Chemistry of Natural Compounds, Vol. 34, No. 4, 1998

OLIGOMERIC PROANTHOCYANIDIN GLYCOSIDES OF *Rhodiola pamiroalaica*

A. É. Ismailov,[b] Z. A. Kuliev,[a] A. D. Vdovin,[a] N. D. Abdullaev,[a] and B. M. Murzubraimov[b]

UDC 547.972

Two oligomeric proanthocyanidin glycosides have been isolated from the roots of Rhodiola pamiroalaica *and their structures and relative configurations have been established: 7-O−[6-O-galloyl−β-D-Glcp←6-O-β-D-Glcp←6-O-β-D-Glcp]-(+)-gallocatechin-(4α-8)-(−)-epicatechin-(4β-8)-(−)-epicatechin-(4β-8)-(+)-catechin-(4α-8)-5-O-[6-O-galloyl-β-D-Glcp←6-O-β-D-Glcp←6-O-β-D-Glcp]-(+)-catechin — RP-1 — and 7-O-[O-D-Glcp←6-O-β-D-Glcp-]-(−)-epicatechin-(4β-6)-7-O-β-D-Glcp-(−)-epicatechin-(4β-6)-3-O-galloyl-(−)-epigallocatechin-(4β-6)-3-O-galloyl-(−)-epigallocatechin-(4β-6)-3-O-galloyl-5-O-β-D-Glcp-(−)-epicatechin —RP-2.*

Many species of plants of the genus *Rhodiola* (fam. Crassulaceae) are rich sources of proanthocyanidins [1] and are used in folk medicine. We have studied the proanthocyanidin composition of the roots of *Rhodiola pamiroalaica* A. Bor. growing in the rocky soils and gravels in the alpine regions in the upper reaches of the R. Akbura, Oshskaya oblast, Kyrgystan, and gathered in the flowering and budding phase [2]. By the CC on microcrystalline cellulose of a butanolic fraction of an aqueous alcoholic extract of the roots of *Rh. pamiroalaica* we have isolated two individual compounds and have called them proanthocyanidins RP-1 and RP-2.

According to its ^{13}C NMR spectra (Table 1), compound RP-1 is a glycosylated proanthocyanidin.

In the ^{13}C NMR spectrum a broad resonance signal in the 157.2 ppm region relates to the C-5, C-7, and C-9 carbon atoms of ring A. A resonance signal at 134.5 ppm (C-4′) witnesses the presence of a gallocatechin block, while signals at 117.0 and 120.4 ppm, relating to the C-2′, C-5′, and C-6′ atoms, respectively [sic], showed that RP-1 contains catechin blocks [3, 4].

TABLE 1. Chemical Shifts (ppm) of the Signals of the Carbon Atoms in the ^{13}C NMR Spectrum of Proanthocyanidin RP-1

