QUANTITATIVE DETERMINATION OF FLAVONOIDS IN SOME REPRESENTATIVES OF THE FAMILY FABACEAE

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The quantitative composition of the combined flavonoids in the epigeal parts of eight representatives of the family Fabaceae (four species of *Onobrychis*, two of *Astragalus*, and two of *Trifolium*) have been studied. For the quantitative estimation of the flavonoids we used a spectrophotometric method permitting the determination of flavonols and isoflavones when they are present simultaneously. A comparatively high content of flavonoids (from 2.37 to 4.13%) was found. The sum of the flavonoid compounds from the species of *Onobrychis*, *Astragalus*, and *Trifolium* investigated possesses a hypolipidemic activity.

A preliminary chromatographic analysis of ethanolic extracts from the epigeal parts of *Onobrychis tanaitica* Spreng., *O. biebersteinii* Siry., *O. bobrovii* Grossh., *O. arenaria* (Kit.) DC, *Astragalus onobrychis* L., *A. lasioglottis* Stev., *Trifolium polyphyllum* C.A.M., and *T. strepens* Crantz., collected in the flowering period in the Northern Caucasus showed that they contained flavonols and isoflavones [1-4].

To esimate the flavonoids in the plant material quantitatively we used a spectrophotometric method permitting the determination of individual groups of flavonoids in mixtures of them (flavonols and isoflavones) [5]. The essence of the method consists in the extraction of the flavonoids from the raw material with 95% ethanol, chromatographic separation on paper, and spectrophotometric determination after elution.

On the basis of properties characteristic for the groups of flavonoids being analyzed, we selected two analytical wavelengths — 262 and 363 nm. Absorption at 363 nm corresponds to the flavonols present, and that at 262 nm to the flavonols and isoflavonones together. The amounts of flavonols were calculated as rutin and that of isoflavones as formononetin. As standard samples we used rutin produced industrially (mp 190-192°C) [6] and formononetin obtained by synthesis (mp 255-257°C) [7].

EXPERIMENTAL

Analysis of the Raw Material. An accurately weighed sample of the air-dry raw material (about 1.0 g) was exhaustively extracted with ethanol in 3- to 5-ml portions. The extracts were collected in a 25-ml measuring flask, and the volume was made up to the mark with ethanol. An accurately measured volume of extract (about 0.2 ml) was deposited on chromatographic paper with a micropipette. The extract was subjected to two-dimensional chromatography in the 15% CH_3COOH and the 1-butanol—CH_3COOH—H_2O (4:1:5) systems. The dried chromatogram was viewed in UV light, and the separated spots were marked, cut out, and exhaustively eluted by being heated on the water bath with 3- to 5-ml portions of ethanol. Each eluate was collected in a 25-ml measuring flask and the volume was made up to the mark with ethanol. The optical density of the eluate obtained was measured on an SF-4A spectrophotometer in a cell with a working length of 1 cm relative to 95% ethanol at a wavelength of 363 nm, which corresponds to the best-defined absorption maximum of the flavonols, and at 262 nm, which corresponds to the most pronounced absorption maximum of the isoflavones.

The percentages of flavonols (X_1) and of isoflavones (X_2) were calculated from the formulas [5, 8].

$$X_1 = \frac{A_{363} \cdot Y_1 \cdot Y_2}{E^{1\%}_{1cm(363)} \cdot a \cdot b}$$

and

$$X_2 = \frac{E^{1\%}_{1cm(363)} \cdot A_{262} - E^{1\%}_{1cm(262,fl)} \cdot A_{363} \cdot Y_1 \cdot Y_2}{E^{1\%}_{1cm(262,if)} \cdot E^{1\%}_{1cm(363)} \cdot a \cdot b},$$

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where A_{363} and A_{262} are the optical densities of the extracts under investigation at 363 and 262 nm; $E^{1\%}_{1cm(363)}$ is the specific absorption index of a sample of rutin at 363 nm ($E^{1\%}_{1cm}$ = 279.8 ± 2.08); $E^{1\%}_{1cm(262,fl)}$ is the specific absorption index of the standard solution of rutin at 262 ($E^{1\%}_{1cm}$ = 338.3 ± 1.59); $E^{1\%}_{1cm(262,if)}$ is the specific absorption index of a standard sample of formononetin at 262 nm ($E^{1\%}_{1cm}$ = 856.6 ± 1.82); Y_1 is the volume of the extract, ml; Y_2 is the volume of eluate, ml; a is the weight of raw material, g; and b is the volume of the extract of the plant material deposited on the chromatogram, ml.

To determine the specific absorption indices of rutin and formononetin we used eluates obtained on chromatography from 0.002 to 0.012 ml of 0.1% solutions of standard samples in precisely the same way as described above for the quantitative determination of the flavonoids in the raw material. The values of the specific absorptiom indices so obtained agreed well with those given in the literature [5].

The amounts of flavonoids in the plant species investigated are given below; in each case the results of six determinations were treated statistically (%):

Plant	Flavonols	Isoflavones
Onobrychis tanaitica	3,73±0,095	0 08±0,008
O. bieberstein ii	3,46±0,131	0,07±0,010
O. bobrovii	3,68±0,159	—
O. arenaria	3,77±0,123	—
Astragalus onobrychis	2,45±0,128	—
A. lasioglottis	2,37±0,116	—
Trifolium polyphyllum	2,41±0,139	1,72±0,092
T. strepens	2,20±0,146	1,88±0,072

Flavonols were present in all the plants investigated, and isoflavones in two species of *Onobrychis* and the two species of *Trifolium*. In view of their wide distribution, the powerful raw materials basis, and also the comparatively high amount of flavonoids (from 0.237 to 4.13%), we then made a biological study of the sum of the flavonoids isolated from the epigeal parts of the plants under investigation. It was established that the sum of the flavonoid compounds from the *Onobrychis*, *Astragalus*, and *Trifolium* species considerably lowered the cholesterol and triglyceride contents in animals under conditions of experimental hyperlipidemia.

SUMMARY

1. The quantitative composition of the sum of the flavonoids from the epigeal parts of eight representatives of the family Fabaceae have been studied.

2. A comparatively high amount of flavonoids in the plants investigated has been shown.

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