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FLAVONOIDS OF GALIUM PALUSTRE

M. I. Borisov and N. F. Komissarenko

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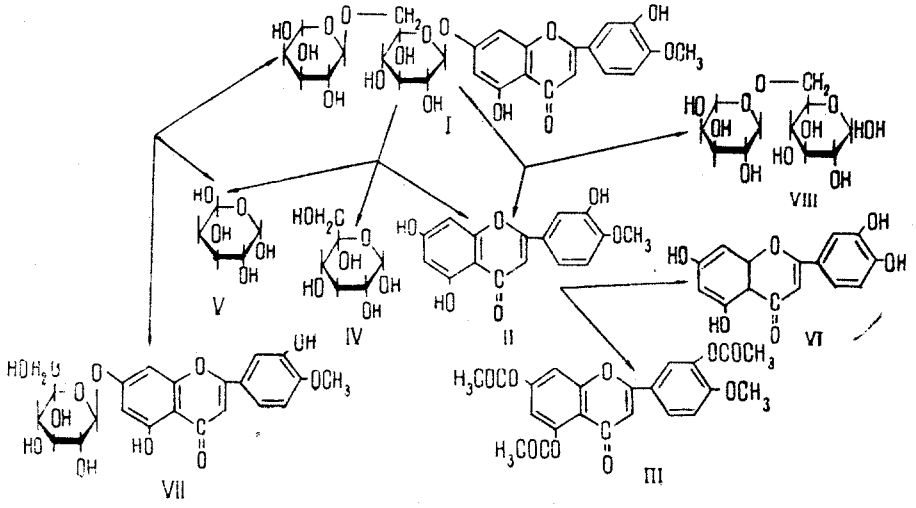
From the roots of Galium palustre L. (marsh bedstraw), family Rubiaceae, by column chromatography on polyamide sorbent we have isolated (together with asperuloside, rutin, and a mixture of two unidentified flavonoids) a new glycoside with the general formula $C_{27}H_{30}O_{15}$ which we have called palustroside (I).



The hydrolysis of I with dilute mineral acids (2-5%) took place with difficulty and lasted several days. An increase in the concentration of the acids (15-20%) led to cleavage into the final products in 2 hr. From the hydrolysate we isolated an aglycone (II) with the composition $C_{16}H_{12}O_6$, and by paper chromatography in various systems we detected the presence of L-arabinose (V) and D-glucose (IV).

On the basis of the molecular weight and elementary composition of II and its triacetyl derivative III, the genin may be assumed to contain three free and one methylated hydroxyls. This was confirmed by a quantitative Zeisel determination [1], which showed the presence of one methoxyl group, while luteolin was isolated from the reaction mixture.

To determine the positions of the free hydroxyls in the molecule of II we recorded its spectra in the UV region with various ionizing and complex-forming reagents [2,3]. It was found that two of them are present at C_5 and C_7. The third hydroxyl was not shown up. This permits the statement that the methoxyl group is located at C_4', since if a free hydroxyl group was present in this position (rather than 3') it would readily be shown up on the addition of sodium ethoxide [3].

Thus, II has the structure of 5,7,3'-trihydroxy-4'-methoxyflavone (diosmetin). The results of a comparison of its physicochemical properties and also the absence of a depression of the melting point of a mixture of II with an authentic sample of diosmetin, confirmed their complete identity [4].

To determine the position and nature of the attachment of the sugar residues in I we used the results of a comparison of the UV spectra of the initial glycoside and its aglycone, enzymatic and acid hydrolyses, and also exhaustive methylation.

The difficulty of acid cleavage, the absence of a bathochromic shift in the UV spectra of I, in contrast to II, on the addition of sodium acetate, and the production of 7-hydroxy-5,3'-4'-trimethoxyflavone as a result of the hydrolysis of the product of complete methylation of I shows that the sugar residue is attached at C_7. On treatment with rhamnodiastase, I was split into II and a biose (VIII). This shows that the sugars are attached to one another by a 1 → 6 glycosidic bond and, probably, that V is terminal.

To confirm this assumption, I was subjected to stepwise hydrolysis with 1% hydrochloric acid. This gave a monoside (VII and V), and the subsequent treatment of VII with the enzymes of the fungus Aspergillus oryzae or with the pancreatic juice of the grape snail led to the splitting off of IV.

