Hydrolysis of (I). A mixture of 0.1 g of the glucoside of 2 ml of 3% HCl was heated in the boiling water bath for 1.5 h. The completeness of hydrolysis was confirmed by thin-layer chromatography on Silufol in the chloroform-methanol (10:1) system. The aglycone that deposited on cooling was filtered off. The filtrate was neutralized with anion-exchange resin, separated from the resin, and extracted with ethyl acetate. An additional amount of aglycone (making a total of 0.06 g) passed into the organic extract. The substance recrystallized from ethyl acetate at mp 184-186°C, M^+ 264. The PMR spectrum, mass-spectrometric fragmentation, and other characteristics coincided with those reported [3] for peucedanol (II).

The mother liquor from the crystallization of (II) contained another two coumarins. In their mobility on Silufol in the solvent system given above they corresponded to authentic samples of (III) and (IV).

SUMMARY

A new coumarin, 3'-β-D-glucopyranoside peucedanol, has been isolated from the epigeal part of *Phlojodicarpus turczaninovii*. Its structure has been established by chemical and spectral methods.

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FLAVONOIDS OF *Haplophyllum perforatum*.

NEW GLYCOSIDES OF LIMOCITRIN

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Two new glycosides of limocitrin have been isolated from the epigeal part of *Haplophyllum perforatum* (M. B.) Kar. et Kir. On the basis of chemical transformations and spectral characteristics the structures of the substances isolated have been established as 7-(6"-acetyl-β-D-glucopyranosyloxy)-3,4',5-trihydroxy-3',8-dimethoxyflavone and 7-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-3,4',5-trihydroxy-3',8-dimethoxyflavone.

We have previously demonstrated the presence of flavonoids in the plant *Haplophyllum perforatum* (M. B.) Kar. et Kir. [1, 2]. A further study of the components of ethyl acetate and butanol fractions of an ethanolic extract of the epigeal part has led to the isolation of another four new flavonoids. In the present paper we report on the determination of the structures of two of them.

The compounds, with the composition $C_{25}H_{26}O_{14}$ (I) and $C_{29}H_{34}O_{17}$ (II), were assigned on the basis of their UV spectra, qualitative reactions with zirconium oxychloride in citric acid, and their bright yellow fluorescence in UV light to the group of flavonols with free hydroxyls at C-3. According to the results of acid hydrolysis, both substances were glycosides. It was established by TLC and GLC that the carbohydrate moiety of (I) consisted of D-glucose and that of (II) D-glucose and L-rhamnose. The aglycones of the glycosides (I) and (II) proved to be the same compound with the composition $C_{17}H_{14}O_8$, M^+ 346 (III). UV spectra with diagnostic additives showed the presence of free phenolic hydroxy groups in the C-3, C-5, C-7, and C-4' positions of the aglycone [3].

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The presence of a methoxy group in the sixth or eighth positions of (III) was shown by its mass spectrum, which contained a strong peak of an ion with m/z 331 $(M - CH_3)^+$ [4]. The relative intensities of the peaks of the ions M^+, M − 1, and M − 15, and the absence of the peak of a M − 18 ion, characterized (III) as a derivative of 5,7-dihydroxy-8-methoxyflavone [5]. The alkaline cleavage of the aglycone formed vanillic acid, and demethylation with pyridine hydrochloride formed gossypetin. These facts and its physical constants positively identified (III) as limocitrin [6, 7]. Thus, flavonoids (I) and (II) are limocitrin glycosides.

A study of the mass and PMR spectra of the acetates of (I) and (II) showed that the former was a monoside and the latter a bioside. Furthermore, (I) was an acylated glycoside, as was shown not only by its IR spectrum (1735 cm^{-1}, ester carbonyl) but also by its PMR spectrum in Py-d_5, which contained a three-proton singlet from $-OCOCH_3$ at 2.02 ppm.

