TRIFOSIDE – AN ISOFLAVONE FROM *Trifolium pratense*

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We have detected by paper chromatography six substances of flavonoid nature in the herb Trifolium pratense L. sp. pl. (red clover) collected in the flowering phase in the Tashkent oblast.

To isolate the flavonoids, the comminuted raw material previously treated with chloroform was extracted with ethanol. The extract was concentrated to small volume, and the flavonoids that precipitated were filtered off and separated on a column filled with polyamide sorbent. When the column was washed with 40% ethanol, a flavonoid was obtained with the composition $C_{22}H_{22}O_{10}$, mp 183-184°C (from methanol), $[\alpha]_D^{25}$ $-50°$ (c 0.3; methanol), mol. wt. 446 (spectrophotometrically), R_f 0.85 in the BAW system, and we have called it trifoside.

The substance is soluble in pyridine, dimethyl sulfoxide, formamide, dioxane, acetone, and ethanol, and is insoluble in chloroform, ether, and carbon tetrachloride.

The UV spectrum of the flavonoid shows absorption maxima at 325 and 264 nm (log ε 3.75, 4.59) which corresponds to the chromophore of 4',5,7-trihydroxyisoflavone. In the presence of zirconyl nitrate, a bathochromic shift of the maximum of the second band by 14 nm, disappearing on the addition of citric acid, was found, which confirms the presence of a hydroxy group in position 5.

Enzymatic and acid hydrolysis (10% H_2SO_4, 2 h, on the boiling-water bath) gave D-glucose and the aglycone (yield about 50%), which shows that the substance is a monoside. This was confirmed by the values of the specific absorption of the glycoside and of the aglycone (for the glycoside, $D_{1cm}^{1\%}$ 880, for the aglycone 1035). The aglycone is a colorless crystalline substance with mp 232-233°C (from methanol). M^+ 284. In its UV spectrum in the presence of sodium ethoxide a bathochromic shift of the maximum of the second band by 12 nm takes place, showing that the glucose is attached at position 4'.

The IR spectrum of the glucoside shows the following absorption bands (cm^{-1}): 3370-3410 (hydroxy group), 1660 (stretching vibrations of a carbonyl group), 1617, 1585, 1520, 1448 (aromatic nucleus). Bands at 1076, 1052, and 1024 cm^{-1}, and also at 955 and 902 cm^{-1}, show that the glucose has the pyranose form and is attached by a β-glycosidic linkage [1]. This is confirmed by the results of enzymatic cleavage of the glycoside by emulsin and by the calculated value of $[M]_D \cdot K_{Ph}$ according to Klyne [2] (found 137°, calculated 182°).

In the NMR spectrum taken at 60 MHz (solution of the substance in DMSO), a singlet with δ 8.6 ppm (1H) corresponds to the H-2 proton; doublets at 7.82 ppm (2H), J = 8 Hz, and 7.34 ppm (2H), J = 8 Hz, are due to the H-2', H-6', and to the H-3', H-5' protons, respectively. Doublets at 6.84 and 6.63 ppm, J = 1.5 Hz (1H each) show the presence of protons in the H-8 and H-6 positions [3], and a singlet at 13.09 ppm (1H) is due to the proton of a 5-OH group. In the spectrum taken in deuteropyridine, a three-proton singlet is found at 3.58 ppm, which is due to a methoxy group attached to an aromatic ring, and multiplets appear at 4.11 ppm (6H) and 5.43 ppm (1H), showing the presence of a glucose residue attached to the aglycone by a β-glycosidic linkage.

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On the basis of physicochemical constants, the results of hydrolysis, UV, IR, and NMR spectroscopy, and optical activity, it was established that the flavonoid isolated is 4'-O-β-D-glucopyranosyl-5-hydroxy-7-methoxyisoflavone. A glucoside of the same aglycone (prunetin) has been isolated previously from the bark of *Prunus* [4], but neither the position of the glucose nor the size of the oxide ring nor the nature of the glycosidic linkage in it was determined. The name trifoside is proposed for the substance of the structure that we have determined.

LITERATURE CITED

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