The size of the oxide rings of the sugars present in the glycoside and the precise nature of the bond between them was determined by the exhaustive methylation [5, 6] of the initial compound. The methylated product was hydrolyzed; from the hydrolysate 7-hydroxy-5,3',4-trimethoxyflavone was isolated while paper chromatography showed the presence of 2,3,4-trimethyl-L-arabinose and 2,3,4-trimethyl-D-glucose.

The configuration of the glycosidic bonds were established from molecular rotation differences.

On the basis of the investigations carried out, the structure of palustroside can be represented as diosmetin 7-O-β-D-glucopyranosideo(6 → 1)-O-α-L-arabinopyranoside.

Experimental

Isolation of the glycosides. One kilogram of the comminuted air-dry herb Galium palustre L. was treated with boiling 96% ethanol (3 × 8 *l*), the extracts were evaporated, the residue was extracted with 0.5 *l* of boiling water, and the aqueous solution was purified with 4 *l* of diethyl ether (8 × 4.5 *l*) and deposited on a Kapron column (80 × 7 cm).

Water eluted a fraction from which 10.9 g of asperuloside [7] was isolated, while 96% ethanol eluted the combined flavonoids. The concentrated ethanolic eluates yielded 5.75 g of pale yellow acicular crystals of I with mp 176–177° C, readily soluble in aqueous ethanol, dimethylformamide, pyridine, and alkalis, less readily in hot water, and sparingly in 96% ethanol, methanol, ethyl acetate and acetone; it was insoluble in diethyl ether, chloroform, benzene, and petroleum ether. With ferric chloride the aqueous solutions gave a brown coloration. $[\alpha]_D^{18}$ −57° (diemthylformamide).

Found, %: C 54.49, 54.51; H 6.03, 5.11. Calculated for $C_{27}H_{30}O_{15}$, %: C 54.54; H 5.09.

The mother liquor after the separation of I was evaporated to a syrup, dissolved in 150 ml of water, and deposited on a Kapron column (60 × 5 cm). A zone containing two unidentified flavonoids (0.47 g) was eluted with 20% ethanol and a zone containing rutin (6.44 g) with 40% ethanol.

Acid hydrolysis of palustroside (I). A solution of 0.250 g of I in 25 ml of 15% H_2SO_4 was heated in the boiling water bath for 2 hr. The completeness of the hydrolysis was checked by paper chromatography in the benzene–ethyl acetate–acetic acid (24.5: 73.5 : 2) system (formamide). From the cooled hydrolysate, 112 mg of II with mp 257–259° C (from methanol) was isolated.

Found, %: C 64.03, 63.97; H 4.07, 4.06. Calculated for $C_{16}H_{12}O_6$, %: C 64.00; H 4.03.

A mixture of II with an authentic sample of diosmetin [4] gave no depression of the melting point.

The acidic aqueous solution after the separation of II was neutralized with barium carbonate and filtered, and the filtrate was evaporated to the consistency of a syrup. Paper chromatography by the descending method in various sytems showed the presence of IV and V.

Cleavage of palustroside (I) with rhamnodiastase. A solution of 50 mg of I in 50 ml of water was treated with 100 mg of rhamnodiastase, obtained from the fruit of the alder buckthorn, and was left at 38–40° C for 16 hr. The precipitate that deposited was separated off and recrystallized from ethanol, giving 22 mg of II.

The aqueous filtrate was evaporated to 7–10 ml and the enzymes were precipitated by the addition of one and a half volumes of 96% ethanol. The precipitate that deposited was discarded, and the solution was treated similarly twice more, after which it was evaporated to 1.5–2 ml and chromatographed on paper. This showed the presence of the biose VIII which, on hydrolysis with 5% acid, was separated into IV and V.

Stepwise acid hydrolysis of palustroside (I). A solution of 1 g of I in 75 ml of 1% HCl was heated in the boiling water bath for 45 min, rapidly cooled, and deposited on a column of Kapron (60 × 5 cm). Residues of the initial glycoside were eluted with 25% ethanol, and the monoside VII with 60% ethanol. The fractions containing the VII were combined, evaporated in vacuum, and crystallized from ethanol.

