DETERMINATION OF THE BIOLOGICALLY ACTIVE COMPONENTS OF THE RHIZOMES OF Rhodiola rosea

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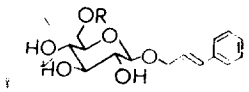
A chromato-spectrometric method for the quantitative determination of the total cinnamyl glycosides and salidroside in the raw material of roseroot stonecrop using a standard sample of rosavin has been developed.

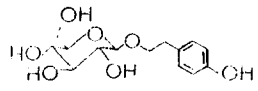
An extract of the rhizomes of roseroot stonecrop Rhodiola rosea L. is being used successfully in medical practice as a tonic and adaptogenic agent. The standardization of this drug and the raw material is done at the present time from its salidroside content. However, as has been established [1, 2], salidroside is a generic characteristic of stonecrops which lessens its worth as an objective factor in the evaluation of the quality of the raw material of a common species – roseroot stonecrop. In this sense, rosavin and other cinnamyl glycosides (rosin, rosarin) possess unique properties and serve as characteristic diagnostic features of just roseroot stonecrop, simultaneously being the bearers of the biological activity of the extract and being present in the rhizomes in considerable amounts [3].

We propose to evaluate the authenticity of the raw material roseroot stonecrop from the presence of rosavin by the TLC method, and this has been reflected in the USSR State Pharmacopeia, XIth edition. Furthermore, procedures have been developed for the quantitative determination of rosavin using chromato-spectrophotometry [4] and high-performance liquid chromatography (HPLC) [5], with the aid of which the component composition of an extract of the rhizomes of roseroot stonecrop has been determined and it has been confirmed that its main components are cinnamyl glycosides (rosin, rosavin, rosarin) and salidroside.

The results of the investigations performed [1-5] led to the conclusion of the desirability of performing the standardization of the raw material and of preparations of roseroot stonecrop from the total amount of cinnamyl glycosides and salidroside.



Rosin : R = H
Rosavin : R = Ara – p
Rosarin : R = Ara – f



Salidroside

The aim of our investigations was to develop a simple and practicable method for the quantitative determination of the sum of the active substances in the raw material roseroot stone crop based on spectrophotometry. In the development of this method, we took as our basis the conditions of extracting the raw material proposed in an earlier paper [4]. To separate all the main active substances from the accompanying components we used column chromatography on silica gel. In the development of the stage of chromatographic separation of the desired substances, monitoring was carried out by the TLC method in combination with UV spectroscopy.

The investigations showed that complete separation of the active substances of roseroot stonecrop with the aid of column chromatography on silica gel is achieved by the use of chloroform–ethanol (6:4) after the washing of the column with chloroform. On further elution with ethanol and acidified ethanol the eluates contain only tanning substances (TLC). The results of TLC showed tht the active substances of the extract were present only in the chloroform–ethanol eluate, while they were absent from the chloroform eluates and the ethanol

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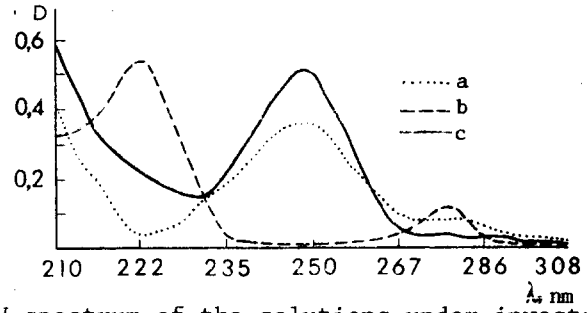

Fig. 1. UV spectrum of the solutions under investigation: a) purified extract of roseroot stonecrop; b) salidroside (0.0019% solution); c) rosavin (0.0012% solution).

eluates. When the optimum conditions of chromatographic purification were used (see the Experimental Section), an eluate was obtained the UV spectrum of which contained an absorption maximum at a wavelength of 252 nm and a shoulder in the 272-282 nm region (Fig. 1) and which contained all the active substances of roseroot stonecrop, as was subsequently confirmed with the aid of HPLC (Fig. 2).

