STRUCTURE OF THE ACYLATED FLAVONOL GLYCOSIDE HAPLOSIDE D

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From the epigeal part of *Haplophyllum perforatum* (MB.) Kar. et Kir. has been isolated the new flavonol glycoside haploside D, $C_{30}H_{32}O_{18}$, mp 225-228°C, $[\alpha]_D^{20}$ −212° (c 0.17; CH_3OH) for which, on the basis of chemical transformations and spectral characteristics the structure of haplogenin 7-O-[6-O-acetyl-2-O-α-L-rhamnopyranosyl-β-D-glucopyranoside] has been established.

Continuing an investigation of the flavonoids of *Haplophyllum perforatum* (MB.) Kar. et Kir. [1, 2], from a butanolic fraction of an ethanolic extract of the epigeal part we have isolated a new flavonoid, haploside D (I).

According to its UV spectrum and qualitative reactions (λ_{max} 261, 276 sh., 348, 390 nm), compound (I) is a flavonol glycoside [3, 4]. The results of UV spectroscopy with diagnostic reagents [3] and a positive gossypetin test have shown the presence of free phenolic hydroxy groups in positions 3, 4', 5, and 8 of the flavonoid. The hydrolysis of (I) with 3% sulfuric acid led to haplogenin (3, 4', 5, 7, 8-pentahydroxy-3'-methoxyflavone) [1]. Paper chromatography and the GLC method revealed the presence of two sugars — glucose and rhamnose.

The presence of the signals of the protons of four aromatic acyl groups (2.24, 2.27, 2.33, and 2.36 ppm) and six aliphatic acetyl groups in the spectrum of the acetyl derivative of (I), (II), showed that haploside D is a bioside. The carbohydrate residue is located in position 7, since the UV spectrum of (I) did not change on the addition of sodium acetate.

The stepwise acid hydrolysis of (I) gave haplogenin 7-glucoside [2]. This shows that the rhamnose is the terminal sugar. To elucidate the structure of the carbohydrate moiety we oxidized (I) with periodic acid followed by further oxidation with nitric acid [5]. The destruction of both sugars excludes a 1 → 3 bond, and the absence of tartaric acid among the oxidation products excludes a 1 → 4 bond, between the sugars [5, 6].

The IR spectrum of haploside D contains, in addition to absorption bands characteristic for the flavonoids, an intense band of an ester carbonyl group at 1732 cm^{-1}, and in the PMR spectrum there is a three-proton singlet of an acetyl group at 1.93 ppm.

The saponification of (I) with 0.5% KOH at room temperature led to the formation of acetic acid and to deacetylhaploside D (III). The stability of (III) to alkaline hydrolysis shows a 1 → 2 bond of the rhamnose with the glucose [7]. The signals of the anomeric protons of the glucose (5.57 ppm, J ≈ 6.5 Hz) and of the rhamnose (4.81 ppm, J ≈ 2 Hz, TMS ether of (III) in CCl_4) are characteristic for the pyranose forms of these sugars in the C1 and 1C conformations, respectively [3, 8, 9]. Consequently, the sugar moiety of (I) consists of monoacetylated neohesperidose.

The PMR spectrum of (II) shows a 7:5 ratio of the protons with δ 4.50-5.50 and 3.40-4.40 ppm (with the exception of the protons of the methoxy group) that is characteristic for neohesperidosides [3, 10].

The site of attachment of the acetyl group was established on the basis of the results of a comparison of the PMR spectra of the TMS ethers of (I) and (III). The spectrum of haploside D lacked the signal of gem-acyl methine proton. However, a two-proton multiplet due to a $-\underline{CH_2}OCOCH_3$ grouping appeared in the 4.06-4.20 ppm region. This signal disappeared (underwent a diamagnetic shift) in the spectrum of (III). This is explained by the assumption that in haploside D the first alcoholic hydroxy group of the glucose is acylated.

