TERPENOIDS OF THE RHIZOMES OF *Rhodiola rosea*

V. A. Kurkin, G. G. Zapesochnaya,
and A. N. Shchavlinskii

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The structures of two new monoterpene compounds isolated from the rhizomes of *Rhodiola rosea* L. (*Crassulaceae*) have been established: 3,7-dimethylocta-2,6-diene-1,4-diol (rosiridol) and 3,7-dimethylocta-2,6-diene-1,4-diol 1-O-β-D-glucopyranoside (rosiridin). Daucosterol has been isolated from the rhizomes of this plant for the first time.

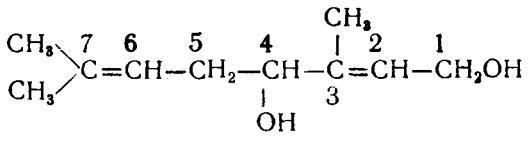
In a study of the flavonoids and cinnamyl glycosides of the rhizomes of roseroot sedum (*Rhodiola rosea* L. family *Crassulaceae*) [1-4], fractions containing substances of terpenoid nature were obtained. As the result of the chromatographic purification of these fractions, two new compounds have been isolated which have been called rosiridol (I) and rosiridin (II), and also daucosterol (IV) and the β-sitosterol (III) that is known for oyster plant (*Scolymus*) [5]. Compounds (I) and (II) were detected on Silufol plates in the form of blue spots on treatment with 20% sulfuric acid (110°C). Compounds (III) and (IV) appeared under these conditions in the form of crimson spots.

The enzymatic hydrolysis of rosiridin (II) with β-glucosidase gave glucose and an aglycone identical with compound (I) which could not be isolated on the acid hydrolysis of (II).

Rosiridol (I) consists of an acyclic monoterpene alcohol. Its mass spectrum lacks the peak of the molecular ion, which is characteristic for compounds of this class [6-8]. The resiridol molecule contains two hydroxy groups giving bands at 3610 and 3380 cm^{-1} in the IR spectrum. On acetylation it formed a diacetate in the IR spectrum of which the bands of OH groups had disappeared, while in its NMR spectrum the signals of two acetoxy groups had appeared (Table 1).

Rosiridol gave singlet signals of three methyl groups located at a double bond (1.64 and 1.72 ppm), of two olefinic protons (δ 5.05 and 5.40) and of a methylene group (δ 2.20), and also the signals of methine (δ 3.90) and methylene (δ 4.06) groups (see Table 1). The chemical shifts of the last two signals were characteristic for protons geminal to OH groups and, as was to be expected, these signals shifted downfield when rosiridol was acetylated (δ 4.90 and 4.50, respectively).

In a comparison with literature information [6-9] the following facts were revealed: the PMR spectrum of rosiridol differed from that of geraniol [9] by the fact that instead of two signals of CH_2 groups at 2.20 ppm only one was present, and the signal of a $\geq$CH—OH group appeared in the weak field (3.90 ppm). Thus, rosiridol (I) is a hydroxygeraniol, and the parameters of its spectra permit the structure of 2,7-dimethylocta-2,6-diene-1,4-diol to be proposed for it:



(I)

In order to establish the structure of rosiridin (II) it was necessary to determine the position of glycosylation of compound (I): at the C-1 or the C-4 hydroxy group. This

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TABLE 1. Characteristics of the ^1H NMR Spectra of Rosiridin and Its Derivatives (δ, ppm, J, Hz; internal standard TMS, 100 MHZ)

