at 80°C for 20 min. After the usual working up and chromatographic purification, 17 mg of a substance with mp 202-204°C, M^+ 192, identical with scopoletin, was obtained.

Acetylation of (I). A solution of 45 mg of (I) in 1 ml of pyridine was treated with 2 ml of acetic anhydride. After a day the reaction mixture was diluted with water and extracted with chloroform. After drying and the removal of the solvent by distillation, an acetyl derivative with mp 98-99°C was obtained.

Acetylation of Isofraxetin. Compound (III) (40 mg) was acetylated with 2 ml of acetic anhydride in the presence of pyridine (1 ml) at room temperature. After the usual working up, a diacetate was obtained with mp 180-182°C (ethanol).

SUMMARY

β-Sitosterol, isofraxetin, and a new coumarin $C_{15}H_{18}O_6$ with mp 135-137°C, which has been called obtusinin, have been isolated from the epigeal part of *Haplophyllum obtusifolium*. The structure of obtusinin has been established on the basis of chemical transformations and spectral characteristics.

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HAPLOSIDE A — A NEW ACYLATED FLAVONOL GLYCOSIDE FROM *Haplophyllum perforatum*

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The epigeal part of *Haplophyllum perforatum* has yielded a new acylated flavonol glycoside, haploside A, for which the structure of 3,4',5,7,8-pentahydroxy-3'-methoxyflavone 7-O-(6"-O-acetyl-β-D-glucopyranoside) has been established. The aglycone of the glycoside isolated — 3,4',5,7,8-pentahydroxy-3'-methoxyflavone — has also proved to be new and it has been called haplogenin. The structures of the compounds mentioned have been established on the basis of UV, IR, PMR, and mass spectra and the products of acetylation and of acid and alkaline hydrolysis.

According to the literature, the flavonoids of the plants of the genus *Haplophyllum* (family Rutaceae) have not been studied [1]. We have begun the study of the flavonoids of *H. perforatum* growing in the foothills of the Alim-Tau mountains (Southern Kazakhstan). From an ethanolic extract of the epigeal part of the plant collected in the flowering period (June, 1978) a flavonoid with the composition $C_{24}H_{24}O_{14}$ (I) has been isolated by adsorption chromatography on a column of silica gel. It has proved to be new, and we have called it haploside A. According to qualitative reactions [2] and UV spectroscopy [3] (λ_{max}, nm, 261, 280 sh, 343, 389), the flavonoid isolated belongs to the flavonol group.

Compound (I) is a glycoside, as was shown by Bryant's qualitative cyanidin reaction, spectral characteristics, and the existence of optical rotation. In actual fact, the acid hydrolysis of (I) gave D-glucose and an aglycone with mp 218-221°C, M^+ 332.

A study of the PMR spectrum of haploside A permitted it to be assigned to the monoglycosides. In the spectrum taken in deuteropyridine (Fig. 1), in addition to the signals of

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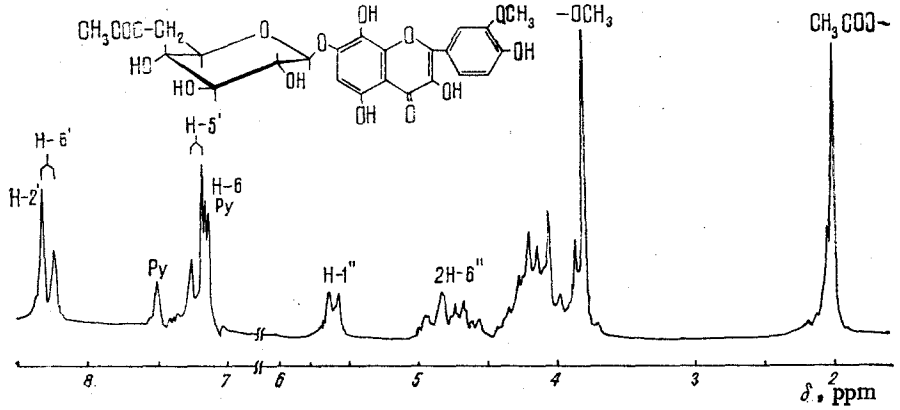

