QUANTITATIVE DETERMINATION OF TRITERPENOIDS IN PLANTS OF THE GENUS Thymus

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Hitherto there have been no comparatively accurate and rapid methods of analysis for the quantitative estimation of triterpenoids in plant material. Generally accepted methods are the gravimetric method, which does not always give objective results, and the Fontan–Candela method.

Brieskorn and Briner have suggested the use of the reaction of triterpenoids with chlorosulfonic acid for colorimetric determination [1]. Although this method is more accurate than the gravimetric method, it is laborious: the performance of an analysis requires a considerable consumption of time (about 2 days). Because not only triterpenoids but also sterols in general react with chlorosulfonic acid, objective results cannot always be obtained. On studying the mechanism of the Liebermann–Burchard reaction, Brieskorn and Herrig established that under the influence of sulfuric acid triterpenoids are oxidized, forming diene derivatives with a characteristic coloration [2].

The reaction of triterpenoids with chlorosulfonic acid apparently has the same mechanism, since in this case analogous products are formed.

In investigations of some Soviet authors it has been shown that all the products of the reaction of triterpenoids with conc. sulfuric acid have a characteristic maximum in the 310 nm region [3-5]. Earlier, Bernstein's investigations had shown that although steroids also react with sulfuric acid the products of their reaction do not have a band at 310 nm [6]. Thus, there is a fundamental difference in the absorption spectra of the products of the reaction of triterpenoids and of steroids with sulfuric acid. On this basis, we suggest that steroids and triterpenoids can be detected spectrophotometrically even when they are present in combination.

V. F. Semenchenko et al. [7] have developed the optimum conditions for the reaction of triterpenoids with conc. sulfuric acid and have established that heating at 70°C for 60 min gives an optical density which is retained for 12 h.

In view of what has been said above, we have studied the possibility of the quantitative determination of triterpenoids in plant raw material by the reaction with conc. sulfuric acid. The materials investigated were a number of species of Thymus growing in the Caucasus (Table 1).

TABLE 1

Raw material	Wt. of sample, g	Moisture content	Neutral triterpenoids	Triterpene acids
		%		
Th. transcaucasicus	4,5610	9,7	2,37	3,22
Th. dimorphus	3,6117	9,9	2,16	3,96
Thyme	5,9893	9,5	1,62	2,96
Thyme after extraction with 40% ethanol	6,6018	10,2	1,60	2,95

EXPERIMENTAL

To construct a calibration curve, accurately weighed samples of ursolic acid were each dissolved in 10 ml of conc. sulfuric acid. The solutions were thermostatted at 70°C for 60 min. Then their optical densities at 310 nm were determined (the optical densities are stable for 12 h). Solutions of ursolic acid obey the Lambert–Beer law at concentrations of from 0.10 to 0.40 mg/ml.

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An accurately weighed sample of the raw material was extracted successively with petroleum ether (40-60 °C) and with chloroform. The first extracts contained the neutral triterpenoids and the chloroform extracts contained the triterpene acids. In both cases, the extractant was distilled off and the residues, after drying (at 105°C), were dissolved in a definite volume of conc. sulfuric acid and, after appropriate dilutions, were thermostatted at 70°C for 60 min.

The optical densities were determined relative to pure sulfuric acid. The error was less than 1.0%. To check the accuracy of the method, 100 ml of a chloroform extract of Th. dimorphus Klok was separated into two 50-ml portions. Ursolic acid (0.040 g) was added to the first portion. Samples of each portion were taken for analysis. The deviations obtained did not exceed the error of the method. The optical densities were determined on an SF-4A spectrophotometer at a wavelength of 310 nm (see Table 1).

SUMMARY

1. On the basis of a study of the UV absorption spectra of pentacyclic triterpenoids in conc. sulfuric acid, a method has been developed for the quantitative determination of triterpenoids in the foliage of wild-growing species of Thymus and cultivated thyme.

2. The method is distinguished from existing ones by its rapidity and low consumption of reagents.

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