Proton	Compound and solvent							
	rosiridol (I)		rosiridol diacetate		rosiridin (II)		rosiridin pentaacetate	
	CCl_4	C_6D_6	CCl_4	C_6D_6	C_5D_5N	$(CD_3)_2CO$	CCl_4	C_6D_6
2H-1	4.06 (d, 2H) J=6	4,10 (d, 2H)	4,50 (d, 2H)	4,50 (d, 2H)	3,8—4,8 (m, 9H)	3,4—4,3 (m, 8H)	3,9—4,3 (m, 4H)	4,0–4,4 (m, 4H)
H-2	5,50 (t, 1H) J=6	5,65	5,50	5,65	6,00	5,57	5,45	5,65
H-4	3,90 (t, 1H) J=6	4,00	4,90	5,10	3,8—4,8 (m, 9H)	4,03	4,7—5,2 (m, 5H)	5,0—5,5 (m, 5H)
2H-5	2,20 (br.t, 2H)	2,28	2,28	2,30	2,46	2,23	2,30	2,33
H-6	5,05 (t, 1H) J=6	5,20	5,00	5,27	5,40	5,16	4,7—5,2 (m, 5H)	5,0—5,5 (m, 5H)
CH_3	1,64 (s, 3H)	1,52	1,62 (s, 3H)	1,50	1,62	1,60 (s, 3H)	1,62	1,56
	1,72 (s, 6H)	1,56	1,68 (s, 6H)	1,54	1,68	1,67 (s, 6H)	1,66	1,60
		1,62		1,62	1,80		1,70	1,68
CH_3CO	—	—	1,97 (s, 6H)	1,68 1,70	—	—	1,96 (s, 3H) 2,00 (s, 9H) 2,04 (s, 3H)	1,78 1,79 1,80 1,81 1,84
H-1' of the glucose residue	—	—	—	—	4,85 (d, 1H) J=7	4,40 (d, 1H) J=7	4,50 (d, 1H) J=7	4,44 (d, 1H) J=7
Other protons of the glucose residue	—	—	—	—	3,8—4,8 m, 9H (2H-1, H-4, 6H of the glucose residue	3,4—4,3 m, 8H (2H-1, 6H of the glucose residue	4,7—5,2 m, 5H (H-4, H-6, H-2', 3', 4') 3,9—4,3 m, 4H (2H-1, 2H-6') 3,60 (m, 1H) (H-5')	5,0—5,5 m, 5H (H-4, H-6, H-2', 3', 4') 4,0—4,4 m, 4H (2H-1, 2H-6') 3,34 M, 1H (H-5')

TABLE 2. Theoretical Distribution of the Signals in the PMR Spectra of the Pentaacetate of Rosiridin (II) with the Two Possible Types of Glycosylation

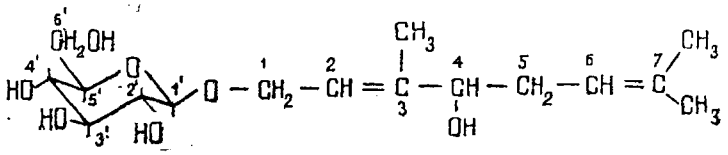
Type of glycosylation	5,0–5,5 ppm	4,4–4,6 ppm	4,0–4,4 ppm
1-Glycosylation	H-4, H-6, H-2',3', 4' total 5H	H-1' 1H	2H-1, 2H-6' 4H
4-Glycosylation	H-6, H-2', 3', 4' total 4H	H-1', 2H-1 3H	H-4, 2H-6' 3H

question was solved by comparing the NMR spectra of the glycoside, the aglycone, and their full acetates (Table 1). In the first case, of the two OH groups of the aglycone it will be the 4-OH group that is acetylated and the signal of its geminal proton (H-4) should shift to ~5.0 ppm. In the second case, the OH at C-1 will be acetylated, and the signals of its two geminal protons (2 H-1) would be located at ~4.5 ppm. Having analyzed the integral intensities

of the three groups of signals of the glycoside pentaacetate in the 4.0-5.5 ppm region for the two possible glycosylation variants (Table 2) and having compared them with the actual values (Table 1), we made our choice in favor of 1-glycosylation.

A pyranoid glucose ring and its $^4C_1$ conformation, and also a β-bond with the aglycone followed from the results of enzymatic hydrolysis and the PMR spectrum (H-1, doublet with J = 7 Hz).

Thus, the structure of 3,7-dimethylocta-2,6-diene-1,4-diol 1-O-β-D-glucopyranoside has been established for rosiridin (II):



II

It must be mentioned that only recently has information appeared in the literature on natural glycosides of acyclic monoterpenes [8, 10-12], although glycosylated monoterpene compounds of the iridoid type are widely represented in plants [13].

Compound (III) was transparent in UV light, and its IR spectrum had the band of an OH group (3420 cm^{-1}). In the 0.6-2.3 ppm region of the NMR spectrum of (III) in deuterochloroform there was a multiplet with an intensity of about 47 protons with the distinct signals of several CH_3 groups and in the weak field appeared the quartet of a proton of a double bond (=CH—CH_2—, 5.3 ppm) and the multiplet of the proton in a ≻CH—O— grouping (3.3-3.7 ppm) which shifted downfield in the acetate.

In the mass spectrum (III), apart from the molecular ion, the strongest ions were those corresponding to the loss of water and to the detachment of the side chain, and also to its cleavage at branching positions (—CH_3, —C_6H_{13}, —$C_{10}H_{21}$). The combination of facts given permitted compound (III) to be identified as β-sitosterol.