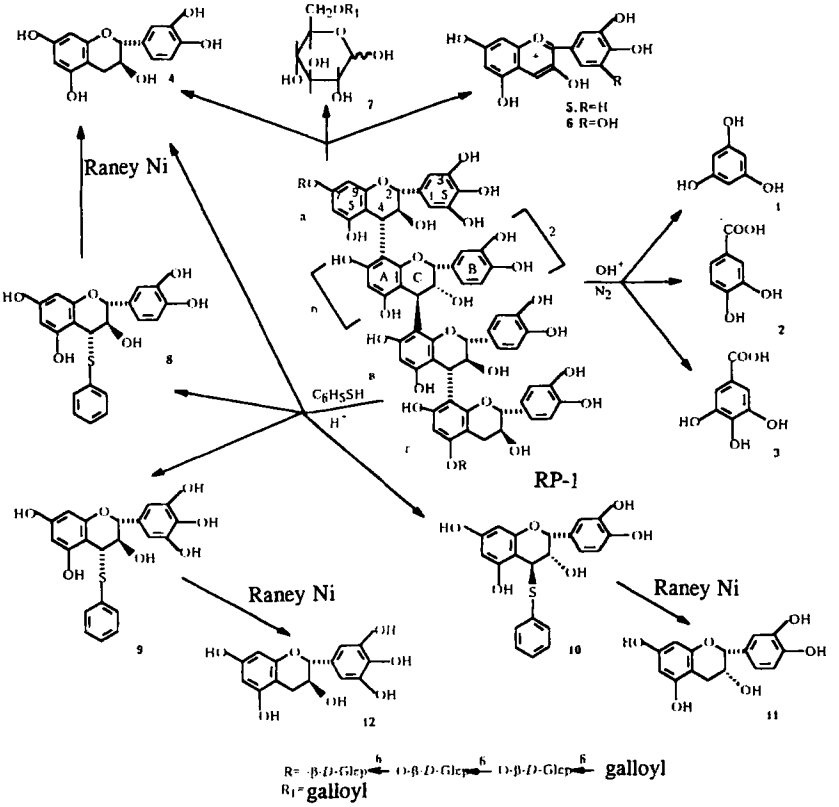
Carbon atom	RP-1 fragment					
	a	b	c	d	galloyl	glucose
C-2	84.5"	78.6"	83.6"	80.3		
C-3	74.4'	72.6*,72.0*	70.2*	68.2		
C-4	38.1	36.1	38.1	30.6		
C-6	98.6	98.6	98.6	98.6		
C-8	97.5	108.0	108.0	108.0		
C-10	101.6#	104.2#	105.0#	104.2#		
C-5, 7, 9	157.2	157.2	157.2	157.2		
C-1′	132.2	132.2	132.2	132.2	122.8	104.2#
C-2′	108.0	117.0	117.0	117.0	111.2	75.0'
C-3′	147.3	147.3	147.3	147.3	147.3	78.6
C-4′	134.5	147.3	147.3	147.3	140.6	71.3*
C-5′	147.3	117.0	117.0	117.0	147.3	77.8"
C-6′	108.0	120.0	120.4	120.4	111.2	
C-6 Gluc.						65.3, 64.8
C-6 Gall.						63.0, 63.3
-COO-					167.4, 168.8	

Note. Signals labeled with the same superscript symbol may be interchanged.

a) Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75; b) Osh State University. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 484-491, July-August, 1998. Original article submitted May 5, 1998.

 0009-3130/98/3404-0450$20.00

Signals from C-2 in fragments of the compound RP-1 appeared at 78.6, 80.3, 83.6, and 84.5 ppm, which unambiguously showed the realization of both the 2,3-*cis* [3, 5] and the 2,3-*trans* [3, 4, 6] stereochemistries of the flavan-3-ols in this compound. Signals in the 101.6-105.0 region, relating to C-10 atoms [3,7] are characteristic for proanthocyanidins with a C-4−C-8 interflavan bond. Signals of the C-1, C-3, and C-5 atoms of glucose at 104.2, 78.6, and 77.8 ppm, respectively, showed that the anomeric center has the β-configuration [8]. The glycoside residues are linked with one another by 1-6 bonds, as shown by the C-6 CSs of 65.3 and 64.8 ppm [10]. The carbohydrate residue is acylated in the sixth position, which is shown by signals at 63.0 and 63.3 ppm, relating to an esterified C-6 atom of a terminal glucose residue.



Scheme 1

The alkaline cleavage of RP-1 in an atmosphere of nitrogen formed three compounds, which were identified from their physicochemical parameters as phloroglucinol (**1**) and protocatechuic and gallic acids (**2**) and (**3**).

The acid hydrolysis of RP-1 led to the formation of (+)-catechin (**4**), cyanidin (**5**), delphinidin (**6**), and β-glucose acylated with gallic acid (**7**).

By mild thiolytic cleavage the "lower" block gave (+)-catechin (**4**) and the "upper" blocks a mixture of three thioethers (**8**)-(**10**), which were subjected to catalytic degradation in the presence of Raney nickel. The substances obtained were identified as (+)-catechin (**4**), (−)-epicatechin (**11**), and (+)-gallocatechin (**12**).