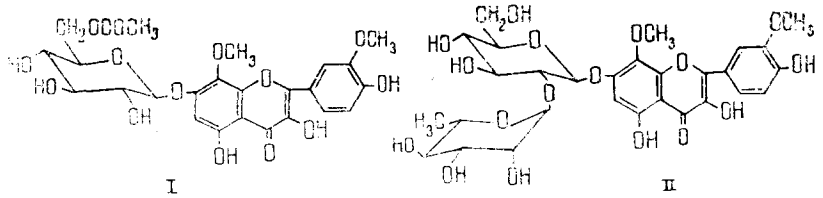
In the PMR spectrum of (I), the signal of the anomeric proton appeared at 5.68 ppm in the form of a doublet with a spin-spin coupling constant ^3J = 7.5 Hz, which showed the C1 conformation of the monosaccharide ring and, consequently, the β configuration of the glycosidic center of the D-glucose residue [8]. A two-proton multiplet with a geminal coupling constant of ~12 Hz resonated in the 4.45-4.96 ppm region which was due to the protons of a gem-acyl methylene group [1, 9]. Consequently, in the molecule of (I) the primary hydroxy group of the D-glucose residue was acylated.

The UV spectra of (I) and (II) taken in methanol with the addition of diagnostic reagents were similar. The absence of a bathochromic shift of the long-wave band in the presence of sodium acetate showed that the position of glycosylation in both compounds was the C-7 hydroxy group.

Thus, glycoside (I) has the structure of 7-(6"-acetyl-β-D-glucopyranosyloxy)-3,4',5-trihydroxy-3',8-dimethoxyflavone.

To determine the structure of its carbohydrate moiety, glycoside (II) was methylated by Hakomori's method [10]. In a hydrolysate of the methylation product 2,3,4-tri-O-methyl-L-rhamnose and 3,4,6-tri-O-methyl-D-glucose were identified by GLC. Consequently, in the (II) molecule the L-rhamnose was the terminal sugar residue and was attached to the D-glucose residue by a 1 → 2 bond, and both sugars had pyranose oxide rings. In the spectrum of the TMS ether of (II) taken in CCl_4, the signals of the anomeric protons of the glucose and rhamnose residues resonated at 5.26 ppm (d, ^3J = 7 Hz) and 4.89 ppm (br.s, $W_{1/2}$ = 2 Hz). The spin-spin coupling constants of these signals showed the C1 conformation of the glucopyranose ring and the 1C conformation of the rhamnopyranose ring and, consequently, the glycosidic center of the D-glucose residue had the β configuration and that of the L-rhamnose residue the α configuration [8, 11, 12].

Thus, the structure of (II) is that of 7-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-3,4',5-trihydroxy-3',8-dimethoxyflavone. This glycoside has previously been called haploside E.



I II

Haplophyllum perforatum contains glycosides of limocitrin and of the 3'-O-methyl ether of gossypetin, which we have called haplogenin.

Later, J. Harborne reported on the isolation of haplogenin 3-O-rutinoside from *Coronilla varia* (family *Leguminosae*) [13]. Since it is mainly 7-O-glycosides of haplogenin and limocitrin that are found in *Haplophyllum perforatum*, they can readily be distinguished by the qualitative reaction with p-benzoquinone (gossypetin test). Furthermore, the glycosides of these aglycones differ in their color on a chromatogram (TLC on Silufol UV-254) without treatment with chromogenic reagents. The yellow spots of haplogenin glycosides become green after 1 h and then acquire a blue-green coloration, while limocitrin glycosides do not change their yellow coloration. Such behavior of the haplogenin glycosides is probably connected with the presence of the readily oxidized 5,8-dihydroxychromone grouping.

It must be mentioned that in the plant *Coronilla varia* [13], as in *Haplophyllum perforatum*, pairs of haplogenin and limocitrin glycosides are found which do not differ even in relation to the position of attachment of the sugar moiety to the aglycone. This fact shows a common scheme of biosynthesis and a possible mutual transition of the above-mentioned flavonoids and their glycosides.

EXPERIMENTAL

For general remarks, see [1]. The following solvent systems were used: 1) butanol—methanol—water (5:3:1); 2) chloroform—hexane (9:1); and 3) chloroform—methanol (50:1). For column chromatography we used type L silica gel (Czechoslovakia) with a grain size of 100/250 μm.

The sugars and their derivatives were detected on TLC by spraying with o-toluidine salicylate followed by heating at 100-105°C for 2-5 min.

Gas-liquid chromatography was performed on a Chrom-5 chromatograph. The mass spectra of the glycoside acetates were taken on an MKh-1310 instrument with an ionizing voltage of 50 V.