On acid hydrolysis, compound (V) gave glucose and an aglycone identical with β-sitosterol in composition, chromatographic mobility, and NMR and mass spectra. In the NMR spectra of the TMS ether of (IV) there was a multiplet with an intensity of about 47 protons in the strong field (0.6-2.3 ppm) and in the weak field at 5.24 ppm a poorly resolved quartet of a proton at a double bond (=CH—CH_2—) and at 4.2 ppm a doublet (J = 7 Hz) of the anomeric proton of a β-D-glucose residue, while six H of the glucose residue and 1 H of the aglycone (in a ≻CH—O— grouping) resonated in the 2.9-3.7 ppm region. In the spectrum of the tetraacetate of (IV) the signals of four acetyl groups could be seen (1.98-2.08 ppm). Thus, compound (IV) was β-sitosterol β-D-glucoside (daucosterol).

EXPERIMENTAL

The spectral information was obtained on the following instruments: Varian HA-100D at 100 MHz with tetramethylsilane as internal standard (PMR); Varian CH-8 at 70 eV (mass spectra); Hitachi EPS-3T (UV); and UR-20, paraffin oil (IR). Melting points were determined on a Kofler stage, elementary analyses were performed on a Hewlett-Packard 185B automatic CHN analyser and angles of rotation on a Polamat A polarimeter at 546 nm with calculation by means of the formula $[\alpha]_D = [\alpha]_{546} \cdot 1{,}17543$. Chromatographic monitoring was performed by TLC (Silufol UV-254) in the following systems: 1) chloroform—methanol—water (26:14:3) and 2) chloroform—methanol (6:1), and PC (for the identification of the sugars) in 3) butanol—acetic acid—water (4:1:2) and 4) water-saturated phenol.

Isolation. Fractions with substances (I)-(IV) were obtained in the isolation of the flavonoids and cinnamyl glycosides [1-3]. Compounds (I) and (III) were purified by chromatography on a column of silica gel with a mixture of petroleum ether and chloroform (the yield of each of these substances was 0.05%). The fractions containing compound (II) were purified on a column of polyamide, and then on silica gel with elution by mixtures of chloroform and methanol (99:1—93:7) (yield 1.0%). Compound (IV) was purified by recrystallization from chloroform—ethanol (1:1) (yield 0.1%).

3,7-Dimethylocta-2,6-diene-1,4-diol (rosiridol) (I). Colorless syrupy substance with the composition $C_{10}H_{18}O_2$, $[\alpha]_D^{20}$ —7.7° (*c* 1.3 ; acetone), R_f 0.50 (system 2) and 0.80 (system 1). For the PMR spectrum, see Table 1. Mass spectrum at 30°C, m/z (intensity, %): 153(1), 152(2), 139(2), 101(29), 84(8), 83(60), 73(8), 71(13), 70(100), 69(20), 59(6), 57(4), 56(4), 55(54), 53(5), 43(5), 41(11).

Diacetate of (I). Colorless syrupy substance. For the PMR spectrum, see Table 1.

3,7-Dimethylocta-2,6-diene-1,4-diol 1-O-β-D-Glucopyranoside (Rosiridin) (II). Colorless syrupy substance with the composition $C_{16}H_{28}O_7 \cdot 0.5H_2O$, $[\alpha]_D^{20}$ —32.7° (*c* 1.1 ; acetone); R_f 0.52 (system 1) 0.16 (system 2). For the PMR spectrum, see Table 1. Mass spectrum at 130°C, m/z (intensity, %): 262(7), 170(1), 163(17), 153(11), 152(3), 145(30), 127(21), 101(46), 97(16), 93(10), 91(13), 87(15), 85(43), 84(34), 83(50), 81(13), 73(58), 71(18), 70(100), 69(88), 62(28), 61(20), 57(29), 55(49), 45(18), 43(20), 41(53).

Pentaacetate of (II). Colorless crystals with mp 61-64°C (from ethanol), $[\alpha]_D^{20}$ —28.4° (*c* 1.0; acetone), for the PMR spectrum, see Table 1.

β-Sitosterol (III). Colorless crystals with the composition $C_{29}H_{50}O$, mp 132-135°C (from methanol); R_f 0.95 (system 1), 0.65 (system 2). Forms a green coloration with acetic anhydride in concentrated sulfuric acid (Liebermann—Burchard reaction). Mass spectrum at 120°C, m/z (intensity, %). M^+ 414(100), 396(91), 381(50), 329(73), 303(73), 273(43), 255(49), 231(31), 213 (42), 145(41), 81(49).

Monoacetate of (III), $C_{31}H_{52}O_2$, mp 120-121°C (from ethanol).

Daucosterol (IV). Colorless crystals with the composition $C_{35}H_{60}O_6$, mp 315-319°C (from chloroform—methanol (1:1)), $[\alpha]_D^{20}$ —41.7° (*c* 0.65 ; chloroform—methanol (1:1)); R_f 0.32 (system 2), 0.73 (system 1). The substance was transparent in UV light and gave a positive Liebermann—Burchard reaction.

