20. M. Lienne, M. Caude, and A. Tambute, Analysis, 15, 431 (1987).
21. V. A. Davankov, J. D. Navratil, and H. E. Walton, Ligand-Exchange Chromatography, CRC Press, Boca Raton, Fla (1988).
22. V. P. Georgievskii, The Use of Thin-Layer Chromatography for the Identification and Quantitative Determination of Biologically Active Substances of Plant Origin [in Russian], Central Bureau of Scientific and Technical Information, Moscow (1975).

PHENYLPROPANOIDS OF A CALLUS CULTURE OF Rhodiola rosea

V. A. Kurkin, G. G. Zapesochnaya
A. G. Dubichev, E. D. Vorontsov,
I. V. Aleksandrova, and R. V. Panova

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Using 11 phenylpropanoids isolated from the biomass of a callus culture of roseroot stonecrop, the component compositions in the biomasses of callus and suspension cultures have been studied and their triandrin contents have been determined by the HPLC method.

We have previously [1, 2] reported the isolation of phenylpropanoids from the biomass of a tissue culture of roseroot stonecrop (Rhodiola rosea L.) possessing stimulating properties [3].

In the present paper we give experimental results confirming the structures of the compounds isolated (I-XIII). In addition, we have determined the component compositions of the biomasses of tissue and cell cultures of roseroot stonecrop by the method of high-performance liquid chromatography (HPLC) and have developed a method for the quantitative determination of triandrin (III) – one of the main biologically active substances of the biomass [4].

CHEMICAL STUDY

In the course of an investigation of the chemical composition of the biomass of a callus culture of roseroot stonecrop we isolated compounds having a phenylpropane skeleton that were derivatives of p-hydroxycinnamyl alcohol (I-IV), of p-coumaric acid (V)-(VII), and of caffeic acid (VIII and IX), or belonged to the lignan group (X and XI). Among the accompanying substances, β-sitosterol (XII) and its glucoside daucosterol (XIII) were identified.

In a study of the structures of the compounds isolated we used the results of chemical investigations [acetylation, methylation, enzymatic and acid hydrolysis, and the qualitative reaction with diazotized sulfanilic acid (DSA)], and also a comparison of physicochemical constants and spectral characteristics with those given in the literature; in some cases a comparison with authentic samples was used.

On methylation with diazomethane, triandrin (III) is converted into vimalin (IV). Their enzymatic hydrolysis with β-gluosidase gave, respectively, p-coumaryl (I) and p-methoxycinnamyl (II) alcohols, which were isolated in the free form. The acetylation of triandrin (III) gave a pentaacetate including an aromatic acetoxy group (δ 2.30 in the PMR spectrum) which showed the glycosylation of the alcohol group of the p-coumaryl alcohol. Doublets with J = 16 Hz in the PMR spectra characterized compounds (I-IV) as derivatives of trans-cinnamyl alcohol.

Triandrin and vimalin with the cis configuration of the double bond, isolated from willow bark (Salix trianda and S. viminalis), have been described in the literature [5]. We repeated this experiment. The substances isolated from the willow bark had constants corresponding to this given in the literature [5] but they had the trans configuration of the double bond, like compounds (III) and (IV). In view of this, the structures of triandrin and vimalin given in the literature [5] must be corrected.

β-Glucosidase readily hydrolyzed compounds (VI) and (VII) to p-coumaric acid (V), and compound (IX) to caffeic acid (VIII), and these were also isolated from the biomass. The

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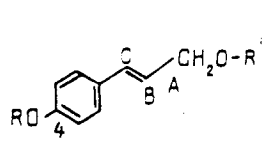
TABLE 1. Retention Times (R_t) of Individual Substances and Various Peaks of an Extract of the Biomass of Roseroot Stonecrop

