CHROMATO-SPECTROPHOTOMETRIC METHOD FOR DETERMINING FERUTININ, TEFERIN, AND FERUTIN

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A preparation has been obtained from the roots of Ferula tenuisecta Eug. Korov. the basis of which consists of esters of phenolcarboxylic acids and the sesquiterpene diol ferutinol [1]: ferutin [2], ferutinin [3], and teferin [4].

The methods of analysis proposed previously [5] for this preparation are unsuitable, in the first place, because of the inadequacy of the chromatographic system (there is no sharp separation of these esters and the accompanying substances, which adsorb intensively in the same region of the UV spectrum), and, in the second place, because under the desorption conditions described the esters are desorbed incompletely from the silica gel.

To separate the esters from one another and from ballast substances we have used thin-layer chromatography on a plate with a fixed layer of type KSK silica gel (150-200 mesh) in the chloroform – ethyl acetate (30:1) system. The R_f values of the substances concerned in this system are: ferutin 0.42 ± 0.05, teferin 0.49 ± 0.05, and ferutinin 0.24 ± 0.05.

The esters were detected on the chromatogram after spraying it with a 1% solution of vanillin in sulfuric acid (blue-green spots), under which conditions the ballast substances were colored pinkish lilac. The sensitivity of the chromatographic method for ferutinin and teferin was 0.2 μg and for ferutinin 0.1 μg.

The ferutinin was eluted from the silica gel with 95% ethanol, and the ferutin and teferin with acetic acid (Fig. 1).

The esters in the eluate were determined spectrophotometrically at 260 nm. The intense absorption of ferutin, teferin, and ferutinin at this wavelength is due to the isovanillic acid, vanillic acid, and p-hydroxybenzoic acid residues (secondary benzene band).

Ferutinol is transparent in the accessible UV region of the spectrum. Its presence in the molecule of an ester causes only an increase in the intensity of absorption and a bathochromic shift. This corresponds to the known fact that the UV spectra of esters differ little from the spectra of the corresponding acids if the alcohol moiety of the ester does not contain multiple bonds [6].

The absorption spectra of ferutin, teferin, and ferutinin in acetic acid scarcely differ from the corresponding spectra in ethanol. The spectral characteristics of the esters of Ferula and of the acids composing them are given below:

	95% Ethanol			Acetic Acid		
	λ, nm	lg ε	$E^{1\%}_{1cm}$	λ, nm	lg ε	$E^{1\%}_{1cm}$
Ferutin	260	4.07	318	260	4.08	315
Isovanillic acid	250	3.97	543	260	4.27	660
Teferin	260	4.06	300	260	4.04	291
Vanillic acid	256	4.02	611	263	4.06	690
Ferutinin	260	4.25	502	260	4.24	495
p-Hydroxybenzoic acid	250	4.18	1071	258	4.25	1262

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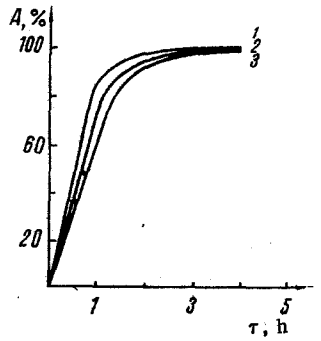

Fig. 1. Kinetic curves of the desorption (A) of: 1) ferutinin; 2) ferutin; 3) teferin.

Since the values of R_f for ferutin and teferin are close and their desorption from silica gel takes place similarly, and, moreover, their values of log ε at 260 nm differ little, these substances were determined together. The sensitivity of the spectrophotometric method for ferutin is 7 μg and for ferutinin 3 μg. The accuracy of the method was evaluated by a quantitative determination of standard samples of esters and synthetic mixtures of them. The relative accuracy of the method (confidence level 95%) for ferutin was ±1.13, for teferin ± 0.82, and for ferutinin ±1.06.

The amounts of ferutin with teferin and of ferutinin in five samples of the preparation were determined by the given method (%):

Sample	Total	Ferutinin	Ferutinin and Teferin
1	88.0	59.7	28.3
2	80.6	60.2	20.4
3	81.4	59.2	22.2
4	84.6	63.7	20.9
5	80.0	56.1	23.9