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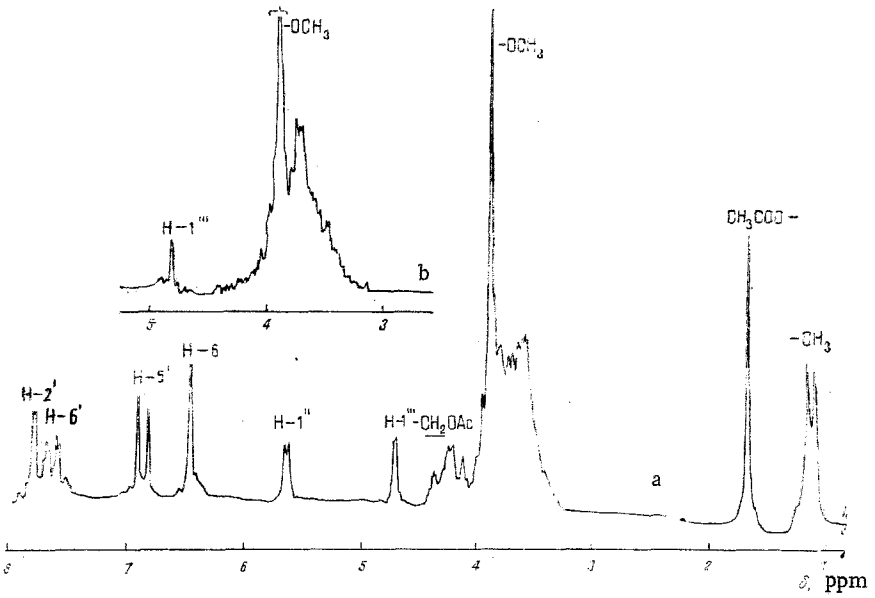
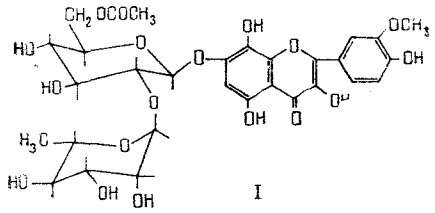


Fig. 1. PMR spectrum of the TMS ether of haploside D (a) and a fragment of the spectrum of the TMS ether of deacetylhaploside D (b) in CCl_4.

Thus, haploside D has the structure of haplogenin 7-O-[6-O-acetyl-2-O-α-L-rhamnopyranosyl-β-D-glucopyranoside] (I).

The configurations of the anomeric protons of the glucose and rhamnose were also confirmed by calculation using the method of molecular rotation differences [11].



I

The anomeric proton of the glucose in the spectrum of (I) taken in deuteropyridine appears in the form of a broad signal with a halfwidth of 6.5 Hz, while in the spectrum of the TMS ether it forms a doublet with a SSCC of 2.5 Hz. This is apparently connected with the fact that in the formation of the TMS ether inversion of the glucopyranose ring takes place. A similar phenomenon has been observed previously for the arabinopyranosides of certain flavonoids [12]. Haploside D belongs to the small group of acylated flavonoid biosides [13-16].

EXPERIMENTAL

UV spectra were recorded on a Hitachi ERS-3T spectrometer, IR spectra on a UR-20 instrument (tablets with KBr), and PMR spectra on JNM-4H-100 and Varian-HA-100 instruments (δ scale, 0 — TMS). The individuality of the substances was checked by the TLC method on Silufol in the ethyl acetate—ethanol—water (13:5:2) system and by paper chromatography (PC) in the systems butan-1-ol—pyridine—water (6:4:3) (1) and butan-1-ol—diethylamine—water (50:0.5:7.5) (2).

Isolation. The butanolic fraction [17] (35.0 g) was chromatographed on a column (5 × 130 cm) containing KSK silica gel (700 g). The substances were eluted with chloroform (fractions 1-22) and with chloroform—propanol (9:1) (22-37) and (8:2) (38-255). The combined fractions 96-119 yielded a mixture of haploside D and a coumarin glycoside. The flavonoid was separated by chromatography on Sephadex LH-20 in methanol. The yield of (I) was 243 mg.

