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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Substance (XIV): mp 251-252°C, λ_{max}^{MeOH} 220, 272 nm.

These substances were not revealed in UV light but gave bright colorations with caustic potash solution and with diazo reagents, which enabled them to be assigned to the hydroxybenzoic acid group. Absorption maxima in the 255-295 nm region are characteristic for p-substituted benzoic acid compounds [5]. The rapid breakdown of the UV spectrum of substance (XIV) in the presence of alkalies showed the side-by-side arrangements of three hydroxy groups. A direct comparison of the physical constants, chromatographic behaviors, colorations with diagnostic reagents, and spectral properties of the compounds isolated with those of authentic samples permitted them to be identified as p-hydroxybenzoic (XII), protocatechuic (XIII), and gallic (XIV) acids.

Substance (XV) with mp 206-208°C and λ_{max}^{MeOH} 240 sh., 303 sh., 329 nm was characterized as chlorogenic acid [6].

Substance (XVI) could not be obtained in crystalline form. It was studied only chromatographically. On comparison with an authentic sample, it was identified as caffeic acid.

Substance (XVII) was eluted from the polyamide column after all the glycosides and aglycons had been washed out with 50-95% ethanol. This compound was purified additionally on a column of polyamide sorbent. Substance (XVII) was a monoglycoside with mp 182-184°C, M^+ 478, $C_{21}H_{18}O_{13}$, $E_{1\,cm}^{1\%}$ 351. IR spectrum: λ_{max}^{KBr} (cm^{-1}) 3300-3500 (OH), 1730 (COO^-), 1650 (C=O), 1085, 1050, 1010 (pyranose form of a sugar). UV spectrum: λ_{max}^{MeOH} 257, 362 nm ($\log \varepsilon$ 4.33, 4.22). Free OH groups were detected with the aid of diagnostic reagents at C_5, C_7, C_3', and C_4'. The aglycon quercetin and glucuronic acid were found in the products of its hydrolysis (5% sulfuric acid, 90 min). Consequently, substance (XVII) was quercetin 3-O-glucopyranoside, or miquelianin [7, 8].

Substance (XVIII) was detected in the mixture of C-glycosides (orientin and homoorientin) [3]. Because of its small amount, it was impossible to isolate it in the crystalline form. It had an orange coloration in UV light and did not undergo acid hydrolysis. By comparison with an authentic sample in several solvent systems, (XVIII) was identified as isomangiferin (4-C-glucosyl-1,3,6,7-tetrahydroxyxanthone) [9].

This is the first time that (±)-catechin, hydroxybenzoic acid, and miquelianin have been isolated from representatives of the genus Hypericum L. Isomangiferin has recently been isolated from H. sampsonii Hance [10].

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