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FLAVONOIDS OF *Haplophyllum perforatum*.

STRUCTURE AND HYPOAZOTEMIC ACTIVITY OF HAPLOSIDE C

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A new acylated glycoside of limocitrin has been isolated from the epigeal part of *Haplophyllum perforatum* (M.B.) Kar. et Kir. — haploside C — which has the structure of 3,4',5,7-tetrahydroxy-3',8-dimethoxyflavone 7-O-[O-α-L-rhamnopyranosyl-(1 → 2)-(6"-O-acetyl-β-D-glucopyranoside)]. It has been established that haploside C possesses a pronounced hypoazotemic action.

We have reported previously on the isolation of two new limocitrin glycosides from the epigeal part of the *Haplophyllum perforatum* (M. B.) Kar. et Kir. (family Rutaceae) and the determination of their structures [1].

In the present paper we consider the structure and hypoazotemic activity of new flavonol glycoside, haploside C (I). The presence in the UV spectrum of maxima at 260, 275*, 343*, and 385 nm (log ε 4.38, 4.20, 4.05, and 4.26) and qualitative reactions with magnesium in hydrochloric acid and with zirconium oxychloride in citric acid has permitted compound (I) to be assigned to the flavonol derivatives with a free hydroxy group in the C-3 position [2, 3]. A confirmation of this was the formation of limocitrin (3,4',5,7-tetrahydroxy-3',8-dimethoxyflavone (II)) on the acid hydrolysis of haploside C [1]. D-Glucose and L-rhamnose were detected in a hydrolysate of glycoside (I) by thin-layer and paper chromatography. It followed from UV spectra in the presence of diagnostic additives that haploside C had free hydroxy groups in the 3,4',5 positions [2].

Absorption bands in the IR spectrum at 1730 and 1264 cm^{-1} showed that the substance under consideration contained an ester functional group. In the PMR spectrum of the glycoside (I) at 2.02 ppm a singlet signal was observed corresponding to three proton units. The facts given indicated that haploside C contained one acetyl group.

The alkaline hydrolysis of compound (I) led to substance (II), which was found to be identical with haploside E [1] in its physicochemical constants and by a comparison of IR spectra. The acetyl derivative (IV) obtained by the reaction of glycoside (I) with acetic anhydride in pyridine was likewise identical with the peracetyl derivative of haploside E.

In the PMR spectrum of the TMS derivatives of haploside C, the signals of protons geminal to an acetyl group appeared at 4.14-4.45 ppm in the form of a two-proton multiplet with a geminal coupling constant of ~12 Hz. On passing from compound (I) to its de-aceylated analogs

*Inflection.

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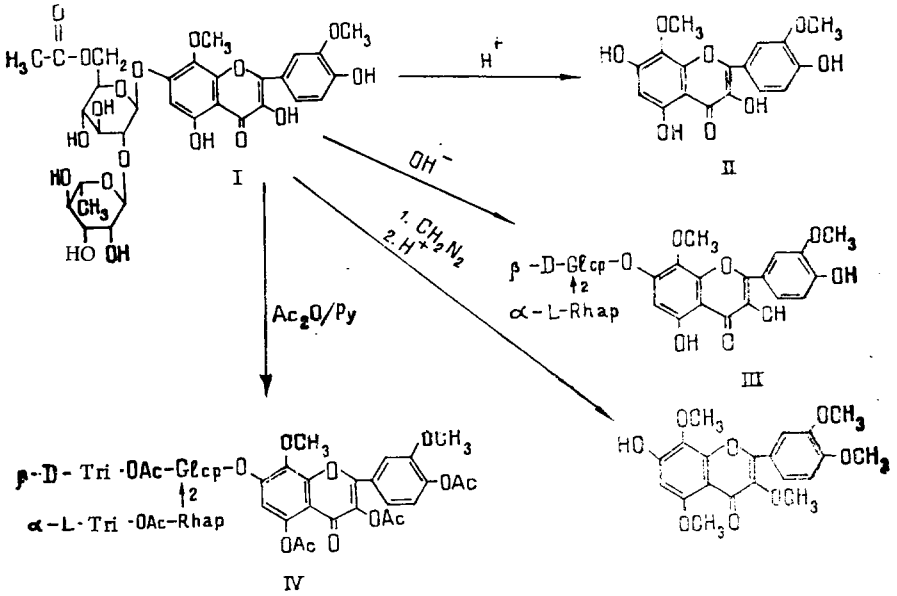
(III), as a result of a diamagnetic shift, this signal fused with the signals of the other protons of the sugar moiety. Consequently, the acetyl group was attached to the primary hydroxy group of the D-glucose residue [4].



In the PMR spectrum of haploside C, taken in deuteropyridine, the signal of the anomeric proton of the D-glucose residue appeared at 5.62 ppm in the form of a doublet with the SSCC ^3J = 7 Hz, which corresponds to the C1 conformation of the monosaccharide ring and the β-configuration of the glycosidic center.