Tetraacetate of (IV), $C_{43}H_{68}O_{16}$, mp 166-168°C (from ethanol).

Acetylation was carried out in acetic anhydride in the presence of pyridine (24 h at room temperature), and the reaction mixture was poured into ice water. The precipitate of the acetates of compounds (II), (III), and (IV) was washed with water and was recrystallized from ethanol. The acetate of (I) was extracted from the reaction mixture with chloroform, and the chloroform extract was evaporated and purified on a column of silica gel (with benzene as eluent).

Enzymatic Hydrolysis. A mixture of 50 mg of rosiridin (II) with an aqueous solution of 15 mg of β-glucosidase was kept at 38°C for 24 h. Glucose was detected in a hydrolysate by PC. A chloroform extract from the hydrolysate was passed through a layer of silica gel, giving 20 mg of rosiridol (I).

Acid Hydrolysis. 1. A mixture of 10 mg of (II) and 5 ml of 1% HCl was heated to 100°C for 30 min. Under these conditions the algycone broke down and only glucose was detected in the hydrolysate (PC).

2. A solution of 10 mg of (IV) in 2 ml of chloroform-methanol (1:1) was heated with 5 ml of 10% HCl at 100°C for 2 h. Glucose (PC) and β-sitosterol (TLC) were detected in the acid hydrolysate.

SUMMARY

Daucosterol has been isolated for the first time from the rhizomes of *Rhodiola rosea* L. together with two new compounds of monoterpene nature for which the following structures have been established: rosiridol — 3,7-dimethylocta-2,6-diene-1,4-diol; and rosiridin — 3,7-dimethylocta-2,6-diene-1,4-diol 1-O-β-D-glucopyranoside.

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SHONACHALIN B — A NEW EUDESMANOLIDE FROM *Artemisia fragans*

S. V. Serkerov and A. N. Aleksarova

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The results are given of an investigation of a new eudesmanolide from *Artemisia fragans* Willd., shonachalin B, with the composition $C_{15}H_{22}O_4$, mp 127-129°C.

Continuing a study of the sesquiterpene lactones of *Artemisia fragans* Willd. [1, 2] growing in various soil and climatic conditions, by chromatography on a column of alumina of an acetone extract of plant material collected in the village of Shona-chala, Lerik region of the Azerbaidzhan SSR, we have isolated 20 mg of a crystalline substance with the composition $C_{15}C_{22}O_4$, mp 127-129°C (ether—hexane). The molecular weight determined by mass spectroscopy was 266.

The IR spectrum of the substance contained, in the region of characteristic frequencies, absorption bands of a γ-lactone ring (1760 cm^{-1}) and of a hydroxy group (3480 cm^{-1}).

The NMR spectrum of the substance showed the singlet of an angular methyl group (1.26 ppm, 3 H, $CH_3—\overset{|}{C}—$), and the doublet of a secondary methyl group attached to a lactone ring (1.28 ppm, J = 7 Hz, 3 H, $CH_3—CH<$), one component of the doublet being superposed on the singlet of the angular methyl group. A one-proton triplet at 4.11 ppm (J = 10 Hz) belonged to a gem-hydroxylic proton and indicated the secondary nature of the hydroxy group. The lactone proton appeared in the form of a quartet at 3.78 ppm (J_1 = 11.5, J_2 = 10 Hz).

The presence of an OH group in the molecule of the compound was also shown by peak at 248 in the mass spectrum due to the splitting out of one molecule of water ($M - H_2O$).

The substance contained one OH group, as was confirmed by the results of the oxidation of the lactone with chromium dioxide: a keto derivative was formed with the composition $C_{15}H_{20}O_4$, mp 166-168°C (ether—hexane). The IR spectrum of this ketone had the absorption bands of a CO group in a γ-lactone ring (1788 cm^{-1}) and of a ketone ring in a six-membered ring (1725 cm^{-1}). There was no band of an OH group in the spectrum.

The UV spectrum contained a maximum characteristic for an isolated ketone group (λ_{max} 283 nm, log ε 1.61).

Thus, of the four oxygen atoms present in the molecule of the lactone under consideration two formed the lactone ring, one was present in the form of an OH group, and the fourth formed an oxide ring. The presence of the oxide ring was shown by a three-proton singlet in the NMR spectrum of a methyl group of 1.40 ppm ($CH_3—\overset{|}{C}—O—$) [3-5] and of a one-proton doublet at 3.66 ppm (J = 5 Hz, $HC—O—\overset{|}{C}—$) [6] and the molecular weight of the compound.

On comparing the facts given with literature information, we came to the conclusion that

V. L. Komarov Institute of Botany, Academy of Sciences of the Azerbaidzhan SSR, Baku.
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