No. of the peak of the extract	R_f, mm	Substance	R_t, min
1	6,0	p-Coumaric acid 4'-glucoside (VII)	6,0
2	8,9	Caffeic acid 3'-glucoside	9,0
3	10,1	Triandrin (III)	10,2
4	10,8	p-Coumaric acid 1-glucoside (VI)	10,8
5	14,8	Caffeic acid (VIII)	14,8
6	19,0	p-Coumaryl alcohol (I)	19,0
7	28,1	p-Coumaric acid (V)	27,0
8	40,5	Lariciresinol 4-glucoside (XI)	40,0
—	—	Vimalin (IV)	48,5
—	—	Salidroside	6,6
—	—	Tyrosol	9,8
—	—	Rosarin	23,0
—	—	Rosarin	24,5
—	—	Rosin	26,5

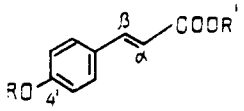
positions of glycosylation were determined from their UV spectra and by the reaction with DSA, which was positive (red coloration) for compounds (VI) and (IX) and negative for substance (VII). Compound (VII) (p-coumaric acid 4'-O-β-D-glucopyranoside) has not been described in the literature.

The lignan glucoside (XI) was hydrolyzed by β-glucosidase with the formation of substance (X), which was identified as (−)-lariciresinol [6, 7]. According to NMR spectroscopy, the hexaacetate obtained on the acetylation of (XI) contained only one phenolic acetoxy group. Consequently, one of the phenolic groups of lariciresinol was glycosylated. The choice was made on the basis of a comparative analysis of the mass spectra of the aglycon of the initial substance and of the methyl ether of lariciresinol obtained by treating compound (XI) with diazomethane, followed by enzymatic hydrolysis. The mass of the benzyl fragment (m/z 137) had not changed, while that of the benzoyl fragment had increased from m/z 151 to 165. Thus, the 4-position of the glycosyl fragment was established. This glycoside is a new natural compound. Only the 9'-O-β-D-glucoside of lariciresinol, in which the aliphatic hydroxy group is glycosylated, has been described in the literature [7].

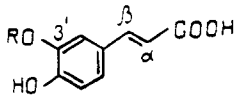
It must be mentioned that neither the salidroside nor the phenylpropanoids that are characteristic for the rhizomes of roseroot stonecrop (rosin (XIV), rosavin (XV), and rosarin (XVI)) [8, 9] were found in the samples of biomass.



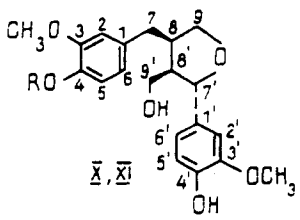
I - IV



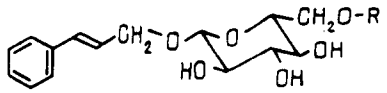
V - VII



VIII, IX



X, XI



XIV - XVI

I. R=R'=H
II. R=CH_3, R'=H
III. R=H, R'=β-*D*-Glc
IV. R=CH_3, R'=β-*D*-Glc
V. R=R'=H
VI. R=H, R'=β-*D*-Glc
VII. R=β-*D*-Glc, R'=H