EXPERIMENTAL

The preparation (20 mg, accurately weighed) was dissolved in 4 ml of chloroform. The silica gel for chromatography was boiled three times with concentrated hydrochloric acid, washed with water to neutrality and then with chloroform and methanol, and was dried at 120°C for 48 h. Plates (18×24 cm) with a fixed layer of silica gel were separated into three equal bands. On each of the first and second bands was deposited 0.05 ml (250 μg) of the chloroform solution of the preparation under investigation, and the third band served as a background for spectrophotometry (blank sample) and was chromatographed in the system given above (length of the run 21 cm). The plates were dried to free them from solvent in the air and the first band was sprayed with a 1% solution of vanillin in sulfuric acid. From the spots that had appeared the corresponding sections of silica gel in the second and third bands were marked. The sections of silica gel containing the ferutinin and the ferutin with teferin were transferred into flasks and were eluted with 10 ml of solvent (95% ethanol for the ferutinin and acetic acid for the ferutin and teferin). The zones of pure silica gel from the third band were treated correspondingly (blank samples). The esters were desorbed from the silica gel by heating in a thermostat at 40-50°C for 3 h. After the eluates had been cooled to room temperature, they were filtered and were subjected to spectrophotometry at 260 nm. Standard solutions of ferutin in acetic acid and of ferutinin in 95% ethanol were subjected to spectrophotometry in parallel.

The percentages of the components x in the preparation calculated on the absolutely dry weight were calculated from the formula

$$x = \frac{C_{st} D_x \cdot V_1 \cdot V_3 \cdot 10000}{D_{st} a \cdot V_2 (100 - h)},$$

where D_X is the optical density of the preparation under investigation; D_{st} is the optical density of standard solutions of ferutin or ferutinin; C_{st} is the concentration of the standard solution of ferutin or ferutinin, mg/ml;

V_1 is the volume of eluate, ml; V_2 is the volume of solution of the preparation of the investigation deposited on the chromatogram, ml; V_3 is the volume of solvent used to dissolve the sample of preparation, ml; a is the weight of the sample of preparation under investigation, mg; and h is the amount of moisture in the weighed sample, %.

The samples of ferutin, teferin, and ferutinin were obtained from A. I. Saidkhodzhaev and G. K. Nikonov.

SUMMARY

A chromatospectrophotometric method for determining ferutinin and teferin + ferutin in a preparation has been developed.

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CAULOSIDE G – A NEW TRITERPENE GLYCOSIDE FROM *Caulophyllum robustum* IDENTIFICATION OF CAULOSIDE C

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Continuing a chemical study of the glycosides (caulosides) of Caulophyllum robustum [1-4], we now report the results of investigations of the structure of caulosides G(I) (earlier called E [1]) and C.

The permethylated product (II) of cauloside G(I) was synthesized by Purdie's method [5]. Acid hydrolysis of (II) gave the methyl ester of 23-O-methylhederagenin and a mixture of methylated methyl glycosides. The latter, after acetylation were identified by the GLC–MS method [6] as the methyl pyranoside derivatives of: 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4,6-tetra-O-methyl-D-glucose, 3,4-di-O-methyl-L-arabinose, 6-O-acetyl-2,3,4-tri-O-methyl-D-glucose, and 4-O-acetyl-2,3,6-tri-O-methyl-D-glucose.

The reductive cleavage of (II) with lithium tetrahydroaluminate gave a methylated progenin (III) and a methylated oligosaccharide (IV). The hydrolysis of (III) gave 23-methoxyerythrodiol and the methyl pyranoside derivatives of: 2,3,4,6-tetra-O-methyl-D-glucose and 3,4-di-O-methyl-L-arabinose (GLC–MS method). In a hydrolyzate of (IV) by TLC in the presence of markers, 2,3,4-tri-O-methyl-L-rhamnose, 2,3,6-tri-O-methyl-D-glucose, and 2,3,4-tri-O-methyl-D-sorbitol were identified. The reduction of the 2,3,4-tri-O-methyl-D-glucose showed that the latter was attached to the carboxy group of the hederagenin.

The structure of the oligosaccharide (IV) was established on the basis of the mass spectrum of the corresponding acetate (V): the presence of peaks of ions with m/e 640 ($M^+ - 60$) and 567 confirmed that (IV) was a trisaccharide. The peaks of ions with m/e 627, 539, and 117 are due to the fragmentation of a trisaccharide in which 1,5-di-O-acetyl-2,3,4-tri-O-methyl-D-sorbitol is the reduced end [7]. The peaks of ions with m/e 189 (A_1), 157 (A_2), 125 (A_3), and 72 (K_1) are due to the fragmentation of terminal 2,3,4-tri-O-methyl-L-rhamnose.

Hydrolysis of cauloside G by the digestive juice of the snail Eulota maackii formed five progenins (VI-X) and hederagenin. From the results of acid hydrolysis and methylation with diazomethane, progenins (VII) and

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