The absorption maximum at a wavelength of 252 nm was due to the presence of rosavin, rosarin, and rosin, and the shoulder at 272-282 nm to salidroside (Fig. 1). In view of this, the content of active substances was determined at wavelengths of 252 and 278 nm. At these wavelengths alcoholic solutions of the compounds under investigation obey the main law of the absorption of light within the range of working concentrations.

The percentage content of active substances was calcualted by means of the formula for the basic law of the absorption of light using the optical density of the solution under investigation measured at the two wavelengths of 252 and 278 nm. The relative error of a single determination with a confidence level of 95% is ±9.3%. The absence of a systematic error was checked by the method of adding rosavin and salidroside to an extract and to the raw material.

A number of samples of the raw material of wild-growing and cultivated roseroot stonecrop have been analyzed with the aid of the procedure developed (Table 1).

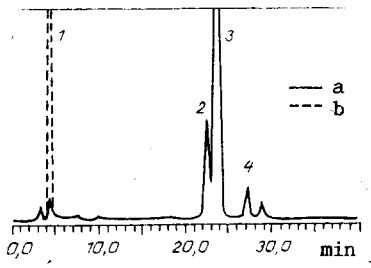

Fig. 2. HPLC analysis of a purified extact of roseroot stonecrop: a) at 252 nm (sensitivity 1.15 O.D.); b) at 278 nm (0.17 O.D.). Peaks: 1) salidroside; 2) rosarin; 3) rosavin; 4) rosin.

TABLE 1. Results of the Analysis of Samples of Roseroot Stonecrop Raw Material.

Sample No.	Place and time of collecting the raw material	Amount of cinnamyl glycosides and salidroside, %
1	Gorno-Altai AO, Ust'-Kansk region, August 17, 1984.	11,8
2	East Kazakhstan province, R. Kurchum, storage base, October 20, 1986	8,3
3	Industrial batch of raw material (Tomsk pharmaceutical chemical factory), 1985.	5,9
4	Moscow province, VILR* (cultivated plants, 5th year of life), August 27, 1984.	7,0
5	Moscow province, VILR* (cultivated plants, 6th year of life), September 20, 1985.	8,0
6	Moscow province, VILR* (cultivated plants, 7th year of life), September 26, 1986.	7,8

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EXPERIMENTAL

For TLC we used Silufol UV 254 plates in the solvent system chloroform–methanol–water (26:14:3). The substances were detected by viewing the chromatogram in UV light at wavelengths of 254 and 360 nm followed by development with a solution of diazotized sulfanilic acid in saturated sodium carbonate solution, and also with a 16% solution of sulfuric acid and heating (110°C).

HPLC analysis was carried out under the conditions described in [5] with a slight modification of the eluent mixture.

The optical densities of the solutions under investigation were measured in a SF-26 spectrophotometer. UV spectra were taken on a Specord M40 instrument.

Procedure for Analyzing the Raw Material. A flat-bottomed flask with a capacity of 100 ml was charged with 2.00 g of the ground raw material (particle size 2 mm), and 40 ml of 60% ethanol was added. The flask was closed with a ground-in stopper and the raw material was extracted at room temperature with shaking for 1.5 h.

The filtered extract (1 ml) was deposited on a column of silica gel. The column was washed with 20 ml of chloroform, and the resulting eluate was discarded. The active substances were eluted from the column with 100 ml of chloroform–ethanol (6:4) into a round-bottomed falsk, and the solvent was distilled off under vacuum to dryness. The dry residue was dissolved in 50 ml of 95% ethanol (solution A). By pipette, 5 ml of solution A was transferred into a 25-ml flask, 10 ml of 95% ethanol was pipetted in, and the contents were carefully mixed (solution B). The optical density of the solution obtained was measured on a spectrophotometer at wavelengths of 252 and 278 nm in a cell with a layer thickness of 10 mm.

