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CHEMICAL COMPOSITION OF THE RHIZOMES OF THE Rhodiola rosea BY THE HPLC METHOD

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The component composition of extracts of the rhizomes of Rhodiola rosea has been studied by the HPLC method; the amount of rosavin has been determined and its lability on the autofermention of the raw material has been established.

The chemical composition of the rhizomes of roseroot stonecrop Rhodiola rosea L. (family Crassulaceae) is represented by substances of various natures: flavolignans (rhodiolin), flavonoids (rhodionin, rhodiosin, acetylrhodalgin, 8-methylherbacetin, kaempferol, kaempferol 7-O-rhamnoside, tricin, and tricin 5-O- and 7-O-glucosides), phenolic compounds (salidroside, tyrosol, gallic acid, methyl gallate), phenylpropenoids (cinnaymyl alcohol and its glycoside rosin, rosavin, rosarin), sterols (β-sitosterol, daucosterol), and monoterpenes (rosiridin, rosidirol) [1-8].

On the basis of results of chemical investigations a number of new biologically active substances have been discovered (cinnamyl alcohol glycosides, rosiridin) [6-10], which, together with salidroside, are among the main components of the plant. On the basis of these facts, new approaches have been proposed to the problem of the standardization of the raw material and of roseroot stonecrop preparations [11, 12]. In partiuclar, procedures have been developed for the quantitative determination of salidroside [11] and of rosavin (rosavidin) [12] which have been used for the study of the dynamics of their accumulation in the rhizome during the ontogenesis of the plant [13] and also for choosing the temperature conditions for drying the raw material [14].

The aim of the present investigations was to determine the component composition of an extract of the rhizomes of roseroot stonecrop by high-performance liquid chromatography (HPLC) and to develop a method for the quantitative determination of rosavin in raw material from this plant.

In the development of a method of qualitative and quantitative analysis with the aid of reversed-phase HPLC on silica gel-C18, we made a choice of the chromatographic conditions (composition of the organic phase of the eluent, the buffer, the rate of elution, and the method of detection). The detection of the separated substances was made from the absorption of UV light at two wavelengths simultaneously: λ_1 = 254 nm (the absorption maximum of rosavin) and λ_2 = 280 nm (the absorption maximum of salidroside). The assignment of the peaks of the substances on chromatograms of an extract of roseroot stonecrop was made on the basis of the retention times of the individual components isolated previously from roseroot stonecrop rhizomes (Table 1).

Chromatograms of the extract (1) contained peaks corresponding in their retention times to rosavin, rosarin, rosin, cinnamyl alcohol, salidroside, tyrosol, gallic acid, and methyl gallate (Table 1). The noncoincidence of the elution profiles of an extract at λ_1 = 254 nm and λ_2 = 280 nm shows that the components separated had different chromophores and, consequently, differed in chemical structure.

A separation of the main active substances (rosavin, rosarin, rosin, and salidroside) optimum with respect to time of chromatography and resolution was achieved by the use as elu-

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TABLE 1. Retention Times (R_t) of the Individual Substances and Various Peaks of an Abstract of Roseroot Stonecrop

Substance	R_t, min	Number of the peak of	R_t, min
Gallic acid	3,6	1	3,6
Salidroside	6,1	2	6,1
Tyrosol	8,2	3	8,3
Methyl gallate	10,0	4	10,1
Rosarin	25,7	5	26,2
Rosavin	27,8	6	28,2
Rosin	30,6	7	31,0
Cinnamyl alcohol	55,4	8	56,7
Rhodiosin	>120	—	—
Rhodionin	>120	—	—

