

Continuing an all-sided investigation of various species of clover [1], we have studied the flavonoid compounds from the epigeal parts of *Trifolium sativum* Crome, *T. pratense* L., *T. alpestre* L., and *T. medium* L., family Leguminosae, growing in the northern Caucasus. The combined flavonoids were obtained by extraction with ethyl acetate from a purified ethanolic concentrate. Chromatography on a polyamide sorbent with subsequent fractional crystallization and preparative chromatography on paper yielded six flavonoid substances from each species.

To determine the nature of these compounds, we studied the physicochemical properties of the initial substances and of their hydrolysis products.

For glycosides, the aglycones were isolated from the products of alkaline hydrolysis and were identified. The filtrates remaining after the separation of the aglycones were neutralized and evaporated, and the nature of the carbohydrate was determined. The position of attachment of the sugar residues was determined by UV spectroscopy with ionizing and complex-forming additives, and also by stepwise hydrolysis. The configurations of the glycosidic bonds were determined by enzymatic hydrolysis and were confirmed by differential IR spectroscopy.

Substance (I), from *T. sativum* Crome and *T. pratense* L.: $C_{16}H_{12}O_7$, mp 305–307°C, $\lambda_{\max}^{CH_3OH}$ 254, 265 sh, 371 nm proved to be isorhamnetin; (II) – $C_{15}H_{10}O_7$, mp 312–314°C, $\lambda_{\max}^{CH_3OH}$ 256, 264 sh, 372 nm was similar to quercetin; (III) – $C_{15}H_{10}O_6$, mp 274–275°C, $\lambda_{\max}^{CH_3OH}$ 267, 368 nm proved to be kaempferol; (IV) – $C_{21}H_{20}O_{11}$, mp 270–271°C, $\lambda_{\max}^{CH_3OH}$ 265, 365 nm is kaempferol 7-O- β -D-glucopyranoside (populnin); (V) – $C_{21}H_{20}O_{12}$, mp 238–240°C, $\lambda_{\max}^{CH_3OH}$ 255, 265, 362 nm was characterized as quercetin 3-O- β -D-glucopyranoside (isoquercitrin); (VI) – $C_{21}H_{20}O_{11} \cdot 2H_2O$, mp 228–230°C, $\lambda_{\max}^{CH_3OH}$ 267, 354 nm was identified as kaempferol 3-O- β -D-galactopyranoside (grifolin); and (VII) – from *T. alpestre* and *T. medium*, $C_{27}H_{30}O_{16}$, mp 189–190°C, $\lambda_{\max}^{CH_3OH}$ 256, 354 nm was identified as quercetin 3-O-rutinoside (rutin).

Quantitative determination of the flavonoids spectrophotometrically after their paper-chromatographic separation [2] showed that *T. sativum* and *T. pratense* contained the largest amounts of isoquercitrin (0.92%), and *T. alpestre* and *T. medium* the largest amounts of rutin (0.89%).

The other glycosides and aglycones were found in somewhat smaller proportions.

The flavonoids from *T. sativum*, *T. alpestre*, and *T. medium* have not been studied previously. This is also the first time that rutin has been found in the genus *Trifolium*.

LITERATURE CITED

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