Fig. 1. PMR spectrum of haploside A in deuteropyridine.

methoxy and acetyl groups and of four aromatic protons, the signals of six glucose protons resonate at 3.75-4.90 ppm (with the deduction of the three protons of a $-OCH_3$ group), and at 5.50 ppm there is the signal of the anomeric proton in the form of a doublet with a spin–spin coupling constant (SSCC) of 7 Hz, which is characteristic for glucose in the C1 conformation attached to an aglycone by a β-glycosidic bond [3].

The acetylation of (I) with acetic anhydride in pyridine gave its octaacetate (II). The results of a study of the weak-field region of the PMR spectrum of the flavonoid itself and of its acetate showed that compound (I) is trisubstituted in ring A and disubstituted in ring B. Haploside A has two maxima in the shortwave region of the UV spectrum that are characteristic for3',4'-disubstituted flavonoids [3].

The substituting groups in ring A occupy the C-5,7,8 positions, since in the PMR spectrum of (II) the H-6 signal appears at 6.73 ppm [4, 5]. In the high-field region of the spectrum of (II) there are the signals of the protons of four aliphatic acetyl groups (1.95-2.05 ppm, 12 H) and of four aromatic acetyl groups (2.24, 2.28, 2.36 ppm; intensity ratio 1:2:1), present in the C-4',3,8,5 positions, respectively [6, 7]. The presence of hydroxy groups in these positions of (I) was also established by UV spectroscopy with ionizing and complex-forming additives, and also by a positive gossypetin test. Under the action of sodium acetate the spectrum of (I) did not change, and its aglycone decomposed under these conditions. This indicates that the glucose is attached to the aglycone through the hydroxyl at C-7.

The position of the methoxy group at C-3' was established from the results of UV spectroscopy and the formation of vanillic acid on the alkaline cleavage of the aglycone.

The presence in the IR spectrum of, apart from the absorption bands characteristic for flavonoids, a strong band of an ester group (1720 cm^{-1}) and also the presence in the PMR spectrum of the signal of the protons of an aliphatic acetoxy group (1.99 ppm) show that (I) is a flavonol glycoside acylated in the carbohydrate moiety.

The alkaline saponification of (I) under mild conditions gave acetic acid (identified by PC in the form of the diethylammonium salt) and the monoglucoside (III). The IR spectrum of the latter lacked the absorption band of an ester grouping.

The position of the acyl residue follows from the characteristics of the PMR spectrum. In the weak field, the spectrum of (I) contains a two-proton multiplet with its center at 4.66 ppm, one of the spin–spin coupling constants of which is 12 Hz, which permits the signal to be assigned to two geminal protons in the C-6" position of the glucose [8].

Thus, haploside A has the structure of 3,4', 5,7,8-pentahydroxy-3'-methoxyflavone 7-O-(6"-O-acetyl-β-D-glucopyranoside). The aglycone of (I), 3,4',5,7,8-pentahydroxy-3'-methoxyflavone is also new, and we have called it haplogenin.

We are the first to have detected the presence of flavonoids in plants of the genus *Haplophyllum*.

EXPERIMENTAL

The homogeneity of the substances was checked by thin-layer chromatography (TLC) on Silufol plates and on silica gel L 5/40 μ in the following systems: 1) ethyl acetate–ethanol–water (13:5:2) and 2) toluene–ethyl acetate–ethanol (1:1:1).