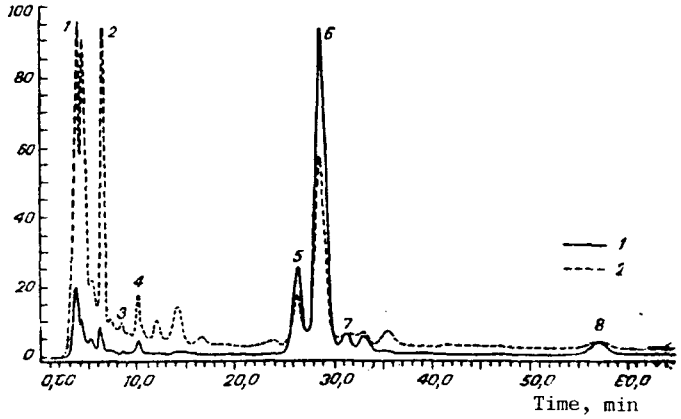


Fig. 1. HPLC separation of the components of the rhizomes of Rhodiola rosea (the numbers of the peaks correspond to those given in Table 1): 1) at 254 nm (sensitivity 0.3 O.D.): 2) at 280 nm (0.05 O.D.).

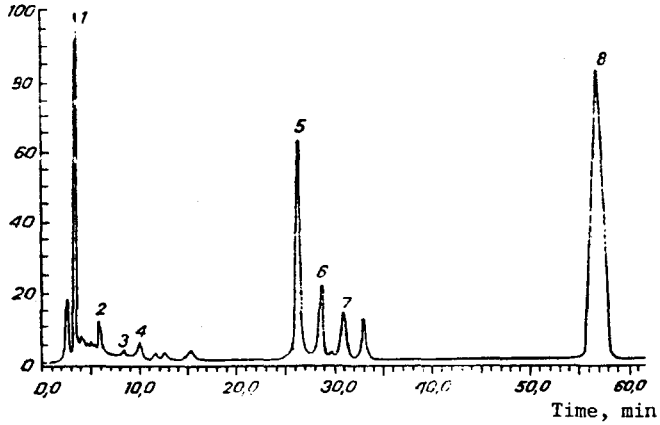


Fig. 2. HPLC separation of the components of the rhizomes of the Rhodiola rosea after autofermentation (40°C, 30 min) at 254 nm (0.11 O.D.).

ent of 15% of ethanol in an acetate buffer with pH 5.5 (Fig. 1). Under these conditions, a resolution of the peak of rosavin (peak 6) from the peaks of the other cinnamyl glycosides (peaks 5 and 7, Fig. 1) sufficient for the determination of this component was achieved. Complete separation of these peaks took place when the ethanol content in the eluent was lowered to 12%, but this greatly increased the time of chromatography.

According to TLC and the preparative separation of the components of roseroot stonecrop, of the ten flavonoids of the rhizomes that were isolated only herbacetin glycosides (rhodionin, rhodiosin) were present in th appreciable amounts, although ther levels in the plant were tens of times lower than the levels of the cinnamyl glycosides and slidroside. However, the absence from the HPLC chromatorams of the peaks of flavonoids is explained not only by their small amounts but also by the fact that in the system used (15% of ethanol in acetate buffer) rhodionin and rhodiosin were not eluted from the column. The chromatograms also lacked the peaks of sterols and monoterpenes, especially rhosiridin, in spite of its high concentration (the vield on its isolation was more than 1% of the weight of the raw material). This is explained by the fact that these terpenoids do not contain chromophoric groups and are transparent in UV light of the working wavelengths.

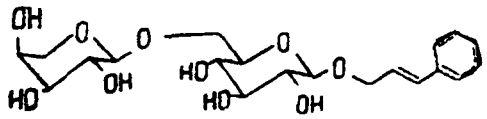
In view of these factors, the HPLC determination of the sterols, monoterpenes, and flavonoids in the raw material from roseroot stonecrop requires different methodological approaches, and this is the object of independent investigations.

To develop a procedure for the quantitative determination of rosavin, a calibration curve was plotted by the use of an authentic sample of this substance, using detection at 254 nm. The calibration relationships of peak area versus concentration and peak intensity versus concentration were linear in the range C = 2-50 μg/ml.