Enzymatic cleavage in the presence of β-glucosidase and acid hydrolysis of a permethylate of RP-1 showed that the glucan moiety in the proanthocyanidin consists of glucose molecules linked with one another by 1-6 bonds, one of them being acylated with gallic acid in the sixth position, while they are bound to the aglycon by a β-glycosidic bond.

On the basis of the results of alkaline, acid, and thiolytic cleavages (Scheme 1) and ^{13}C NMR spectral characteristics and their comparison with the literature, the structure and relative configuration of the oligomeric proanthocyanidin RP-1 that had been isolated was established as: 7-O-[6-O-galloyl-β-D-Glc*p*$\xrightarrow{6}$O-β-*D*-Glc*p*$\xrightarrow{6}$O-β-*D*-Glc*p*]-(+)-gallocatechin-(4α-8)-(−)-epicatechin-(4β-8)-(−)-epicatechin-(4β-8)-(+)-catechin-(4α-8)-5-O-[6-O-galloyl-β-*D*-Glc*p*$\xrightarrow{6}$O-β-*D*-Glc*p*$\xrightarrow{6}$O-β-*D*-Glc*p*]-(+)-catechin.

According to its UV, IR, and ^{13}C NMR spectra (Table 2), RP-2 is also an oligomeric proanthocyanidin glycoside.

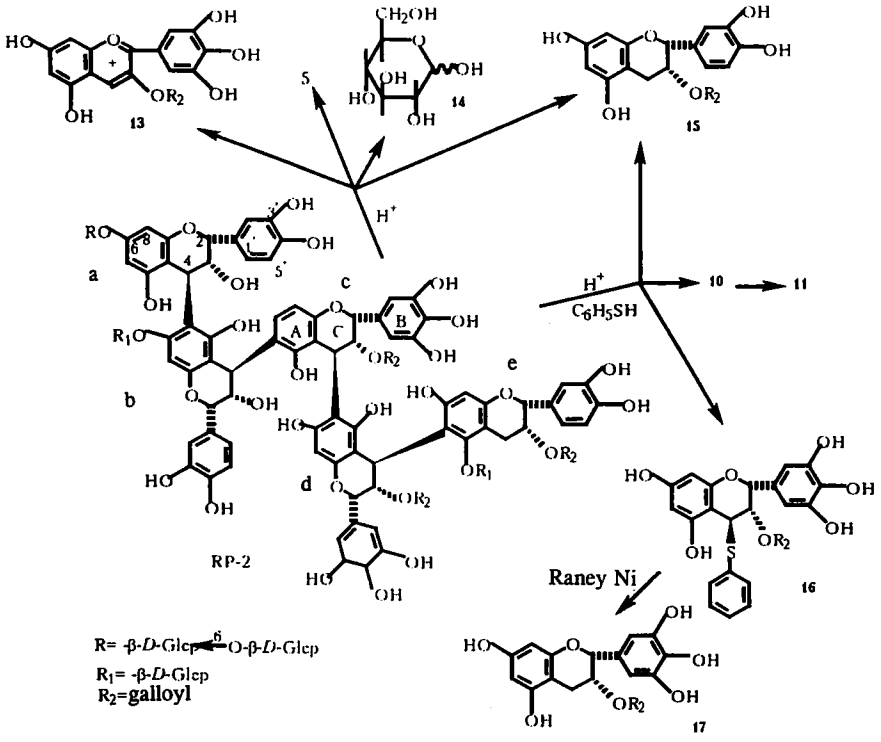
The ^{13}C NMR spectrum of RP-2 contained signals of epicatechin, epigallocatechin, and gallic acid and glucose residues. The distribution of the chemical shifts of the carbons of ring *C* showed that this proanthocyanidin molecule consists

TABLE 2. Chemical Shifts (ppm) of the Signals of the Carbon Atoms in the ^{13}C NMR Spectrum of Proanthocyanidin RP-2