Haploside D (I). This formed a greenish yellow amorphous substance soluble in methanol and pyridine with mp 225-228°C, composition $C_{30}H_{32}O_{18}$, $[\alpha]_D^{20}$ −212° (c 0.17; CH_3OH), R_f 0.75 (TLC), ν_{max} (cm^{-1}) 3140-3560 (OH), 2928 (OCH_3), 1732 (ester C=O), 1659 (C=O of a γ-pyrone).

UV spectrum, nm, λ_{max}: (C_2H_5OH) 261, 276 sh., 348 sh., 390; (+NaOAc) 262, 393; (+$AlCl_3$) 272, 450; (+$AlCl_3$ + HCl) 273, 447; (+NaOAc + H_3BO_3) 261; 390; (+MeONa) — the substance decomposed.

PMR spectrum in C_5D_5N (ppm): 1.64 (d, 6 Hz, rhamnose CH_3), 1.93 (s, CH_3COO—); 3.66 (s, —OCH_3), 3.75-4.80 (11 H of the sugar moiety), 5.57 (J ≈ 6.5 Hz, H-1"), 6.99 (s, H-6), 7.08 (d, 9 Hz, H-5'), 8.12 (q, 2.5 and 9 Hz, H-6'), 8.18 (H-2"), 12.39 (5-OH).

PMR spectrum of the TMS ether in CCl_4 (ppm): 1.11 (d, 5.5 Hz, —CH_3), 1.68 (s, CH_3COO—), 3.36-4.04 (8 H of the sugar moiety); 3.88 (s, —OCH_3), 4.06-4.20 (m, —CH_2OAc), 4.70 (br. s, J ≈ 2 Hz, H-1"), 5.63 (d, 2.5 Hz, H-1'"), 6.46 (s, H-6), 6.86 (d, 8 Hz, H-5'), 7.63 (q, 8 and 2 Hz, H-6'), 7.78 (d, 2 Hz, H-2').

The Acid Hydrolysis of (I). A mixture of 15 mg of compound (I) and 6 ml of 3% H_2SO_4 was heated in the boiling water bath for 5 h. The precipitate of the aglycone that deposited was filtered off, recrystallized, and shown to be identical with haplogenin. Glucose and rhamnose were detected in the $BaCO_3$-neutralized and evaporated filtrate by PC in system 1.

Acetylation of (I). Compound (I) (35 mg) was acetylated with acetic anhydride in pyridine. The decaacetate with mp 129-131°C was obtained.

PMR spectrum in $CDCl_3$ (ppm): 1.08 (d, 6 Hz, —CH_3), 1.91-2.00 (12 H), 2.07 (s, 6 H), 2.24, 2.27, 2.33, 2.36 (all s, 3H), 3.77 (s, —OCH_3), 3.65-4.15 (5 H), 4.80-5.20 (7 H), 6.80 (s, H-6), 7.04 (d, 8 Hz, H-5'), 7.23 (m, 2H, H-2', 6').

Alkaline Hydrolysis of (I). A solution of 60 mg of the substance in 5 ml of 0.5% KOH was left for 25 min and was then neutralized with 5% HCl and extracted with diethyl ether. The ethereal extract was distilled, and the residue was made alkaline with diethylamine. Diethyl ammonium acetate was detected by PC in system 2. Deacetylhaploside D with R_f 0.68 was isolated from the aqueous solution on a polyamide column.

The partial hydrolysis of (I) was performed as described by Chari et al. [16]. From the hydrolysis products, haplogenin 7-O-glucoside with mp 210-213°C was isolated.

Periodate Oxidation. Compound (I) (50 mg) was oxidized by a known method [5]. No glucose or tartaric acid was detected among the reaction products.