The amounts of rosavin in extracts were calculated from the peak intensity and the peak area (automatic separation of the peaks and integration). The results of the calculations from intensity and area coincided. Reproducbility was checked by three determinations for each concentration (σ = ±2%). The amount of rosavin in the raw material from cultivated plants (Moscow province) was about 2%.

The method for the HPLC determination of rosavin that has been developed has been used to study the autofermentation of the raw material of roseroot stonecrop (Figs. 2 and 3), since an instability of rosavin during the storage of some (incompletely dried) samples of the rhizomes and also on the extraction of the fresh raw material with ethanol by the method of steeping at room temperature has bene reported [11]. The results presented in Fig. 3 indicate that the amount of rosavin fell sharply even int ne stage of the conversion of the raw material into a pulp at room temperature: from 1.2% (control sample of the rhizomes) to 0.29% (control-2). A fall in the level of this component took place during subsequent autofermentaiton, most rapidly at a temperature of 40°C (Fig. 3).

In the course of autofermentation, it is mainly rosavin that is broken down (Fig. 2) while the other glycosides, including rosarin, are scarcely affected. It is obvious that in the enzyme system of roseroot stonecrop rhizomes the most active enzyme is vicianosidase: rosavin (cinnamyl vicianoside) is split almost completely (see Figs. 1 and 2), while rosarin, differing from rosavin only by the fact that the L-arabinose residue in it has the furanose form, undergoes no changes, and nor do the glucosides (rosin, salidroside), the peaks of which can be seen on the chromatogram Fig. 2).



As the result of autofermentation, the chemical composition of roseroot stonecrop rhizomes changes sharply and, as can be seen from Fig. 2, the main component of the extract becomes cinnamyl alcohol: in this process the amount of tyrosol (the aglycon of salidroside) does not increase.

EXPERIMENTAL

HPLC was performed in the isocratic regime on a Gilson chromatograph (France) with a μ-Bondapak C_{18} column (10 μm, 3.9 × 300 mm), Waters (USA). A mixture of 0.16 M acetate buffer, pH 5.5, and 95% ethanol in a ratio of 85:15 was used as the mobile phase. The rate of elution was 1.0 ml/min. The detection of the substances being separated was achieved by a UV detector at two wavelengths simultaneously: λ_1 = 254 nm, λ_2 = 280 nm.

Preparation of the Extract. A weighed sample of raw material was extracted with 60% ethanol in a ratio of 1:20 under the conditions described in [11]. The extract was diluted with water in a ratio of 1:3, and 25 μl was introduced into the chromatograph.

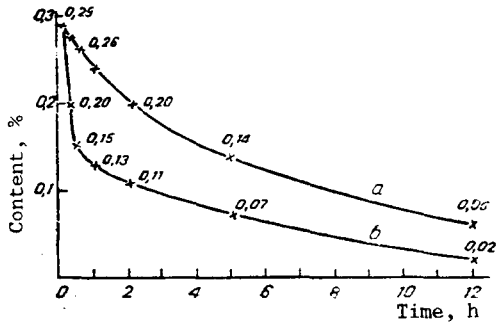


Fig. 3. Amount of rosavin in Rhodiola rosea rhizomes during autofermentation: a) at 50°C; b) at 40°C.

Autofermentation of the Raw Material. A piece of washed fresh roseroot stonecrop rhizome (grown in the Moscow region) weighing about 70 g was finely cut, a sample of about 5 g (control sample of the rhizome) was taken and the remainder of the raw material was ground in a meat grinder and another sample was taken (control-2), and then the residue was separated into 12 parts (approximately 5 g each) which were placed in bottles with stoppers, and 6 samples were kept in thermostats at 40°C and 6 at 60°C. During fermentation, samples of the raw material (after 15 min, 30 min, 1 h, 2 h, 5 h, and 12 h) were taken from the thermostats and were rapidly transferred to a drying chest for drying at 80°C. The control samples were also dried under these conditions.

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