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ISOLATION OF A TOTAL PREPARATION FROM THE ROOTS OF *Ferula tenuisecta*

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The optimum solvent has been selected for the extraction of the total esters (TEs) from the roots of Ferula tenuisecta. *The kinetics of the extraction of the TEs from the raw material have been studied and conditions for the chromatographic purification of the preparation have been determined. The optimum conditions for the separation of the TEs have been found by the method of mathematical planning of experimentation.*

The isolation of a number of esters of sesquiterpene alcohols with aromatic acids from the roots of the perennial plant *Ferula tenuisecta* Korov (fam. Apiaceae) and the determination of their structures have been reported previously [1-8].

Pharmacological investigations conducted in the Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, have permitted the creation from the terpenoid esters of this plant of a new drug [9] possessing estrogenic activity which has been approved for wide medical use.

We have investigated the isolation of the total esters (TEs) in the various stages of the technological cycle. In order to choose an effective extractant we studied the solubility of the preparation in a number of solvents (solubilities in g/100 ml are shown in parentheses): 95% alcohol (27.06), 90% alcohol (22.80), 80% alcohol (13.94), 70% alcohol (12.91), 60% alcohol (12.18), methanol (26.76), acetone (29.30), *n*-butanol (24.20), chloroform (29.83), ethyl acetate (28.70), and diethyl ether (29.52). The best of the solvents studied proved to be alcohol, acetone, diethyl ether, chloroform, and ethyl acetate.

The extraction of the raw material by the chosen solvents was studied under static conditions (Table 1).

Almost identical results were obtained in the extraction of the TEs with methyl and ethyl alcohols and with chloroform.

To determine the time of the process, we studied the kinetics of the extraction of the esters (Fig. 1). Analysis was conducted by a chromato-spectrophotometric method providing for the extraction of the raw material, the separation of the extract by TLC, and spectrophotometric determination of ferutinin and tenuferidin at a wavelength of 260 nm.

It required 4 h to reach the equilibrium concentration at the first phase contact. Phase equilibrium on second contact was achieved in 2 h, and on third contact in 1 h (see Fig. 1). The extraction curves are typical isotherms tending to equilibrium. With a decrease in the amount of extractive substances in the raw material, the relative rate of extraction of the esters rose.

Purification of the esters from accompanying substances of acidic nature was based on the extraction of the latter from the organic solvent with aqueous solutions of potassium or sodium carbonate. The esters remaining in ethyl acetate solution that had phenolic hydroxy groups were extracted on subsequent treatment with solutions of caustic potash or caustic soda. When the alkaline solution was then acidified, the esters that precipitated were extracted with ethyl acetate.

We investigated the process of extracting the accompanying substances from ethyl acetate solution with solutions of potassium or sodium carbonate, and also the extraction of the esters with caustic soda solution (Table 2). The results obtained showed that to eliminate accompanying substances of acidic nature from ethyl acetate solution it is possible to use 2-5% solutions of potassium or sodium carbonate, and, for the extraction of the esters, a 1-3% solution of caustic potash.

To find conditions for the purification of the TEs and the isolation of the esters we determined the dynamics of the extraction of the accompanying substances and esters from ethyl acetate solution (Tables 2 and 3). It can be seen from the results obtained that by a ninefold treament with 5% sodium carbonate it is possible to eliminate about 50% of the accom-

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TABLE 1. Selection of an Effective Extractant

Extractant	Amount, % on the weight of the raw material	
	Extractive substances	TEs
Methanol	18.4	5.2
Ethanol	18.0	5.1
Acetone	15.6	3.6
Chloroform	18.3	5.0

TABLE 2. Kinetics of the Extraction of the Accompanying Substances

Extraction	Amount of accompanying substances isolated from ethyl acetate solution		
	g	% on the weight of the raw material	% on the amount of extractive substances in the ethyl acetate solution
1	32.8	3.28	12.40
2	18.8	1.88	7.11
3	13.8	1.38	5.22
4	12.4	1.24	4.69
5	12.1	1.21	4.57
6	11.7	1.17	4.42
7	11.2	1.12	4.23
8	10.1	1.01	3.82
9	7.0	0.70	2.65
Total	129.9	12.99	49.11

panying substances of acid nature, while for practically exhaustive isolation of the esters six- to sevenfold extraction with 1% caustic potash is sufficient.

The main components of the total esters of *F. tenuisecta* are ferutin, ferutinin, teferin, tenuferidin, tenuferinin, and tenuferin [1-5]. Preliminary experiments showed that to isolate ferutinin and tenuferidin from the TEs it is desirable to use the chromatographic method of separation on silica gel.