Light yellow acicular crystals with mp 266–269° C, $[\alpha]_D^{18}$ −39.0° (dimethylformamide).

Found, %: C 56.2, 56.18; H 4.79, 4.75. Calculated for $C_{22}H_{22}O_{11}$, %: C 56.12; H 4.71.

Enzymatic hydrolysis of the monoside VII. A solution of 20 mg of the monoside in 30 ml of water was treated with 20 mg of an enzyme preparation from Aspergillus oryzae and was left at 38° C for 3 hr. The fermentation mixture was treated with diethyl ether, the ether was evaporated on the water bath, and the residue was crystallized from ethanol, giving 8 mg of II.

The aqueous residue was evaporated to 4 ml and treated with an equal volume of 96% ethanol, the precipitate that deposited was separated off, and the filtrate was concentrated to 1–1.5 ml and chromatographed on paper. The sugar IV was detected.

Acetylation of the aglycone of palustroside (II). 0.2 g of II was acetylated with acetic anhydride in the presence of sodium acetate [8], giving colorless silky acicular crystals with mp 199.5–201.5° C (from ethanol).

Found, %: C 62.01; H 4.23. Calculated for $C_{22}H_{18}O_9$, %: C 61.96; H 4.25.

The acetyl groups were determined quantitatively by the Kuhn-Roth method [9]. The percentage of acetyl groups was 32.08, which corresponds to three such groups.

Demethylation of the aglycone of palustroside (II). A mixture of 50 mg of II, 3 ml of hydriodic acid (d 1.7), 1.4 ml of liquid phenol, and 0.5 ml of acetic anhydride was heated under reflux in a paraffin-oil bath at 150° C for 2 hr. The still-hot reaction mass was poured into water and the mixture was left in the cold. The brown precipitate that deposited was filtered off, dissolved in 5 ml of methanol, and mixed with 1–2 g of Kapron. After the solvent had been evaporated off, the iodine was eluted from the residue with water, while the demethylated flavonoid was eluted with methanol. The eluates were evaporated to dryness and dissolved in 2–3 ml of methanol, and water was added dropwise until an opalescence appeared. In this way 25 mg of yellow crystals of IV with mp 326–327° C was obtained.

Found, %: C 63.01, 62.90; H 3.50, 3.54. Calculated for $C_{15}H_{10}O_6$, %: C 62.94; H 3.52.

A mixture of the demethylated product with an authentic sample of luteolin [4] gave no depression of the melting point.

Methylation of palustroside (I). Palustroside (I); 500 mg (1) was methylated by the method of Mzhel'skaya and Abubakirov [6]. The methylation process was monitored by paper chromatography in the chloroform–formamide system. The partially methylated product was separated on a column of silica gel (30 × 2 cm). The column was washed with benzene, with mixtures of benzene and chloroform with a gradually increasing concentration of the latter, and then with chloroform. The fractions containing the completely methylated product were combined and evaporated, the amorphous residue (97 mg) was dissolved in 5 ml of methanol, and the solution was treated with 5 ml of 20% H_2SO_4 and hydrolyzed in the boiling water bath for 2 hr. After the completion of hydrolysis, 10 ml of water was added and the methanol was driven off in vacuum. The crystals of 7-hydroxy-5,3',4'-trimethoxyflavone that deposited (mp 285–288° C, $C_{18}H_{16}O_6$) were filtered off, and the filtrate was neutralized with the anion exchanger AV-17 and evaporated to a syrup. The syrup was dissolved in acetone and chromatographed on paper in the methyl ethyl ketone saturated with 1% aqueous ammonia [5] and but-1-anol–acetic acid–water (4:1:5) [6] systems. Two methylated sugars were found with R_f values corresponding to those of authentic samples of 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-arabinose.

Conclusions

From the herb Galium palustre L., using adsorption chromatography on Kapron, we have isolated the glucoside asperuloside (1.09%), rutin (0.64%), two unidentified flavonoids (total 0.05%), and a new flavone glycoside (0.57%), which we have called palustroside; on the basis of a study of its chemical and physical properties palustroside has been assigned the structure of diosmetin 7-O-β-D-glucopyranosido-(6 → 1)-O-α-L-arabinopyranoside.

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