7-O-METHYLGOSSYPETIN 3-RHAMNOSIDE FROM Atraphaxis pyrifolia

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Continuing an investigation of the chemical composition of some plants of the genus Atraphaxis growing in the territory of Kazakhstan [1, 2], from the leaves of Atraphaxis pyrifolia Bge., by extraction with acetone followed by separation on silica gel we have isolated a new flavonoid glycoside $C_{22}H_{22}O_{12}$ with mp 158-160°C, $[\alpha]_D^{23} -125°$ (c 0.8; methanol), λ_{max} (in absolute ethanol) 258, 360 nm (log ε 4.66; 4.53); with sodium acetate 258, 360; with sodium ethoxide 266, 394; with boric acid and sodium acetate 262, 384; and with zirconyl chloride 266, 404.

In the products of acid hydrolysis we found rhamnose and an aglycone $C_{16}H_{12}O_8$ with a yield of 66%, which shows the monoside nature of the glycoside. The aglycone had mp 270-272°C, λ_{max} 258, 386 nm (log ε 4.63, 4.51); with sodium acetate 258, 386; with sodium ethoxide 274, 450; with boric acid and sodium acetate 264, 398; and with zirconyl chloride 268, 440.

UV spectroscopy with ionizing and complex-forming reagents showed the presence of free hydroxy groups in positions 3', 4', and 5 for the glycoside and 3, 3', 4', and 5 for the aglycone.

The NMR spectrum of the acetate of the glycoside (Fig. 1) showed [in addition to the signals of aromatic protons [3] – a doublet with its center at δ 7.62 ppm, 1 H, J = 2.5 Hz (H-2'), a double doublet with its center at δ 7.53 ppm, 1 H, J = 2.5 Hz, J_1 = 8 Hz (H-6'), a doublet at δ 7.3 ppm, 1 H, J = 8 Hz (H-5'), and a singlet at 6.64 ppm, 1 H, (H-6)] the signals of a methoxy group – a singlet with an intensity of three proton units, four aromatic acetyl groups at δ 2.24, 2.32, and 2.36 ppm (intensity ratio 2 : 1 : 1) present in positions C-3', -4', -8, and -5, respectively [4, 5], and three aliphatic acetyl groups belonging to rhamnose and res-

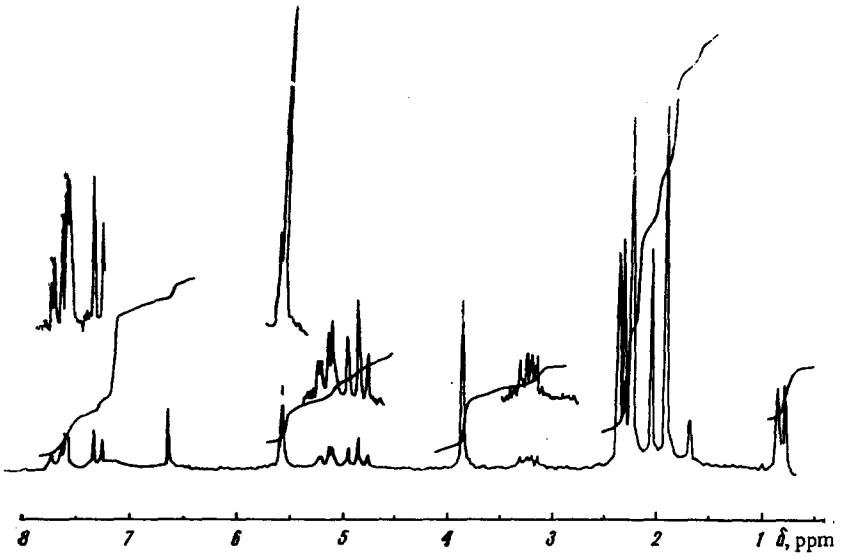

Fig. 1. NMR spectrum of heptaacetate of 7-O-methylgossypetin 3-rhamnoside.