To determine the position of attachment of the carbohydrate moiety unambiguously, haploside C was exhaustively methylated with diazomethane. Acid hydrolysis of the methylation product led to an incomplete methyl ether of gossypetin (V) with mp 249-250°C, identical according to its UV and mass spectra and melting point with 7-hydroxy-3,3',4',5,8-pentamethoxyflavone [5].

Thus, the experimental facts given, in combination, determine haploside C as 3,4',5,7-tetrahydroxy-3',8-dimethoxyflavone 7-O-[O-α-L-rhamnopyranosyl-(1 → 2)-(6''-O-acetyl-β-D-glucopyranoside)].

A pharmacological study of haploside C has shown that it possesses a hypoazotemic action. Thus, a single dose of the compound under investigation led to a fall in the amount of urea [6] and of residual nitrogen [7] by 21-23% ($P < 0.02$). The maximum hypoazotemic effect was observed 2 h after the substance had entered the organism, and then its action weakened and was practically undetectable after 4 h. The prolonged introduction of haploside C into the animal organism led to a more pronounced and stable hypoazotemic effect. This was followed particularly clearly under the conditions of experimental renal pathology [8] accompanied by a fairly pronounced rise in the levels of urea and residual nitrogen in the blood. In rats of the control group, a day after the beginning of the experiment a rise in the level of urea and residual nitrogen by 163-166% was observed ($P < 0.01$), and this effect continued for a long time (6 days). Animals receiving haploside C every day were characterized by lower levels of the final products of the nitrogen metabolism and one day after the induction of the renal pathology this increase was already only 101-104%, while after three days it was 48-49%, by the sixth day the numerical indices had normalized, and by the tenth day there was even some fall in the initial level.

Comparative investigations showed that in its activity haploside C was slightly inferior to the drug lespenephryl.

EXPERIMENTAL

For general observations, see [1]. PMR spectra were taken on a Varian HA-100D instrument at 100 MHz with tetramethylsilane as internal standard. Chromatographic control was performed

by TLC (Silufol UV-254) in the solvent systems ethyl acetate—methanol—water (10:2:1) and chloroform—methanol (4:1), and PC in the systems butan-1-ol—pyridine—water (5:3:1) and butan-1-ol—methanol—water (5:3:1). Type SK silica gel, 160-250 μm was used for column chromatography.

Isolation of Haploside C. Part of the butanolic fraction (35.0 g) of an ethanolic extract of the inflorescences and leaves of *Haplophyllum perforatum* [9] was chromatographed on a column (5 × 130 cm) of silica gel (700 g) in the chloroform—propanol (9:1 → 6:4) system. At a composition of the mixture of 8:2, a flavonoid was eluted which contained coumarin glycosides as an impurity. This was rechromatographed in a column of polyamide solvent with elution by water until the bright blue fluorescence of the eluates in UV light due to the presence of coumarins had disappeared. Then the flavonoid was desorbed with ethanol, the eluate was evaporated in vacuum, and the residue was crystallized from aqueous ethanol. This gave 7.64 g of haploside C.

Haploside C (I). Yellow crystals from aqueous ethanol with the composition $C_{31}H_{36}O_{18}$, mp 143-145°C, $[\alpha]_D^{22}$ 0 ± 3° (c 0.35; methanol), $\lambda_{max}^{ethanol}$ (nm) 260, 275*, 343*, 385; +CH_3COONa 264, 394, 432*; +CH_3COONa + H_3BO_3 260, 277*, 342*, 383; +CH_3ONa 265, 373*, 458; +$AlCl_3$ 271, 374, 448, ν_{max}^{KBr} (cm^{-1}): 3520-3270 (OH groups); 1730, 1650 (C=O of a γ-pyrone); 1620, 1600, 1566. PMR spectrum in Py-d_5 (ppm): 1.73 (3H, d, 6 Hz — CH_3), 2.02 (3H, s, —$OCOCH_3$), 3.87 (3H, s, OCH_3), 4.16 (3H, s, —OCH_3), 3.78-4.96 (protons of the sugar moiety), 5.62 (d, 7 Hz, H-1"), 6.46 (s, OH), 6.98 (s, H-6), 7.31 (d, 8.5 Hz, H-5'), 8.24 (br.s, H-2') 8.28 (dd, 2 and 8.5 Hz H-6').

PMR spectrum of the TMS derivative in CCl_4 (ppm): 1.13 (d, 5.5 Hz, —CH_3), 1.79 (s, —OCO-CH_3), 3.42-4.14 (8H of the sugar moiety), 3.88 (s, OCH_3), 3.96 (s, OCH_3), 4.30 (2H, m, J_{gem} = 12 Hz, —CH_2OCOCH_3), 4.84 (br.s, H-1'''), 5.45 (d, 4 Hz, H-1"), 6.44 (s, H-6), 6.87 (d, 8 Hz, H-5'), 7.74 (dd, 2 and 8 Hz, (H-6'), 7.85 (d 2 Hz, H-2').