Isolation of the Flavonoids. The air-dry comminuted epigeal part of *Haplophyllum perforatum* (3.7 kg) collected in the Surkhandar'inskaya province in the budding stage (May, 1982) was extracted eight times with ethanol at room temperature. The extract was evaporated in a vacuum to 1.5 liters and was diluted with water (1:1) and the resulting precipitate was filtered off. The filtrate was extracted successively with chloroform and with ethyl acetate. The ethyl acetate extract, after the solvent had been distilled off, gave 22.0 g of total extractive substances, which were chromatographed on a column of silica gel in the chloroform-methanol (48:2—75:25) system. From individual fractions, by rechromatography on silica gel and recrystallization from ethanol, 0.15 g of (I) and 0.45 g of (II) were isolated.

Limocitrin 7-O-(6"-acetyl-β-D-glucopyranoside) (I) — $C_{25}H_{26}O_{14}$, mp 231-233°C, $[\alpha]_D^{20}$ — 19.35° (c 0.31; DMFA); ν_{max}^{KBr} (cm^{-1}): 3360-3600 (OH), 2937 (OCH_3), 1735 (ester C=O), 1652 (γ-pyrone C=O), 1605, 1568, 1524 (aromatic C=C bonds), 1105-1007 (C—O vibrations of glycosides); $\lambda_{max}^{CH_3OH}$ (nm); 260, 275 sh., 336 sh., 379; +CH_3COONa 259, 274, 338, 382; +CH_3ONa 264, 295, 380, 460; +$AlCl_3$ 269, 373, 446.

PMR spectrum in C_5D_5N (ppm): 2.02 (3 H, s, —$COCH_3$); 3.80 (3 H, s, 3'-OCH_3); 4.00 (3 H, s, 8-OCH_3); 3.92-4.33 (4 H of the sugar moiety); 4.45-4.96 (2 H, m, —CH_2OCOCH_3); 5.68 (d, 7.5 Hz, H-1''); 7.08 (1 H, s, H-6); 7.26 (d, 8 Hz, H-5'); 8.19 (br. s, H-2'); 8.24 (q, 8 and 2 Hz, H-6').

The Acid Hydrolysis of (I). Glycoside (I) (50 mg) was hydrolyzed with 5% hydrochloric acid in the boiling water bath for 2 h. The precipitate of aglycone was filtered off and recrystallized from methanol and was identified as limocitrin. D-Glucose was found in the neutralized filtrate by TLC (system 1) and GLC.

Limocitrin (III) — $C_{17}H_{14}O_8$, mp 273-274°C (with sublimation), tetraacetate with mp 156-157°C; ν_{max}^{KBr} (cm^{-1}) 3340-3490 (OH); 2930 (OCH_3); 1655 (γ-pyrone C=O); 1628, 1569, 1518 (C=C bonds). UV spectrum, $\lambda_{max}^{ethanol}$, nm: 260, 274 infl., 341 infl., 381 (log ε 4.21, 4.18, 3.95, 4.15); +CH_3COONa 261, 280, 323, 388; +CH_3COONa + H_3BO_3 260, 275, 338, 380; +CH_3ONa 276, 336, 438; +$AlCl_3$ 273, 368, 441. Mass spectrum m/z (%): M^+ 346 (68), 331 (M — CH_3, 100), 317 (M — CHO, 8), 316 (8), 303 (M — CH_3 — CO, 6), 149 (6.5), 111 (6), 109 (5), 97 (8), 83 (6.5), 71 (7), 69 (10).

The limocitrin was demethylated as described by Harborne [13]. Gossypetin was identified in the reaction product by TLC and from its IR spectrum.

Acetylation of (I). A solution of 10 mg (I) in 0.5 ml of pyridine was treated with 1 ml of acetic anhydride. After 24 h, the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with 5% sulfuric acid and then with water to neutrality, and was dried with anhydrous sodium sulfate and evaporated. The residue was purified by preparative TLC in system 2 and gave 5 mg of an acetate $C_{37}H_{38}O_{20}$ with mp 114-116°C.