VIII. R=H
IX. R=β-D-Glc
X. R=H
XI. R=β-*D*-Glc
XIV. R=H
XV. R=α-*L*-Ara-p
XVI. R=α-*L*-Ara-f

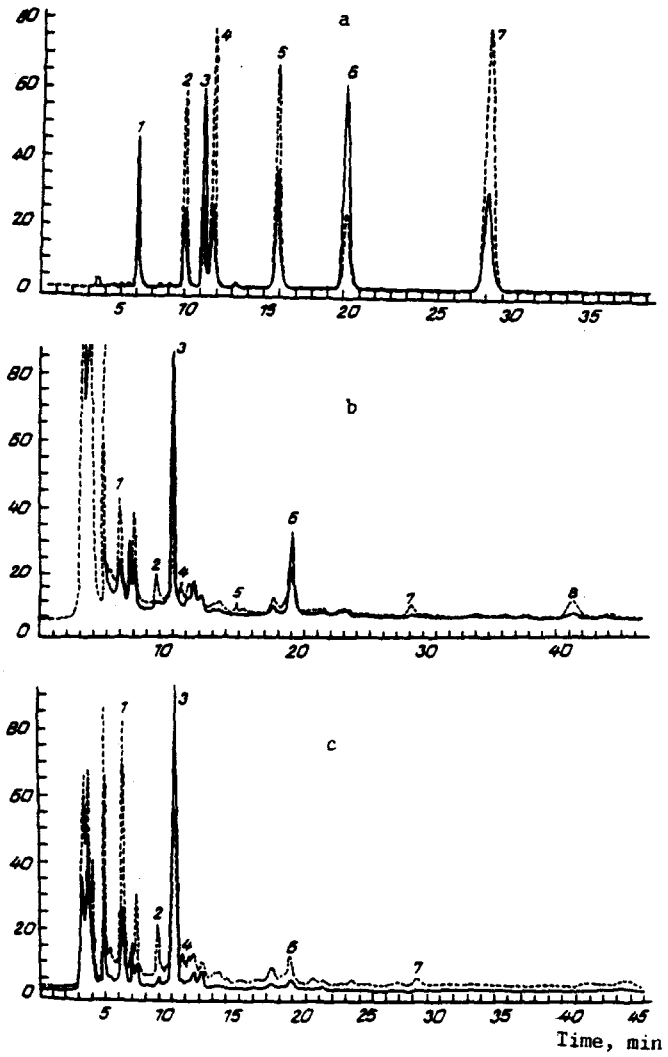


Fig. 1. HPLC separation of the biomass of roseroot stonecrop (the numbers of the peaks correspond to those given in Table 1; the full line shows absorption at 264 nm, and the dotted line that at 280 nm: a) model mixture of individual substances (sensitivity 0.2 O.D. at both wavelengths); b) extract of a callus culture (0.1 O.D); c) extract of a suspension culture (0.1 O. D.).

The investigations performed permit the statements that literature information [10] on the isolation from the biomass of roseroot stonecrop of tyrosol (p-hydroxyphenylethanol) and its glycoside salidroside are erroneous.

On the basis of our chemical study it is proposed to perform the standardization of the biomass of roseroot stonecrop from its phenylpropanoid content using a standard sample of triandrin.

HPLC ANALYSIS

The aim of the present investigations was to determine the component composition of an extract of the biomass of roseroot stonecrop by the HPLC method, to develop a procedure for the quantitative determination of triandrin, and to make a comparative evaluation of its accumulation in callus and suspension cultures.

In the development of a method for qualitative and quantitative analysis with the aid of reversed-phase HPLC on silica gel-C_{18}, a choice of chromatographic conditions was made. The detection of the separated substances was achieved with the aid of a UV detector at two

wavelengths simultaneously: λ_1 = 264 nm (the absorption maximum of triandrin (III), and also of compounds (I), (II), (IV), and (VII)), and λ_2 = 280 nm (the absorption maximum of the lignan (XI), and also of substances (V) and (VI), (VIII), (IX), and (X)).

The assignment of the peaks of the substances on the chromatograms of extracts of the biomass of roseroot stonecrop was made on the basis of the retention times of the individual components (Table 1), and also of model mixtures prepared from them (Fig. 1a). Since we were faced with the task of the quantitative determination of triandrin (III), we studied most carefully the behavior of the substances forming a group of peaks just in this region of the chromatogram (Fig. 1, peaks 2, 3 and 4).

Chromatograms of extracts of two samples of biomass are shown in Fig. 1b and c. As can be seen from the chromatograms, the extracts consisted of mixtures of substances with different absorptions at 264 and 280 nm. From their retention times, eight compounds were identified in the extracts (Table 1). The relative amounts of the various substances differed in the two extracts, this applying particularly to compounds (I), (III), (VII), and (XI).