A comparison solution was prepared in the following way. To a column was added 1 ml of a standard solution of rosavin in 60% ethanol and it was washed with 20 ml of chloroform (this solution was discarded) and then with 100 ml of chloroform–95% ethanol (6:4); the resulting eluate was treated further as described for the extract. The optical density of a rosavin solution was measured in parallel at a wavelength of 252 nm.

The percentage content of active substances (X) calculated as rosavin on the absolutely dry raw material was calculated from the formula for the basic law of the absorption of light using the optical density of the solution under investigation measured at the two wavelengths of 252 and 278 nm [6].

$$X = \frac{46 \cdot V \cdot m_0 \cdot (D_{252} + 14.5\, D_{278})}{V_0 \cdot m \cdot D_0} \cdot \frac{100}{100 - W},$$

where D_0 is the optical dnesity of the standard solution of a sample of rosavin at a wavelength of 252 nm:

V is the volume of the solution under investigation;

D_{252} is the optical density of solution B at a wavelength of 252 nm;

D_{278} is the optical density of solution B at a wavelength of 278 nm;

V_0 is the volume of the rosavin solution;

m is the weight of the raw material, g;

m_0 is the weight of rosavin, g;

W is the loss in mass on drying the raw material, %; and

46 and 14.5 are constants calculated from the specific absorption coefficients of the individual components (rosavin and salidroside) that also take into account the contribution to the optical density of the solution under investigation.

Preparation of the Column. A 50-ml beaker was charged with 5 g of silica gel L40/100 for chromatography (Czechoslovakia), 20 ml of chloroform was added, and the mixture was stirred and was poured through a funnel with a diameter of 3.5 cm into a column (1.5 cm in diameter and 25 cm in height in the lower part of which had been placed a small absorbent cotton plug previously moistened with chloroform. The column was filled with the cock open. On top of the sorbent was placed a small absorbent cotton plug and it was washed with 5 ml of chloroform.

Preparation of the Rosavin Solution. About 0.05 g (accurately weighed) of rosavin was dissolved in a 25-ml measuring flask in 60% ethanol, the volume of the solution was made up to the mark with the same ethanol, and the contents were mixed (solution 1).

Solution 1 (1 ml) was pipetted into the column of silica gel and then the same operations were performed with the plant extract.

Preparation of Samples for HPLC. For HPLC analysis, an extract (1:20 in 60% ethanol) was diluted with water in a ratio of 1:4, and 25 μl was injected into the chromatograph. The eluates from the silica gel column were evaporated to dryness and the residues were dissolved in the HPLC eluent. All the samples were analyzed with the deduction of the peaks of impurities of the corresponding eluates from the control column.

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TERPENOIDS OF THE OLEORESIN OF THE SCHRENK SPRUCE

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Using the scheme for the group separation of the oleoresins of conifers, the quantitative and qualitative composition of the oleoresin of the Schrenk pine Picea shrenkiana Fish. et May has been investigated. Thirty-eight diterpenoids have been identified, one of which (palustradiene) has been detected in conifers for the first time.

The Schrenk spruce Picea schrenkiana Fish et May, which belongs to the Morinda botanical section is distributed in the Tien-shan mountains [1]. There is little literature information on the chemical composition of its oleoresin – results have been published of an investigation of the volatile components of the neutral fraction, which consists of monoterpenoids and one sesquiterpene (bisabolene [2]) and also of the fractions of the polar components of the total acids, which proved to be the acid succinates of diterpene alcohols [3].

In order to obtain information on the quantitative and qualitative chemical compositions of the whole oleoresin, we have analyzed it by the scheme for the group separation of conifer oleoresins [4]. The results for the neutral fraction, amounting to 41.4% of the oleoresin, are given in Table 1.

According to GLC, the diterpenes were represented by at least nine compounds, the main ones being palustradiene, isopimeradiene, abietadiene, and dehydroabietane (I-IV: R = CH_3; amounts 1, 7, 0.9, 0.7, and 0.7%, respectively, on the sum of all the hydrocarbons of the oleoresin). After the elimination of the monoterpenes and bisabolene by vacuum distillation, the residue was separated by chromatography on silica gel impregnated with 5% of silver

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