For paper chromatography we used the following systems: 3) butan-1-ol—pyridine—water (6:4:3), 4) butan-1-ol—diethylamine—water (50:0.5:7.5), and 5) water-saturated butan-1-ol. Column chromatography was performed on Woelm Pharma silica gel (GFR). The UV spectra were taken on a Hitachi spectrophotometer in methanol, the IR spectra on a UR-20 instrument in tablets with KBr, and the PMR spectra on a JNM-4H-100 instrument (100 MHz) with HMDS as internal standard (δ scale). The mass spectrum was obtained on a MKh-1303 instrument fitted with a system for direct introduction into the ion source.

Isolation. The comminuted air-dry raw material (3 kg) was extracted five times with ethanol at room temperature. The extract was concentrated in vacuum, duluted with water, filtered, and extracted successively with chloroform, ether, ethyl acetate, and butanol. After the solvents had been distilled off, 7.34 g of chloroform fraction, 1.46 g of ether fraction, 20.2 g of ethyl acetate fraction, and 55.0 g of butanol fraction were obtained. When the ethyl acetate extract was concentrated, a precipitate (1.75 g) deposited which, according to TLC, consisted of a mixture of two substances. It was chromatographed on a column of silica gel. The flavonoids were eluted with mixtures of chloroform and ethyl acetate. At compositions of the mixtures of 4:1 and 3:2, 0.65 g of substance (I) was eluted from the column.

Haploside A formed light yellow crystals soluble in ethanol and methanol with mp 192-195°C, $[\alpha]_D^{26}$ −145.4° (c 0.22; CH_3OH), R_f 0.83 (system 1), 0.55 (system 2).

IR spectrum, cm^{-1}: 3510, 3200-3400 (OH group), 1720 (ester C=O), 1653 (C=O of a γ-pyrone ring), 1620, 1610, 1567, 1516 (C=C bonds in rings), 1465, 1445, 1375, 1352, 1323, 1277, 1240, 1195, 1150, 1126, 1092, 1048, 1028, 1000, 876, 820, 795, 752. UV spectrum, nm: λ_{max} (MeOH) 261, 280 sh, 343, 389; (+NaOAc) 262, 281 sh, 390; (+$AlCl_3$) 272, 382, 453; (+$AlCl_3$+HCl) 271, 375, 450; (+NaOAc+H_3BO_3) 261, 280, 390; (+MeONa) — the substance decomposed.

PMR spectrum (in Py-d_5), ppm: 1.96 (s, CH_3COO—), 3.72 (s, —OCH_3), 3.75-4.90 (m, 6 H of glucose), 4.43-4.89 (m, J_{gem} = 12 Hz, 2 H-6''), 5.50 (d, 7 Hz, H-1''), 7.09 (s, H-6), 7.16 (d, 8 Hz, H-5'), 8.25 (q, 2.5 and 8 Hz, H-6'), 8.29 (H-2').

Acid Hydrolysis of (I). A mixture of 35 mg of (I) and 10 ml of 2% HCl was heated on the boiling water bath. The course of hydrolysis was followed by TLC (system 2). After being heated for three hours, the substance had hydrolyzed completely. The precipitate of the aglycone that deposited after cooling was filtered off, washed with water to neutrality, and purified on a column of cellulose. Ethyl acetate eluted 15 mg of the aglycone with mp 218-221°C (petroleum ether), M^+ 332, R_f 0.65 (system 2), 0.91 (system 1). The aqueous residue was neutralized on AV-10G anion-exchange resin and evaporated. D-Glucose was detected by PC in system 3.

3,4',5,7,8-Pentahydroxy-3'-methoxyflavone (haplogenin) formed yellow crystals soluble in ethanol and ethyl acetate with mp 218-221°C, composition $C_{16}H_{12}O_8$, M^+ 332.

IR spectrum, cm^{-1} 3250-3470 (OH groups), 2930 (OCH_3), 1656 (C=O of a γ-pyrone), 1628, 1610, 1570, 1520 (C=C bonds in rings), 1469, 1384, 1330, 1286, 1215, 1158, 1127, 1070, 1035, 1000, 884, 828, 794, 726.

