BIOCHANIN A AND ITS GLUCOSIDE IN SOME SPECIES OF CLOVER

A. L. Kazakov

We have previously reported the isolation of flavones from plants of the genus <u>Trifolium</u> [1]. A study of the flavonoid composition of the herbs <u>Trifolium sativum</u> Crome., <u>T. pratense L., <u>T. medium</u> L., <u>T.</u> <u>alpestre L., T. trichocephalum M. B., T. canescens Welld., T. caucasicum Tausch. and T. apertum Bobr., family Leguminosae, by qualitative reactions [2] showed the presence of substances of an isoflavone nature in them.</u></u>

For the isolation of these compounds, the herbage of each species of clover collected in the flowering phase in the Stavropol territory was extracted successively with chloroform and ethanol. The ethanol extracts were evaporated to eliminate the alcohol in vacuum. The residue was dissolved in a small amount of hot water. Chromatography on a column of polyamide sorbent using ethanols of various concentrations as eluents yielded two isoflavones in each case. The first was in the form of crystals with the composition $C_{22}H_{22}O_{10}$, mp 208-210°C (from aqueous ethanol), $[\alpha]_D^{20}-24.4^\circ$ (c 0.41; methanol), $\lambda_{max}^{C_2H_5OH}$ 262, 323 nm. Its pentaacetate $C_{32}H_{32}O_{15}$ had mp 203-205°C. On acid hydrolysis it gave D-glucose and an aglycone identified as biochanin A.

The results of enzymatic hydrolysis and differential IR spectroscopy showed that the glucose was attached to the aglycone by a β linkage and was present in the pyranose form. On the basis of UV and IR spectra, hydrolysis products, and the melting point of the acetate, the compound was identified as $7-\beta$ -D-glucosyloxy-5-hydroxy-4'-methoxyisoflavone [3].

The second flavonoid had the composition $C_{16}H_{12}O_5$, mp 212-214°C (from methanol), $\lambda_{max}^{C_2H_5OH}$ 263 nm, acetate with mp 186-188°C, monomethyl derivative with mp 138-139°C. Alkaline degradation gave homoanisic acid and phloroglucinol. On the basis of its UV and IR spectra, the melting points of derivatives, and alkaline degradation, it was identified as biochanin A (5,7-dihydroxy-4'-methoxyisoflavone).

This is the first time that the isoflavones of <u>Trifolium</u> caucasicum Tausch. and <u>T. apertum</u> Bobr. have been studied.

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