max}, cm^{-1}): 3450 (OH), 1660 (C=O of γ-pyrone), 1618, 1575, 1518 (aromatic C=C), 1075, 1026, 1009 (C–O of glycosides). PMR spectrum (δ, ppm, J/Hz, C_5D_5N): 3.65-4.44 (sugar protons), 5.47 (1H, d, J = 7.0, H-1″), 6.51 (1H, d, J = 2.0, H-6), 6.59 (1H, d, J = 2.0, H-8), 7.21 (1H, d, J = 8.5, H-5′), 7.86 (1H, dd, J = 2.0, J = 8.5, H-6′), 8.11 (1H, br.s, H-2′), 13.78 (1H, br.s, 5-OH).

Acid hydrolysis of **2** (5% HCl, 4 h) formed D-glucose and quercetin (3,5,7,3′,4′-pentahydroxyflavone), $C_{15}H_{10}O_7$ (M^+ 302), mp 313-315°C, UV spectrum (MeOH, λ_{max}, nm): 257, 268, 371; CH_3CO_2Na 270, 405.

Acetylation of **2** (acetic anhydride and pyridine) gave the octaacetate, mp 200-202°C (M^+ 770 and peaks for fragments of tetraacetylhexose with *m*/*z* 331, 271, and 169).

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