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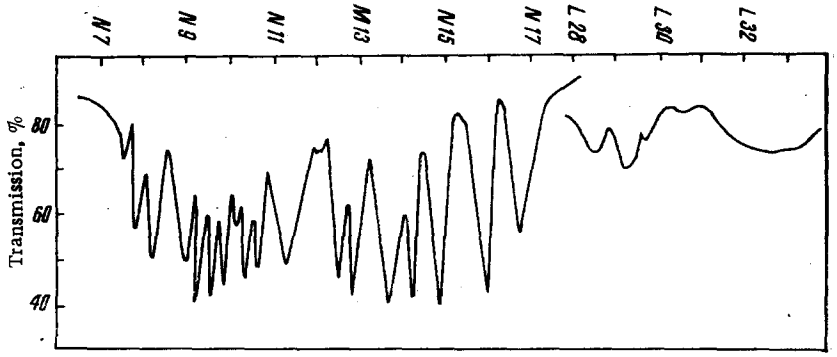

Fig. 2. Differential IR spectrum of 7-O-methylgossypetin 3-rhamnoside

onating at δ 1.92 and 2.06 ppm (intensity ratio 2 : 1). The protons of the rhamnose appeared as follows: triplet with its center at δ 4.80 ppm, 1 H, $J_{4,3}=J_{4,5}=9$ Hz (C-4); quartet with its center at δ 5.18 ppm, 1 H, $J_{3,2}=3$ Hz, $J_{3,4}=9$ Hz (C-3); quartet with its center at 3.20 ppm, 1 H, J = 3 Hz, $J_1=9$ Hz (C-5); and a poorly resolved doublet at δ 5.50 ppm, 2 H, J = 2 Hz, corresponding to the anomeric proton of the rhamnose. The spin–spin coupling constant showed the α-configuration of the bond of the rhamnose with aglycone [6, 7]. The signal of the C-2 proton of the rhamnose was superposed on the signal of the anomeric center. A doublet at δ 0.83 ppm, 3 H, J = 6 Hz was given by the methyl group of the rhamnose attached to the 3-OH group of the flavonoid [8, 9].

Absorption bands in the 840, 1030, 1065, and 1098 cm^{-1} regions in the differential IR spectrum of this glycoside (Fig. 2), and also the results of a polarimetric analysis ($[M]_D \times K_p$ for the glycoside = −298.7°; $[M]_D$ of phenyl α-L-rhamnopyranoside = −254°) showed an α-bond and the pyranose form of the sugar [10].

The results of NMR and IR spectroscopy show the presence of a methoxy group in the glycoside, the position of which at C-7 of ring A is confirmed by UV spectroscopy (absence of a shift with sodium acetate) and by alkaline fusion (formation of protocatechuic acid as the only product detected chromatographically).

The demethylation of the aglycone with pyridinium hydrochloride [11] gave a flavonol coinciding in its physicochemical and spectral properties and color reactions (formation of a red-brown precipitate with an ethanolic solution of p-benzoquinone) with gossypetin [12-14].

The complete methylation of the glycoside with subsequent acid hydrolysis of the methyl derivative and acetylation yielded products with mp 228-230°C and 200-202°C identical with 3-hydroxy-3',4',5,7,8-pentamethoxyflavone and its monoacetate, respectively [15, 16].

Thus, the results obtained have enabled the glycoside isolated to be characterized as 3',4',5,8-tetrahydroxy-7-methoxyflavonol 3-O-α-L-rhamnopyranoside and its aglycone as 3,3',4',5,8-pentahydroxy-7-methoxyflavone [17].

The glycoside is a new natural product.

EXPERIMENTAL METHOD

The melting points of the substances were determined on a Kofler block; the UV spectra were taken on an SF-4A spectrophotometer; the IR spectra (in KBr tablets) on a UR-10 instrument; and the NMR spectrum of the heptaacetate of the glycoside on a Varian HA-100 instrument in deuterated chloroform. For chromatography we used FN-3 paper in the following solvent systems: I) BAW (4 : 1 : 5); II) 15% acetic acid; III) benzene–acetic acid–water (125 : 72 : 3), and for thin-layer chromatography on Silufol plates we used the solvent systems [18]; IV) chloroform–methyl ethyl ketone–methanol (12 : 1 : 2); and V) ethyl acetate–chloroform (1 : 1). The elementary analyses corresponded to the calculated figures.