Of the 13 substances isolated, the peaks of eight compounds were present in the chromatograms (Table 1). The peaks of the other substances were absent for various reasons: vimalin (IV) and its aglycon (II) were present in minor amounts, lariciresinol (X) has a low specific absorption coefficient and was present in comparatively small amount, while the sterols (XII) and (XIII) were transparent in UV light of the working wavelengths.

For the quantitative characterization of the extracts we determined the concentrations of triandrin (III) in them using detection at 264 nm. The peak area–concentration and peak intensity–concentration calibration curves were linear in the range of concentration of triandrin of from 4 to 130 μg/ml. The results of calculations of the amounts of triandrin in the extracts from the intensities and areas of the peaks coincided. Reproducibility was checked by three determinations for each concentration (σ = ±2%).

Together with factory samples of biomass, we made a HPLC comparison of the compositions of a number of samples of roseroot stonecrop biomass obtained under laboratory conditions on agarized and liquid media.

Figure 1c gives a chromatogram of an extract of a suspension of biomass cultivated for 15 days. The concentration of triandrin in it was 0.19%, while the second component by weight was compound (VII) (peak 1). In an earlier phase of cultivation (eight days) the concentration of triandrin differed little (0.15%), but its aglycon (I) was present in considerable amounts (~0.1%). There were practically no lignans in the samples of suspension culture.

A comparison of the samples of callus culture (for example Fig. 1b) the cultivation of which was continued for 25 days, showed that the amount of triandrin in them was somewhat lower (0.02-0.06%) but a lignan glycoside (peak 8) was present in considerable amounts. In spite of the fact that peak 8 of lignan (XI) had a comparatively small area on the chromatogram (Fig. 1b), its amount was comparable with that of triandrin (about 0.15% in the factory callus) since the specific adsorption index of glycoside (XI) at 280 nm is 10 times smaller than the specific absorption index of triandrin at 264 nm.

Thus, in a suspension culture the main component was the phenylpropanoid triandrin, while in a callus culture the process of biosynthesis went further and, together with triandrin, the main components were dimeric phenylpropanoids – lignans – i.e., processes of the "ageing" of the biomass took place.

The results that we obtained in a detailed study of the chemical composition of the rhizomes of roseroot stonecrop [8], including those obtained by the HPLC method [9] permit the statement that chromatograms of biotechnological extracts contain practically no peaks corresponding to tyrosol, salidroside, rosin, rosavin, and rosarin (retention times given in Table 1). For greater certainty, we added a model mixture of these substances to extracts of the biomass, as a result of which the corresponding additional peaks appeared on the chromatograms. These substances, particularly rosarin and salidroside, are present in extracts of the roots [9].

Attention is attracted by the possibility of directing the biosynthesis of phenolic substances in plant cells of roseroot stonecrop grown in vitro – the conditions of cultivation had an appreciable influence on this process. Proposals for the correction of biosyn-

theses could obviously arise, in a study of enzyme systems where, to all appearances, phenol hydroxylases, which convert the rosin (XIV) that is characteristic for the intact plant into p-hydroxyrosin (triandrin, III), are activated.

EXPERIMENTAL

Spectral characteristics were obtained on Varian HA-100D (100 MHz) and Bruker (250 and 500 MHz) instruments for ^1H NMR (δ scale, O – TMS); for the acetates of compounds of (III) and (XI) the assignment of the NMR signals was made by double resonance; a Varian CH-8 instrument at 70 eV was used for the mass spectra; Specord M40 and Hitachi EPS-3T for UV; and a UR-20, with paraffin oil, for IR. Melting points were determined on a Koffler block. Elementary analysis corresponded to the calculated figures. Angles of rotation were determined on a Polamat A polarimeter at 546 nm with recalculation for 589.3 nm. β-Glucosidase from the firm Serva was used for enzymatic hydrolysis.