We have found the optimum conditions for the purification and separation of the total esters by the method of mathematical planning of experimental work using a 3 × 3 Latin square design. We investigated the influence of the following factors on the process: the ratio of the height of the absorption layer and the diameter of the column, the rate of flow of the eluent (liters/h·m^2), and the ratio of the amounts of material deposited on the column and of the sorbent.

The highest yield of technical product was obtained at a rate of elution of 45 liters/h·m^2 with ratio of the height of the absorption layer and the diameter of 2:1, and a ratio of material to sorbent of 1:20.

To obtain a preparation with a purity of not less than 95%, we investigated the conditions of recrystallization in the chloroform—hexane system (Table 4).

Recrystallization from chloroform—hexane (1:3) made it possible to obtain a preparation with a purity of not less than 95%.

EXPERIMENTAL

Selection of the Solvent for Extracting the Esters from the Raw Material. Comparative extraction was carried out under identical conditions by threefold treatment of 1 kg of *Ferula tenuisecta* roots with the solvents under consideration.

Study of the Kinetics of the Extraction Process. To establish phase equilibrium at first phase contact, 1 kg of comminuted roots was charged into each of six 10-liter extractors, the extractant used being ethyl alcohol. In the first extractor the time of extraction was 1 h; in the second, 2 h; in the third, 3 h; in the fourth, 4 h; in the fifth, 5 h; and in the sixth, 6 h. After the lapse of the given time in each case, the extract was decanted off, evaporated, and dried to constant weight.

To establish phase equilibrium on second phase contact, the experiments were performed under the following conditions. In each of six extractors, 1 kg of comminuted raw material was extracted for 4 h. The extracts were decanted off, and fresh portions of solvent were added. The new extracts were decanted off from the first extractor after 0.5 h; from the second, after 1 h; from the third, after 1.5 h; from the fourth, after 2 h; from the fifth, after 2.5 h; and from the sixth, after 3 h, and their contents of extractive substances were determined by drying to constant weight and weighing the dry residues. To determine the time of extraction on third phase contact, 1-kg portions of the roots were extracted for 4 h, the extracts were

TABLE 3. Kinetics of the Extraction of the Esters

Number of extractions	Extraction of esters	
	g	% on the weight of the raw material
1	4.0	0.40
2	18.2	1.82
3	19.8	1.98
4	20.7	2.07
5	10.3	1.03
6	2.2	0.22
7	0.8	0.08
8	0.3	0.03
Total	76.3	7.63

TABLE 4. Selection of Recrystallization Conditions

System	Yield, g	Amount of preparation, % on the amount in the technical product
Chloroform—hexane 1:1	3.2	40.0
" 1:2	5.8	72.5
" 1:3	6.5	81.2
" 1:4	Resinified	–

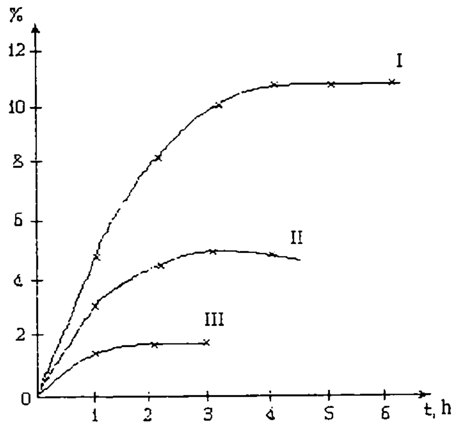

Fig. 1. Kinetics of the extraction of the total esters.

decanted off, and then fresh portions of solvent were added and the raw material was extracted for another 2 h. After this, the time necessary to achieve phase equilibrium on third phase contact was determined.

Finding the Optimum Conditions for Purifying the Total Esters. The concentrated extract from 1 kg of roots was diluted with 300 ml of water, and the esters were extracted with 1 liter of ethyl acetate. The ethyl acetate extract was treated nine times with 300-ml portions of 5% potassium carbonate solution.

Determination of the Dynamics of the Extraction of the Esters from Aqueous Solution. The ethyl acetate extract from an extract of 1 kg of roots after treatment with 5% potassium carbonate solution was extracted with 1% caustic potash solution. The amount of esters in each extract was determined by a chromato-spectrophotometric method.

Chromatographic Separation of the Esters. A column with type KSK silica gel having a particle size of 0.1-0.25 mm was used, with chloroform as the eluent. The separation process was monitored by thin-layer chromatography.

Selection of the Optimum Conditions for Recrystallization. The technical product, in 10-g portions, was crystallized from chloroform—hexane mixtures in ratios of 1:1, 1:2, 1:3, and 1:4. The best results were obtained with the 1:3 mixture.

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