SUMMARY

From the epigeal part of *Haplophyllum perforatum* has been isolated the new acylated flavonol glycoside haploside D, the structure of which has been established as haplogenin 7-O-[6-O-acetyl-2-O-α-L-rhamnopyranosyl-β-D-glucopyranoside].

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INTERPRETATION OF π→π* ELECTRONIC TRANSMISSIONS IN THE ABSORPTION SPECTRA OF FLAVONE AND ITS HYDROXY DERIVATIVES

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The electronic spectra of flavone and its 4'-, 3-, 5-, and 7-monohydroxy derivatives have been calculated by the standard Pople—Pariser—Parr method. The contributions of the fragments to the total molecular excitation have been calculated. It has been shown by computer modeling that the electronic transitions of the flavone spectrum can be reduced to the corresponding transitions of the spectrum of chromone, but not of chalcone. A graphical analysis has been performed of the experimental absorption curves. A four-component system of individual bands in the 230-400 nm region is proposed. Their mutual superposition or resolution is responsible for the features of the spectra of hydroxy-substituted flavones.

The absorption characteristics of flavones in the UV and visible regions of the spectrum are widely used for identifying and quantitatively determining these important biologically active natural compounds [1]. However, in spite of a number of special experimental and theoretical investigations [2-5], the question of the nature of the π→π* electronic transitions in the spectra of flavone and its derivatives cannot apparently be regarded as having been solved In the present paper to describe the electronic transitions a new theoretical approach is used [6], according to which the contribution of an atom (μ) or fragment (Fr) of a molecule to the $\Psi_0 \rightarrow \Psi_i$ excitation is characterized by the so-called localization number $L_\mu^{o\rightarrow i}$, %, or $L_{Fr}^{o\rightarrow i}$, %. For any transition, the sum of the values of L_μ over all the atoms of the molecule is, by definition equal to 100%. A study of the distribution of L_μ is, in our view, the most natural theoretical basis for the widely used empirical "fragmentation approach," according to which absorption bands are assigned to particular parts of the molecule.

The calculation of the energies and intensities of the transitions was performed by the standard Pople—Pariser—Parr (PPP) method according to a published program [7]. The localization numbers were determined with the aid of a special extension.* The molecule of flavone and its hydroxy derivatives were assumed to be planar and the interatomic distances (r_μ) were taken to be equal to the average tabled lengths of the C—C, C=O, C—Ȯ bonds [8]. The "spectroscopic" system of parameters generally adopted was used: $W_C = -11.16$ eV, $W_{\dot{O}} = -17.70$ eV, $W_{\ddot{O}} = -34$ eV, $\gamma_C = 11.13$ eV, $\gamma_{\dot{O}} = 15.23$ eV, $\gamma_{\ddot{O}} = 23$ eV, $\beta_{benz} = -2.4$ eV, $\beta_{C-C} = 2.11$ eV, $\beta_{C=C} = -2.53$ eV, $\beta_{C=\dot{O}} = -3.3$ eV, $\beta_{C-\ddot{O}} = -2.5$ eV. The integrals $\gamma_{\mu\nu}$ ($r_{\mu\nu}$) were calculated by means of the Mataga—Nishimoto formula. A total of 49 single excited configurations was calculated.

The calculated π-electronic spectrum of flavone is shown in Fig. 1. The energy sequence of the transitions and the qualitative ratio of the intensities that are given are stable with a variation in the semiempirical parameters within wide limits. An analysis of the distribution of the localization numbers permits the direct identification of the low-intensity 0—3 transition as a "benzene" transition localized in ring B. For the other transitions analyzed, the contribution of ring A to the corresponding excitations is always considerable ($L_A \cong 35$-60%), while the contribution of ring B is relatively small ($L_B \cong 10$-25%). Even this result casts doubt on the assignment of one of the long-wave π → π* transitions of the spectrum of

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