$\lambda_{max}^{CH_3OH}$ 230, 283, and 341 nm, was identified from the results of acid hydrolysis and ^1H and ^{13}C NMR spectroscopy as scopoletin 7-O-β-D-glucopyranoside (scopolin) [2, 3].

On acid hydrolysis, compound (IV), with the composition $C_{21}H_{26}O_{13}$, mp 234-236°C, and $\lambda_{max}^{CH_3OH}$ 230, 283, and 340 nm, formed compound (II), D-glucose, and D-xylose. The order of attachment of the carbohydrate residues was determined by means of an analysis of the ^{13}C NMR spectrum of compound (IV). The assignment of the signals, which is given in Table 1, was made on the basis of literature information [3-5] and a comparison with the spectra of (II) and (III) and by the use of the INEPT and off-resonance methods. The downfield shift of the signal of C-6 of the glucose residue of (IV) as compared with its resonance in (III) indicated a 1 → 6 interglycoside bond. Measurements of the carbon-proton SSCCs in spectra without decoupling from protons gave values for J_{C1-H_1} of 160.5 and 159.6 Hz for the glucose and xylose residues, respectively. This shows their β-configuration [5].

Thus, the coumarin bioside from Ph. physaloides has the structure of scopoletin 7-O-β-D xylopyranosyl-(1 → 6)-β-D-glucopyranoside (fabiatrin) [2]. Together with scopolin, the latter has been isolated previously [6] from the species Physochlaina infundibulum growing in China.

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DETERMINATION OF COUMARIN IN SWEET CLOVER HERBAGE BY A POLAROGRAPHIC METHOD

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Continuing an investigation of coumarin-containing plants [1, 2], we have developed a polarographic method of determining coumarin in sweet clover herbage, in which the amount of this constituent serves as a criterion of the fodder [3] and medicinal [4] properties of the plant.

For analysis we used samples of the herbage of yellow and white sweet clovers (Melilotus officinalis Desz and Melilotus alba, respectively) gathered in Ryazan' province in 1986. The comminuted sweet clover herbage (5 g) was extracted with boiling ethanol (100 ml for 1 h and 50 ml for 30 min). The ethanol was distilled off from the combined extracts and the residue was transferred quantitatively to a 25-ml measuring flask.

To 1 ml of the extract so obtained was added 4 ml of ethanol and 3 ml of a 1% solution of tetraethylammonium iodide (TEAI) and analysis was performed by a polarographic method, as described in [1, 2, 5]. A 0.1% solution of coumarin was used as standard. The amount of coumarin in the sweet clover herbage was calculated from the formula

$$X = \frac{C_{st} \cdot H_x \cdot V_x}{H_{st} \cdot A},$$

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where X is the coumarin content, % (calculated on the air-dry raw material); H_x and H_{st} are the heights of the waves of the analytical and the standard solutions, respectively, mm; A is the weight of sweet clover, g; and V_x is the volume of sweet clover extract, ml.

The completeness of extraction of the coumarin from the sweet clover herbage was checked by the TLC method on Silufol UV-254 plates in the benzene–ether (1:1) system from the absence of the coumarin wave ($E_{1/2}$ = −1.44 V). By this method 0.54% of coumarin was found in the herbage of yellow sweet clover (five samples) and 0.34% in that of white sweet clover (five samples). E_{rel} = ±2.5%. The polarographic measurements were performed on a LP-7 polarograph with a dropping mercury electrode.

The validity of the procedure that we have developed was checked by an analysis of an ethanolic extract of sweet clover herbage by the method of chromatographic separation on Silufol UV-254 plates followed by the polarographic determination of the coumarin in ethanolic eluates. The polarographic method that we propose permits the determination of coumarin in the presence of the dihydrocoumarin and the glycoside, melilitoside, that are also present in sweet clover herbage [6].

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MIQUELIANIN AND OTHER POLYPHENOLS FROM Hypericum hirsutum

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We have previously isolated from the epigeal part of Hypericum hirsutum L., and identified, ten flavonoid compounds: (±)-catechin, (−)-epicatechin, quercetin, hyperoside, rutin, myricetin, luteolin, orientin, homoorientin, and 2"-acetylorientin [1-3]. In the present paper we give information on the isolation and study of eight polyphenolic substances (XI-XVIII).

The total catechins were obtained by the procedure of [1]. The mixture of (−)-epicatechin and substance (XI) was separated on a column of silica gel in a current of nitrogen [eluent: diethyl ether–ethyl acetate (1:1)].

Substance (XI) had mp 211-215°C, $[\alpha]_D^{20}$ 0° (c 0.20; acetone–water 1:1). A green coloration with ferric chloride solution and the absence of a coloration with potassium cyanide solution characterized it as a pyrocatechol derivative. With the vanillin reagent it gave a bright red coloration. On the basis of these facts and the results of chromatography with an authentic sample, substance (XI) was identified as (±)-catechin [4].

The polyphenols from Hypericum hirsutum were extracted and purified as described in [2]. The phenolic carboxylic acids were extracted from the aqueous eluates with diethyl ether. They were separated preparatively by PC in the 0.1 N hydrochloric acid, 2% acetic acid, and butan-1-ol–acetic acid–water (40:10:22) systems. They were purified on polyamide columns. Five individual substances (XII-XVI) were obtained.

Substance (XII): mp 210-212°C, λ_{max}^{MeOH} 217, 256 nm.

Substance (XIII): mp 202-203°C, λ_{max}^{MeOH} 220, 260, 296 nm.

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