Chromatographic monitoring was carried out by TLC on Silufol UV 254 in the chloroform–methanol–water (26:14:3) (1) and chloroform–methanol (4:1) (2) systems and by PC (identification of the sugars) in the butanol–acetic acid–water (4:1:2), ethyl acetate–propanol–water (7:2:1), and butanol–pyridine–water (6:4:3) systems. Detection (TLC): UV at 254 nm, diazotized sufanilic acid in an alkaline medium, and 16% sulfuric acid with heating; in the case of PC: aniline phthalate with heating.

HPLC was conducted in the isocratic regime on a Gilson chromatograph (France) with a TSK ODS-120T column (5 μm, 0.46 × 25 cm, LKB, Sweden). The mobile phase was CH_3CN–0.2% CH_3COOH (125:875). The rate of elution was 1.0 ml/min. Detection was carried out with a UV detector at two wavelengths simultaneously (264 and 280 nm). As reference materials for the identification of the peaks in the chromatograms of the extracts under investigation we used the individual substances shown in Table 1. Retention times were determined for each substance separately and in model mixtures. In a number of case, individual substances and model mixtures were added to the extracts.

Preparation of Extracts for HPLC. A weighed sample of air-dried biomass was extracted with 80% ethanol in a ratio of 1:20 at the boil for 30 min. After cooling, the extract was filtered through filter paper and was centrifuged at 3000 rpm on a Janetzki T32 centrifuge for 10 min. The supernatant liquid was separated off and was diluted with double-distilled water in a ratio of 1:3, and 2020 μl was injected into the chromatograph.

Samples of Biomass. For the isolation of the substances we used the biomass from a commercial strain of roseroot stonecrop cultivated by the surface method in the Volgograd Biochemical Factory. Under laboratory conditions, a number of samples of biomass were obtained on agarized and liquid media (times of cultivation 25 and 15 days, respectively).

Isolation of the Substances. The air-dried biomass from roseroot stonecrop (callus, Volgograd factory, 1983) (2.2 kg) was exhaustively extracted with a 80% ethanol at a ratio of 1:10 (twice at 20°C and twice at the boil). The combined extract was evaporated in vacuum to a syrupy residue, which was diluted with water to 0.7 liter and was then treated with chloroform (6 × 0.3 liter).

Compounds (XII), (X), (I), and (II), and (V) were obtained from the chlorofrom extract by chromatography on silica gel L 40/100 (chlf–MeOH (100:0 → 98:2) system).

The aqueous residue after treatment with chloroform was evaporated in vacuum to the syrupy state and was then mixed with polyamide and the mixture was dried in the air. The powder so obtained was chromatographed on polyamide (Olaine, 0.1-0.25 mm) using as eluents water and 50 and 96% ethanols. Subsequent chromatography of the evaporated 50% eluate on a column of silica gel (chlf–MeOH, 100:0 → 70:30) led to fractions in which compounds (III), (IV), (XI), (VI), (VII), (IX), and (XIII) predominated.

The final purification of daucosterol (XIII) was achieved by recrystallization from chlf–MeOH (1:1). Compound (XI) was purified by successive chromatography on Sephadex LH-20 (chlf–MeOH (97:3)) and on silica gel (chlf–MeOH (100:0 → 93:7)).

Compounds (III), (IV), (VI), (VII), (VIII), (IX) and (XI) were separated on a column of Woelm polyamide using mixtures of chloroform and MeOH in ratios of 95:5 (VIII), 90:10 (VI and VII), 88:12 (III), and 85:15 (IX). When the mixture of compounds (VI) and (VII) was rechromatographed on Sephadex LH-20 with chlf–MeOH (90:10 and 88:12), these substances were obtained in the individual form.

p-Coumaryl alcohol (I), yield 0.001% on the weight of the biomass. Lustrous white crystals with the compositions $C_9H_{10}O_2$, mp 116-118°C (water), λ_{max}^{EtOH} 264 nm. R_f 0.71 (system 1) and 0.50 (system 2). Mass spectrum, m/z (%): M^+ 150 (66), 107 (100), 94 (70).

p-Methoxycinnamyl alcohol (II), yield 0.0001%. Lustrous colorless crystals with the composition $C_{10}H_{12}O_2$, mp 75-78°C (acetone), λ_{max}^{EtOH} 261 nm. R_f 0.90 (1) and 0.69 (2). Mass spectrum, m/z (%): M^+ 164 (25), 121 (100), 108 (38).