UV spectrum, nm: λ_{max} (MeOH) 262, 279, 386; (+NaOAc) 280, 376 — the substance decomposed; (+$AlCl_3$) 268, 370, 446; (+$AlCl_3$+HCl) 266, 370, 444; (+NaOAc+H_3BO_3) 260, 283, 388; (+MeONa) 408 — the substance decomposed.

Alkaline Hydrolysis of (I). A solution of 40 mg of (I) in 6 ml of 0.5% NaOH was left at room temperature for 40 min. Then it was neutralized with 10% HCl and extracted with diethyl ether (4 × 15 ml). The ethereal extract was evaporated, and the residue was brought to pH 10 with diethylamine.

Diethylammonium acetate was identified by paper chromatography with markers in systems 4 and 5. Then the saponification product was extracted from the aqueous residue with butanol (3 × 15 ml). The solution was distilled and the residue was purified by column chromatography on silica gel.

Saponification Product (III). R_f 0.34 (TLC, system 2). IR spectrum, cm^{-1}: 3200-3500 (OH group), 2935 (OCH_3), 1664 (C=O of a γ-pyrone), 1605, 1574, 1525 (C=C bonds in rings), 1467, 1439, 1380, 1280, 1215, 1180, 1128, 1077, 1035, 827, 796, 730.

Acetylation of (I). A solution of 25 mg of (I) in 0.5 ml of pyridine was treated with 2 ml of acetic anhydride and the mixture was left at room temperature.

After a day, it was poured into ice water and the mixture was stirred. The reaction product was extracted with chloroform, and the solution was washed with water, dried, and evaporated. After recrystallization from ethanol, the acetyl derivative of (I) with mp 197-198°C (II) was obtained.

PMR spectrum (in $CDCl_3$): 1.96-2.03, 12H (four aliphatic CH_3COO groups at C-2",3",4",6"), 2.24 (s, aromatic CH_3COO group at C-4'), 2.28, 6 H (s, CH_3COO groups at C-3, 8); 2.36 (s, CH_3COO– at C-5); 3.65 and 3.90 (dd, $J_{4",5"}$ = 6.5 Hz, H-5"); 3.80 (s, OCH_3); 4.05-4.25 (m, 2H-6"), 4.95-5.29, 4 H (m, H-1",2",3",4"); 6.73 (s, H-6), 7.04 (d, 8.5 Hz, H-5'); 7.21 (d, 2.5 Hz, H-2'); 7.25 (q, 2.5 and 8.5 Hz, H-6').

SUMMARY

The presence of flavonoids in *Haplophyllum perforatum* has been revealed. From the epigeal part of this plant a new acylated flavonoid glycoside haploside A has been isolated, and its structure has been determined as 3,4',5,7,8-pentahydroxy-3'-methoxyflavone 7-O-(6"-O-acetyl-β-D-glucopyranoside).

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FLAVONOID GLYCOSIDES OF SPORE-BEARING STEMS OF *Equisetum arvense*

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In an extract of spore-bearing stems of *Equisetum arvense* L. (field horsetail) saponaretin, apigenin 5-glucoside, luteolin 5-glucoside, kaempferol 3-sophoroside, quercetin 3-glucoside, 4-hydroxy-6-(2-hydroxyethyl)-2,2,5,7-tetramethylindanone, and a compound of ketonic nature, isolated previously from an extract of the herbage of the field horsetail, have been identified by high-performance liquid chromatography. The characteristics of the chromatographic behavior of glycosylated flavones under the conditions of reversed-phase liquid chromatography have been studied and it has been shown that the glycosylation of flavones at position 5 of the molecule causes a greater fall in the affinity for a nonpolar stationary phase than in position 7 of the molecule.

Phenolic acids and flavonoid aglycones have been identified in the form of their TMS ethers previously in an extract from spore-bearing stems of *Equisetum arvense* (field horsetail) by the gas–liquid chromatography (GLC) method [1]. Using high-performance liquid chromatography, we have now identified flavonoid glycosides in this extract without their

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