FLAVONOIDS OF *Ononis leiosperma*

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Continuing a study of plants of the genus *Ononis* L. [1], we have investigated the epigeal parts of *O. leiosperma* Boiss., collected in July 1980, in the region of the town of Alushta. On two-dimensional paper chromatography [1) butan-1-ol—glacial acetic acid—water (4:1:2), and 2) 15% acetic acid] of ethanolic extracts from the epigeal part of the *Ononis* we detected not less than 20 substances of phenolic nature belonging to the groups of flavonoids, phenolcarboxylic acids, and coumarins.

The air-dry raw material (1 kg) was exhaustively extracted with 80% ethanol. The extracts were concentrated to eliminate the ethanol, hot water was added, and the precipitate that formed was filtered off. The filtrate was purified by chloroform, and the combined phenolic compounds were extracted with ethyl acetate. From the ethyl acetate extract by column chromatography on polyamide with elution by chloroform and with chloroform—ethanol with increasing concentrations of the latter, six substances assigned to the flavones, flavonols, and their glycosides were isolated.

The structures of the compounds isolated were shown by the use of physical and physicochemical characteristics.

Substances I — $C_{15}H_{10}O_6$, mp 279-280°C, $\lambda_{max}^{CH_3OH}$: 266, 367 nm was kaempferol [2].

Substance II — $C_{16}H_{12}O_6$, mp 287-290°C, $\lambda_{max}^{CH_3OH}$: 275, 335 nm: + CH_3COONa: $\Delta\lambda$ = +25 + H_3BO_3 + CH_3COONa: $\Delta\lambda$ = 0; + KOH: $\Delta\lambda$ = +70; + $Zr(NO_3)_2$; $\Delta\lambda$ = +55; + $Zr(NO_3)_2$ + citric acid, $\Delta\lambda$ = 0. The UV spectra with ionizing and complex-forming additives show the presence of free OH groups in the 4', 5, and 7 positions. IR spectrum, ν_{max}^{KBr}, cm^{-1}: 3300-3100 (—OH); 2950, 2850 ($-OCH_3$); 1655 (>C=O); 1610, 1570, 1490 (>C=C<); 840, 810 (1,4- substitution of ring B). PMR spectrum of the acetate (II) ($CDCl_3$, δ, ppm): 7.83 (d, J = 8.5 Hz, H-2',6'); 7.20 (d, J = 9.5 Hz, H=3',5'); 7.39 (s, H-8); 6.56 (c, H-3); 2.46, 2.36, and 2.31 — the signals of three acetyl groups at C-5, C-7, and C-4', respectively. The presence of the signal of a methoxy group at δ 3.85 ppm permits the assumption that it is located at C-6.

On the basis of the facts obtained, it was established that the substance is hispidulin (4',5,7-trihydroxy-6-methoxyflavone) [3].

Substance III — $C_{15}H_{10}O_7$, mp 310-312°C, $\lambda_{max}^{CH_3OH}$: 256, 370 nm, was characterized as quercetin [4].

Substance IV — $C_{21}H_{10}O_{12}$, mp 235-237°C, $[\alpha]_D^{20}$ −59° (c 0.1; dimethylformamide); $\lambda_{max}^{CH_3OH}$ 258, 365 nm. On hydrolysis with 3% H_2SO_4, quercetin and D-galactose were detected. The substance gave no depression of the melting point in admixture with an authentic sample of hyperoside [4].

Substance (V) — $C_{21}H_{20}O_{11}$, mp 270-272°C, $[\alpha]_D^{20}$ −46° (c 0.15; methanol); $\lambda_{max}^{CH_3OH}$ 270, 368 nm. In the products of acid hydrolysis and in those of enzymatic hydrolysis with emulsion, kaempferol and D-glucose were detected. The substance gave no depression of the melting point with an authentic sample of populnin [4].

Substance (XI) — $C_{21}H_{20}O_{11}$, mp 173-176°C; $[\alpha]_D^{20}$ −56° (c 0.1; dimethylformamide); $\lambda_{max}^{CH_3OH}$, nm: 270, 375; + CH_3COONa, $\Delta\lambda$ = +15; + H_3BO_3 + CH_3COONa, $\Delta\lambda$ = 0; + $Zr(HO_3)_2$, $\Delta\lambda$ = +45; + $Zr(NO_3)_2$ + citric acid, $\Delta\lambda$ = 0. On enzymatic hydrolysis with emulsin, kaempferol and D-glucose were detected.

The substance was identified as astragalin (kaempferol 3-O-β-D-glucopyranoside) [5].

The flavonoids of *O. leiosperma* have not been studied previously.

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DIACETYLISOSTACHYFLASIDE AND ACETYLISOSTACHYFLASIDE FROM *Stachys atherocalyx*

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From the herbage of *Stachys atherocalyx* C. Koch. we have isolated two new acylated flavone glycosides which we have called diacetylisostachyflaside and acetylisostachyflaside.

Diacetylisostachyflaside ($C_{31}H_{34}O_{18}$, mp 186-188°C) and acetylisostachyflaside ($C_{29}H_{32}O_{17}$, mp 174-178°C) have R_f values of 0.67 and 0.57, respectively, in solvent system 1) butan-1-ol–acetic acid–water (4:1:2), and 0.36 and 0.47, respectively, in system 2 (50% acetic acid).

Qualitative chemical reactions, chromatographic behavior, and the results IR and PMR spectroscopy characterized the compounds under investigation as glycosides of a flavone nature.

The PMR spectrum of diacetylisostachyflaside has the signals of two acetyl groups (signals at δ 1.85 and 1.62), of which the first belongs to an axial and the second to an equatorial acetoxy group of a carbohydrate component [1]. In the spectrum of acetylisostachyflaside, only the signal at δ 1.85 appears. The presence of acetyl substituents was also confirmed by the formation of acetohydroxamic acid [2]. Diacetylisostachyflaside and acetylisostachyflaside were hydrolyzed by 5% sulfuric acid with the formation of an aglycone, D-glucose, D-mannose, and acetic acid. From its physicochemical properties and the results of UV, IR, and PMR spectroscopy, the aglycone $C_{15}H_{10}O_6$, mp 298-301°C) was identified as 4',5,7,8-tetrahydroxyflavone (isoscutellarein) [3].

Both glucosides were stable to the action of rhamnodiastase and emulsin, but the esterases of the grape snail hydrolyzed them to the de-acetyl derivative – isostachyflaside [4]. An intermediate product in the enzymatic hydrolysis of diacetylisostachyflaside was acetylisostachyflaside. Different rates of the stripping off of the acetyl groups attached to equatorial and axial hydroxyls was also observed on mild alkaline hydrolysis with a 1.5% solution of potassium bicarbonate.

The position of the equatorial acetoxy group in the diacetylisostachyflaside molecule was determined from the products of periodate oxidation [5]. The absence of D-glucose and D-mannose in the degradation products excluded the presence of acetyl groups at C-3 and C-4 of both sugar residues. Attachment of the acetyl derivatives was possible at the C-6 hydroxyls of the D-glucose or D-mannose residues.

The bioside nature of the compounds studied was shown by oxidative degradation according to Chandler and Harper [6].

Neither glycoside was hydrolyzed by 0.5% caustic soda solution which shows the 1→2 order of the bond between the sugars [7].

The position of the carbohydrate component, the order of attachment of the D-glucose and D-mannose residues and the configuration of the glycosidic bonds were determined as described for isostachyflaside [4].

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