Carbon atom	RP-2 fragment							
	a	b	c	d	ë	glucose	glucose	galloyl
C-2	77.4	77.4	75.0*	75.0	77.4			
C-3	71.4#	71.4#	75.8*	75.8**	69.4			
C-4	35.1	35.1	32.0	32.0	26.4			
C-6	95.6	106.0	106.0	106.0	106.0			
C-8	94.9	94.9•	94.9•	94.9•	95.6•			
C-10	101.5	98.7	98.7	98.7	98.7			
C-5,7,9		153.8-154.5						
C-1'	129.2	129.2	129.2	129.2	129.2	101.5	101.5	119.2
C-2'	114.0	114.0	108.5	108.5	114.0	75.0*	75.0*	108.5
C-3'	144.0	144.0	144.0	144.0	144.0	77.4	77.4	144.0
C-4'	144.0	144.0	132.9"	133.0'	144.0	69.4	72.8#	137.2
C-5'	114.0	114.0	144.0	144.0	114.0	75.8*	77.4	144.0
C-6'	118.5	118.5	108.5	108.5	118.5	64.7	60.6	108.5
COO-								162.6
								164.1

Note. Signals labeled with the same superscript symbol may be interchanged.

of epicatechin and epigallocatechin blocks with the 2,3-*cis*-configuration. The positions of the C-2, C-3, and C-4 signals in the spectrum showed that some of the blocks, including the "lower" block, are galloylated. The presence of a signal at 98.7 ppm from the C-10 carbon atom permitted the conclusion that the interflavan bond in RP-2 is of the C-4−C-6 type [3, 7]. The majority of the signals of the carbon atoms of the sugar moiety are overlapped by the signal of the carbon atoms of ring C. The signal of the C-6 atom of one glucose residue lay outside this region and appeared at 60.6 ppm. The chemical shift of a glucose C-6 atom at 64.7 ppm showed that two glucose molecules were linked to one another by a 1-6 bond. Enzymatic cleavage and acid hydrolysis of a permethylate of RP-2 showed that the sugar residues consist of D-molecular linked to one another and to the aglycon by β-bonds. The results of chemical degradations (Scheme 2) and of spectral methods of investigation showed that the monomeric composition consisted of (−)-epicatechin (**11**), epicatechin 3-O-gallate (**15**), and epigallocatechin 3-O-gallate (**17**). Presumably, in the RP-2 molecule the "upper" blocks a and b are glycosylated in the C-7 position and the "lower" block also in the C-5 position.



Thus, proanthocyanidin RP-2 has the structure and relative configuration of 7-O-[O-*D*-Glc*p*$\overset{6}{\leftarrow}$O-β-*D*-Glc*p*]-(−)-epicatechin-(4β-6)-7-O-β-*D*-Glc*p*-(−)-epicatechin-(4β-6)-3-O-galloyl-(−)-epigallocatechin-(4β-6)-3-O-galloyl-(−)-epigallocatechin-(4β-6)-3-O-galloyl-5-O-β-*D*-Glc*p*-(−)-epicatechin

EXPERIMENTAL

The UV spectra of the proanthocyanidins and their derivatives were taken in alcoholic solution on a Perkin−Elmer Lambda-16 instrument, and IR spectra on a Perkin−Elmer System 200 BT IR instrument in tablets with potassium bromide. ^{13}C NMR spectra were obtained on a Tesla BS 567A instrument (25 MHz) in $Me_2CO-d_6-D_2O$ (1:1) solution under conditions of complete suppression of spin−spin coupling with protons, internal standard TMS, δ scale, length of a ninety-degree pulse 8 msec, delay — 1, number of accumulations: for RP-1, 18,300; for RP-2, 24,400. The concentration of the substances was about 20%. Molecular masses were determined on a MOM 3170 ultracentrifuge and by gel filtration in a calibrated column of Sephadex LH-20. To check the homogeneity of the substances we used PC and TLC on Silufol plates. The elementary analyses of all the compounds corresponded to the calculated values.