Mass spectrum, m/z (%): 760 (M — 42) (0.2), 718 (0.3), 676 (0.6), 574 (0.6), 472 (0.6), 430 (2), 388 (4.5), 347 (6), 346 (18.5), 332 (6), 331 (26), 317 (10), 271 (3), 229 (6), 211 (3.5), 169 (100), 153 (4), 151 (7), 145 (8), 139 (11), 127 (28.5), 115 (6), 109 (86), 103 (6), 98 (11).

Haploside E (II) – $C_{29}H_{34}O_{17}$ (II), mp 184-186°C, $[\alpha]_D^{20}$ – 25° (c 0.4; CH_3OH), ν_{max}^{KBr} (cm^{-1}): 3150-3570 (OH), 2937 (OCH_3), 1654 (γ-pyrone C=O), 1605, 1571, 1520 (aromatic C=C bonds), 1100-1000 (C–O vibrations of glycosides); $\lambda_{max}^{CH_3OH}$ (nm): 259, 274 sh., 338 sh., 380 (log ε 4.24, 4.12, 3.94, 4.10); +CH_3COONa 260, 273, 341, 382; +CH_3ONa 265, 297, 382, 458; +$AlCl_3$ 271, 374, 448.

PMR spectrum (DMSO-d_6), ppm: 1.08 (3 H, d, 6 Hz, –CH_3); 2.80-4.00 (10 H of the sugar moiety); 3.78, 3.82 (each 3 H, s, 2 × OCH_3); 4.14-5.40 (H-1", H-1'", and the signals of the OH groups); 6.54 (1 H, s, H-6); 6.89 (1 H, d, 8 Hz, H-5'); 7.66 (1 H, m, H-6'); 7.70 (br. s, H-2').

Acid Hydrolysis of (II). Glycoside (II) (20 mg) was hydrolyzed with 5% sulfuric acid on the water bath for 4 h. The precipitate of aglycone that deposited was filtered off and recrystallized from methanol and was shown to be identical with limocitrin. D-Glucose and L-rhamnose were detected in the $BaCO_3$-neutralized and evaporated filtrate by TLC with markers in system 1.

Acetylation of (II). Compound (II) (50 mg) was acetylated with acetic anhydride in pyridine. After the usual working up and purification by preparative TLC in system 3, an acetate $C_{47}H_{52}O_{26}$ with mp 193-195°C was obtained.

PMR spectrum ($CDCl_3$): 1.12 (d, 5.5 Hz, –CH_3); 1.92-2.16 (18 H, 6 × $COCH_3$); 2.37 (6 H, s, 2 × $ArOCOCH_3$); 2.40 (3 H, s, 5-$COCH_3$); 3.82 (3 H, s, 3'-OCH_3); 3.95 (3 H, s, 8-OCH_3); 3.50-4.35 (5 H); 4.82-5.30 (7 H); 6.72 (1 H, s, H-6); 7.09 (d, 8.5 Hz, H-5'); 7.40 (2 H, m, H-2', 6').

Mass spectrum, m/z (%): 990 (M – 42) (1.2), 948 (990 – 42) (13), 906 (0.6), 602 (0.7), 561 (7), 500 (1.5), 472 (0.4), 458 (6), 416 (14.5), 398 (4), 374 (20), 346 (10), 332 (73), 331 (54.5), 273 (9), 246 (9), 213 (3), 177 (10), 169 (100%), 153 (10), 149 (54.5), 109 (91).

Determination of the Structure of the Sugar Moiety of (II). Glycoside (II) (20 mg) was methylated by Hakomori's method [10]. This gave 12 mg of the methylation products, in the IR spectrum of which there were no absorption bands of hydroxy groups. Part of the methylation product (6 mg) was dissolved in 3 ml of 6% methanolic hydrogen chloride and the solution was boiled in the water bath for 4 h. Then the reaction mixture was neutralized with silver carbonate, the precipitate that had deposited was filtered off, and the filtrate was evaporated to dryness. 3,4,6-Tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose were identified in the residue by GLC.

SUMMARY

Two new limocitrin glycosides have been isolated from the epigeal part of *Haplophyllum perforatum*: 7-(6"-acetyl-β-D-glucopyranosyloxy)-3,4',5-trihydroxy-3',8-dimethoxyflavone and 7-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyloxy]-3,4',5-trihydroxy-3',8-dimethoxyflavone.

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