Acid Hydrolysis of Haploside C (I). Glycoside (I) (75 mg) was hydrolyzed with 5% sulfuric acid on the water bath for 2 h. The precipitate that deposited was separated off, washed several times with water, dried, and recrystallized from methanol. This gave 31 mg of an aglycon with mp 271-273°C (with sublimation), M^+ 346; $\lambda_{max}^{ethanol}$ (nm) 260, 274, 333, 382, which was identified as limocitrin. The filtrate was neutralized with AV-10G anion-exchange resin, filtered, and evaporated. D-Glucose and L-rhamnose were found in the residue by TLC and PC.

Alkaline Hydrolysis of Haploside C (I). Glycoside (I) (50 mg) was saponified with a 0.5% solution of KOH at room temperature for 40 min. After neutralization with 5% HCl solution, the reaction mixture was extracted with n-butyl alcohol. The butanolic extract was washed with water and was evaporated to dryness. The residue was chromatographed on a column of polyamide in the chloroform—methanol (7:3) system. This gave 27 mg of an individual substance with mp 183-184°C which was identified from its IR spectrum and by TLC in comparison with an authentic sample of haploside C.

Acetylation of Haploside C (I). A solution of 45 mg of glycoside (I) in 1 ml of pyridine was treated with 2 ml of acetic anhydride. After a day, the reaction mixture was worked up in the usual way, giving a peracetate with mp 195-196°C (from ethanol), identical with the acetate of haploside E according to its IR spectrum and a mixed melting point.

7-Hydroxy-3,3',4',5,8-pentamethoxyflavone (V) from Haploside C (I). A solution of 70 mg of glycoside (I) in absolute methanol was mixed with an excess of an ethereal solution of diazomethane and the mixture was left in the refrigerator for 24 h. After the solvent had been driven off, the methylation process was repeated twice more. The reaction product was hydrolyzed with 5% HCl on a water bath for 3 h. The reaction mixture was diluted with water, and was exhaustively extracted with chloroform. The chloroform extract was washed with water dried with anhydrous sodium sulfate, filtered, and concentrated. The residue was subjected to preparative TLC on silica gel in the chloroform—methanol (11:1.5) system. This yielded 15 mg of an individual compound with mp 249-250°C, $C_{20}H_{20}O_8$, $\lambda_{max}^{ethanol}$ (nm), 254, 273, 352 (log ε 4.73, 4.22, 4.18).

Mass spectrum, m/z (%): M^+ 388 (100%), 387 (66.5), 373 $(M - 15)^+$ (80), 359 (6.5), 358 (9), 357 $(M - CH_3O)^+$ (14.5), 355 (7), 343 $(M - CH_3 - CO)^+$ (6), 327 (6), 315 (5), 197 (A^+) (4), 181 (5), 180 (8), 165 (B^{2+})(10), 149 (8.5).

SUMMARY

The new glycoside haploside C has been isolated from the epigeal part of the plant *Haplophyllum perforatum*, and its structure has been established as 3,4',5,7-tetrahydroxy-3',8-dimethoxyflavone 7-O [O-α-L-rhamnopyranosyl-(1 → 2)-(6"-O-acetyl-β-D-glucopyranoside)].

When this compound is introduced into the animal organism, a fall in the level of urea and residual nitrogen in the blood serum is observed, as on the use of lespenephryl.

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REACTIVITY OF CARBONYL-CONTAINING DERIVATIVES OF 1-MONO- AND 1,3-DIPHENYLPROPANES.

I. KINETICS OF THE OXIMATION REACTION OF FURANOCHROMONE, DIHYDROFLAVONONE, AND CHALCONE DERIVATIVES

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The rate constants of the oximation reaction of more than twenty compounds belonging to the 1-mono and 1,3-diphenylpropane derivatives, and their activation energies have been determined. The oximation reaction depends on the structure and position of the substituents in the γ-pyrone nucleus where such is present, and on the degree of oxidation (reduction) of the pyrone fragment of 2-phenylbenzo-γ-pyrone, and also on the temperature.

Carbonyl compounds belonging to derivatives of 1-phenylpropane — benzopyrones (furanochromones) — and of 1,3-diphenylpropane — chalcones (chalcone, isoliquiritigenin and its glycosides — licuroside), flavones (luteolin), flavanones (liquiritigenin, naringenin) and others — are widely distributed in the vegetable kingdom [1]. Some of them are used in medical practice as vessel-strengthening, cholagogic, antiinflammatory, antiulcer, spasmolytic, antimicrobial, and other agents [2-5]. Analysis of these compounds is carried out mainly by spectral methods [6]. However, the most selective method for the quantitative estimation of these substances is analysis from functional groups (aldehyde, ketone, etc., groups). The hydroxylamine method is frequently used to determine aliphatic and aromatic aldehydes and ketones, sugars, and steroid hormones [7, 8]. This method is also used for the analysis of such a spasmolytic drug as khellin [7]. However, the influence of the position, structure, and number of substituents in the molecules of furanochromone, flavone, flavanone, and 1,3-diphenylpropane derivatives on their reactions with hydroxylamine has not been investigated.

The present paper gives the results of a study of the kinetics of the oximation reaction at the carbonyl group of the γ-pyrone ring for benzopyrone derivatives and for 1,3-diphenylpropane derivatives containing such a ring and of a determination of the dependence of the rate

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