Triandrin (III) yield 0.001%. White acicular crystals with the composition $C_{15}H_{20}O_7 \cdot H_2O$, mp 178-180°C (water). $[\alpha]_D^{20}$ -62.3° (water). λ_{max}^{EtOH} 264 nm (log ε 4.34). R_f 0.52 (1) and 0.14 (2).

^1H NMR spectrum in deuteropyridine at 250 MHz (ppm): 7.40 (d, 8.5 Hz, H-2.6), 7.14 (d, 8.5 Hz, H-3.5), 6.73 (d, 16 Hz, H_C), 6.35 (dt, 16 and 6 Hz, H_B), 5.00 (d, 7.8 hHz, H-1'), 4.76 (ddd, 12.5, 6.0 and 1.5 Hz, H_A), 4.60 (dd, 12 and 2.5 Hz, H-6'), 4.44 (dd, 12.5 and 6 Hz, H_A), 4.42 (dd, 12 and 5.5 Hz., H-6'), 3.9-4.35 (m, 4H of glucose).

Pentaacetate of (III). White crystals with the composition $C_{25}H_{30}O_{12}$, mp 107-108°C (ethanol). ^1H NMR spectrum in $CDCl_3$ at 500 MHz (ppm): 7.38 (d, H-2.6), 7.06 (d, H-3.5), 6.58 (d, H_C), 6.17 (dt, H_B), 5.22 (t, 9.5 Hz, H-3'), 5.12 (t, 9.5 Hz, H-4'), 5.05 (d, 9.5 and 7.0 Hz, H-2'), 4.62 (d, 7.0 Hz, H-1'), 4.50 (ddd, 13.0, 5.5 and 1.5 Hz, H_A), 4.28 (m, H_A) 4.26 (dd, 12 and 4.5 Hz, H-6'), 4.17 (dd, 12 and 2 Hz, H-6'), 3.72 (m, H-5'), 2.30 (s, 3H, arom. Ac), 2.08, 2.06, 2.03, 2.01 (singlets, 4 Ac).

Hydrolysis of (III). The hydrolysis of 10 mg of (III) with β-glucosidase was carried out in the usual way. p-Coumaryl alcohol (I) and glucose were obtained.

Methylation of (III). Compound (III) (15 mg) was methylated with diazomethane by the usual method. The product was purified on a column of silica gel using chlf–methanol (94:6). Vimalin (IV) was obtained.

Vimalin (IV), yield 0.0002%. Elongated white needles with the composition $C_{26}H_{32}O_{12} \cdot H_2O$, mp 74-77°C (water); melting point of the anhydrous substance 143-144°C, $[\alpha]_D^{20}$ -60.6° (MeOH), λ_{max}^{EtOH} 262 nm. R_f 0.58 (1), 0.29 (2).

Hydrolysis of (IV). When 5 mg of (IV) was hydrolyzed with β-glucosidase, p-methoxycinnamyl alcohol (II) and glucose were obtained.

The substance did not react with DSA, which showed the substitution of the aromatic hydroxyl at C-4°.

Hydrolysis of (VII). On hydrolysis with β-glucosidase, compound (VII) (5 mg) formed p-coumaric acid (V) and glucose.

Caffeic aicd (VIII), yield 0.0005% . Light yellow crystals with the composition $C_3H_8O_3$ (M^+ 180). λ_{max}^{EtOH} 235, 299 sh., 326 nm; mp 220-222°C (water–acetone). R_f 0.78 (1) and 0.46 (2).