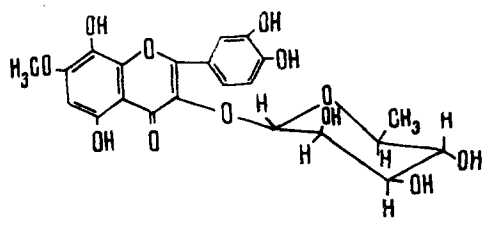

Fig. 3. 7-O-Methylgossypetin 3-O-α-L-rhamnopyranoside.

The combined flavonoids were isolated by extraction of the leaves of Atraphaxis pyrifolia with acetone. The dry evaporated extract was dissolved in a small amount of water and the solution was extracted successively with petroleum ether, chloroform, diethyl ether, and ethyl acetate. In the treatment with the last-mentioned solvent a precipitate de-

posited, the paper chromatography of which in systems I and II showed the presence of three flavonoids with R_f 0.85, 0.60, and 0.80 (I) and 0.75, 0.77, and 0.61 (II). For their separation we used column chromatography on silica gel containing 10% of water, with elution by mixtures of chloroform and methanol in the ratios 48 : 1, 24 : 1, 12 : 1, and 6 : 1. Monitoring of the separation was performed by thin-layer chromatography in system IV. As the result of repeated separation of the intermediate fractions, we isolated a flavonoid $C_{22}H_{22}O_{12}$ with mp 158-160°C (from 50% aqueous methanol) in the form of lustrous light yellow crystals with R_f 0.80, 0.77, and 0.57 (systems I, II, and IV), respectively.

Acid Hydrolysis of the Glycoside. A mixture of 0.2 g of the glycoside and 5 ml of 0.1% hydrochloric acid was heated in the boiling-water bath for two hours. The appearance of the aglycone was observed in the first few minutes of hydrolysis. After the separation of the aglycone, the hydrolyzate was found to contain rhamnose, which was identified by chromatography with a marker. The aglycone, $C_{16}H_{12}O_8$, formed green crystals with mp 270-272°C (from ethanol), R_f 0.82, 0.76 (I, III); sparingly soluble in ethyl acetate and ethanol, readily soluble in acetone.

The aglycone gave a green coloration with $FeCl_3$, was reduced by Mg/HCl, and with a 2% ethanolic solution of p-benzoquinone it formed a brown precipitate only on long standing. In UV light, it had a golden yellow fluorescence.

The acylation of the glycoside was performed with acetic anhydride in pyridine at room temperature (12 h) giving colorless crystals with the composition $C_{36}H_{36}O_{19}$ (V), mp 182-183°C (from ethanol); R_f 0.58.

The acylation of the aglycone was performed under similar conditions; colorless crystals deposited with mp 120°C (from ethanol); composition $C_{26}H_{27}O_{13}$.

Demethylation of the Aglycone. A mixture of 0.03 g of the aglycone and 3 g of pyridinium hydrochloride was heated at 170°C for an hour. Then the reaction mixture was diluted with 50 ml of water, and the precipitate that had deposited was filtered off. From ethyl acetate it formed green crystals with mp 310-314°C (decomp.), R_f 0.41 and 0.26 (I, III). With $FeCl_3$ it gave an olive-green coloration and with a 2% ethanolic solution of p-benzoquinone a brown-red precipitate. In UV light it showed a brown fluorescence.

The methylation of the glycoside [19] was performed with methyl iodide and silver oxide until the reaction with $FeCl_3$ was negative. This led to a brown resinous mass which was hydrolyzed by being boiled with 2% sulfuric acid for two hours. The product was purified on silica gel with elution by chloroform. Recrystallization from ethyl acetate gave bright yellow acicular crystals of 3-hydroxy-3',4',5,7,8-pentamethoxyflavone with mp 228-230°C.

Acetylation with acetic anhydride in absolute pyridine gave colorless crystals of 3-acetoxy-3',4',5,7,8-pentahydroxyflavone with mp 200-202°C (from 80% aqueous methanol).

SUMMARY

From the leaves of Atraphaxis pyrifolia Bge. we have isolated a new flavonoid glycoside. By chemical and spectroscopic investigations its structure has been established as 3',4',5,8-tetrahydroxy-7-methoxyflavonol 3-O-α-L-rhamnopyranoside.

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