Caffeic acid 3'-β-D-glucopyranoside (IX), yield 0.01%. Light yellow crystals with the composition $C_{15}H_{18}O_8$, mp 195-198°C (chlf–MeOH), λ_{max}^{EtOH} 227, 295 sh., 311 nm. R_f 0.46 (1) and 0.41 (2). ^1H NMR spectrum, 250 MHz, pyridine-d_6): δ 7.96 (d, 160.0 Hz, H-β), 7.90 (d, 1.7 Hz, H-2'), 7.27 (dd, 8.5 and 1.7 Hz, H-6'), 7.14 (d, 8.5 Hz, H-5'), 6.77 (d, 16 Hz, H-α), 5.53 (d, 7.5 Hz, H-1"), 4.46 (dd, 12 and 2 Hz, H-6"), 3.9-4.4 (m, 5 H of glucose). IR spectrum (cm^{-1}): 3350, 1690, 1625, 1595, 1510.

Hydrolysis of (IX). The hydrolysis of (IX) (5 mg) with β-glucosidase formed caffeic acid (VIII) and glucose.

(–)-Lariciresinol (X), yield 0.001%. Colorless syrupy substance with a yellowish tinge having the composition $C_{20}H_{24}O_6$, λ_{max}^{EtOH} 230, 282 nm $[\alpha]_D^{23}$ 24.2° (c 0.21; ethanol). R_f 0.90 (1) and 0.56 (2). Mass spectrum at 50 eV (m/z, %): M^+ 360 (17), 151 (100), 137 (62).

(–)-Lariciresinol 4-O-β-D-glucopyranoside (XI), yield 0.02%. Light yellow amorphous powder with the composition $C_{26}H_{34}O_{11}$, $[\alpha]_D^{20}$ –25.9° (c 1.1; ethanol). λ_{max}^{EtOH} (log ε): 227 (3.97), 280 nm (3.57). R_f 0.60 (1) and 0.15 (2). ^1H NMR spectrum (250 MHz, pyridine-d_5): δ 7.53 (d, 8,7 Hz, H-5'), 7.25 (d, 2 Hz, H-2'), 7.14 (d, 8.7 Hz, H-5), 7.08 (dd, 8.7 and 2 Hz, H-6'), 6.93 (d, 2 Hz, H-2), 6.82 (dd, 8.7 and 2 Hz, H-6), 5.63 (d, 6.5 Hz, H-1"), 5.27 (d, 6 Hz, H-7'), 4.47 (dd., 12 and 2 Hz, H-6"), 4.4-3.9 (m, 5H of glucose + 2H-9 + 2H-9'), 3.70 (s, CH_3O), 3.66 (s, CH_3O), 3.17 (dd, 14 and 5 Hz, H-7), 2.95 (m, H-8), 2.7 (m, H-7, H-8').

Hexaacetate of (XI). Colorless vitreous substance with the composition $C_{38}H_{46}O_{17}$, $[\alpha]_D^{23}$ −7.2° (c 3.2; ethanol). ^{1}H NMR spectrum (500 MHz, $CDCl_3$): δ 7.08 (d, 9 Hz, H-5'), 6.95 (d, 9 Hz, H-5), 6.89 (d, 2 Hz, H-2'), 6.81 (dd, 9 and 2 Hz, H-6'), 6.77 (d, 2 Hz, H-2), 6.74 (dd, 9 and 2 Hz, H-6'), 5.27 (m, H-2", H-3"), 5.17 (t, 9.5 Hz, H-4"), 4.95 (d, 7 Hz, H-1"), 4.80 (d, 6 Hz, H-7'), 4.36 (dd, 10 and 7 Hz, H-9'), 4.28 (dd, 12 and 5 Hz, H-6"), 4.20 (dd, 10 and 7 Hz, H-9'), 4.16 (dd, 12 and 2 Hz, H-6"), 4.09 (dd, 8 and 6 Hz, H-9), 3.83 (s, CH_3O), 3.82 (s, CH_3O), 3.76 (m, H-5", H-9), 2.88 (dd, 14 and 5 Hz, H-7), 2.74 (m, H-8), 2.60 (dd, 14 and 11 Hz, H-7), 2.55 (m, H-8'), 2.30 (s, 3H, arom. Ac), 2.08 (s, 6H, 2Ac), 2.03 (s, 9H, 3Ac).

Hydrolysis of (XI). The hydrolysis of (XI) (10 mg) with β-glucosidase under the usual conditions led to (−)-lariciresinol (X) and glucose.

Methylation of (XI) and Hydrolysis. Compound (XI) (10 mg) was methylated with diazomethane, and the product obtained was hydrolyzed with β-glucosidase. The lignan was purified on silica gel using chlf–methanol (95:5). A colorless amorphous powder was obtained with the composition $C_{21}H_{26}O_6$ (lariciresinol 4'-monomethyl ether); mass spectrum (m/z, %): M^+ 374 (70), 165 (52), 137 (100).

β-Sitosterol (XII), yield 0.15%. Lustrous white crystals with the composition $C_{29}H_{50}O$ (M^+ 414), mp 138-140°C (MeOH). R_f 0.95 (1) and 0.70 (2).

Daucosterol (XIII) yield 0.01%. White crystals with the composition $C_{35}H_{60}O_6$, mp 315-319°C (chlf–MeOH). R_f 0.73 (1) and 0.36 (2).

Hydrolysis of (XIII). On the acid hydrolysis of (XIII) (20 mg) with 10% HCl at 100°C for 5 h, β-sitosterol (XII) and glucose were obtained.

LITERATURE CITED

1. G. O. Zapesochnaya, V. A. Kurkin, I. V. Aleksandrova, and R. V. Panova, in: Abstracts of Lectures at the Vth All-Union Symposium on Phenolic Compounds (Tallin (1987), Section B, p. 37.
2. V. A. Kurkin and G. G. Zapesochnaya, in: Fifth International Conference on the Chemistry and Biotechnology of Biologically Active Natural Products, Varna, Bulgaria (1989). Conference Proceedings, Vol. 4, p. 204.
3. L. V. Levina, F. P. Krendal', I. V. Arkavyi, and A. N. Vanyushkin, in: Medicinal Plants in Traditional and Folk Medicine; Abstracts of Lectures of a Scientific Conference [in Russian], Ulan-Udé (1987), p. 89.
4. S. Ya. Sokolov, V. P. Boiko, V. A. Kurkin, G. G. Zapesochnaya, et al., Khim.-farm. Zh., 24, No. 10, 38 (1990).
5. W. Karrer, Konstitution und Vorkommen der organischen Pflanzenstoffe. Ergänzingsband 2, Teil 2, Birkhäuser Verlag, Stuttgart (1985), No. 6673.
6. M. M. Badawi, S. S. Handa, A. D. Kinghorn, G. A. Cordell, and N. R. Farnsworth, J. Pharm. Sci., 72, No. 11, 1285 (1983).
7. T. Satake, T. Murakami, Y. Saiki and C.-M. Chen, Chem. Pharm. Bull., 26, No. 5, 1619 (1978).
8. V. A. Kurkin, A Chemical Study of Roseroot Stonecrop (Rodiola rosea L.), Dissertation for Candidate of Pharmaceutical Sciences [in Russian], Moscow (1985).
9. A. A. Kir'yanov, L. T. Bondarenko, V. A. Kurkin, G. G. Zapesochnaya, A. A. Dubichev, and E. D. Vorontsov, Khim. Prir. Soedin., No. 3, 320 (1991).
10. T. I. Poletaeva, I. V. Aleksandrova, and E. A. Krasnov, Current Problems of Pharmacology and the Search for New Drugs [in Russian], Tomsk, Vol. 1 (1984), p. 149.