Isolation of the Total Proanthocyanidins. Comminuted roots of *Rh. pamiroalaica* (6 kg) were extracted six times with 36% aqueous alcohol. The extracts obtained were combined and evaporated in vacuum at 50°C. The viscous extract was diluted with water (1:3) and was exhaustively treated successively with ethyl acetate and n-butanol. This gave 216 and 330 g of the corresponding fractions. The aqueous residue after evaporation of the solvent yielded 1464 g of proanthocyanidins.

Separation of the Proanthocyanidins. The butanolic extract (70 g) was mixed with cellulose (70 g) and transferred to a column of microcrystalline cellulose (6 × 140 cm, 1680 g), and elution was carried out with chloroform−methanol, methanol, and methanol−water, 100-ml fractions being collected. Similar fractions were combined and were rechromatographed on a column of Sephadex LH-20 (5 × 160 cm). The similarity of fractions was checked by TLC.

Proanthocyanidin RP-1, 1.20 g, composition $C_{125}H_{130}O_{69}$, M 2734, UV spectrum: λ_{max} 209, 244, 274 nm, λ_{min} 255 nm. IR spectrum: ν_{max} 3310, 1698, 1610, 1541, 1517, 1456, 1340, 1230, 1101, 1032, 825, 805, 773, 734 cm^{-1}. For the ^{13}C NMR spectrum, see Table 1.

Proanthocyanidin P-2, 0.940 g, composition, $C_{120}H_{114}O_{64}$, M 2578, UV spectrum: λ_{max}, 209, 246, 275, 306 nm, λ_{min} 256 nm. IR spectrum: ν_{max}, 3216, 1696, 1611, 1515, 1536, 1440, 1341, 1207, 1147, 1101, 1032, 827, 804, 775, 734 cm^{-1}. For the ^{13}C NMR spectrum, see Table 2.

Alkaline Cleavage of RP-1. With the passage of a slow current of nitrogen, a 20-ml four-necked round-bottomed flask was charged with 50 mg of RP-1, and then 5 ml of a 50% solution of potassium hydroxide was added. With constant stirring, the lower part of the flask was immersed in a bath of low-melting alloy at a temperature of 150-160°C. Over five minutes the temperature of the bath was raised to 230°C and then the reaction mixture was rapidly cooled by immersing the flask in ice water, and the contents were acidified with 20% sulfuric acid and were then extracted with ethyl acetate. The ethyl acetate extract was dried with anhydrous sodium sulfate, and the solvent was distilled off. The residue was chromatographed on a column of polyamide. Three compounds were obtained, and they were identified from their physicochemical and spectral characteristics as phloroglucinol (**1**) and protocatechuic and gallic acids (**2**) and (**3**) [10, 11].

Alkaline cleavage of RP-2 was carried out by the method described for RP-1. The same three compounds were obtained — phloroglucinol (**1**) and protocatechuic and gallic acids (**2**) and (**3**).

Acid Cleavage of RP-1. A solution of 0.08 g of RP-1 in 4 ml of ethanol was treated with 1.5 ml of 2 N hydrochloric acid and the mixture was heated on the water bath under reflux in a current of nitrogen for 2 h. Then it was diluted with water and extracted with ethyl acetate.

The extract was washed with sodium bicarbonate solution and dried with anhydrous sodium sulfate, and the solvent was distilled off. The residue was chromatographed on a column of Sephadex LH-20. This gave 5 mg of (+)-catechin, $C_{15}H_{14}O_6$, mp 178-180°C, $[\alpha]_D^{22}$ +21° (0.51; acetone−water (1:1)), cyanidin (**5**), delphinidin (**6**), and glucose 6-gallate (**7**), $C_{13}H_{16}O_{10}$, mp 137-138°C, $[\alpha]_D^{23}$ +22° (c 0.32; acetone) [9-11].

Acid cleavage of RP-2 was conducted by an analogous method, with the formation of (−)-epicatechin 3-gallate (**15**), mp 210-211°C, $[\alpha]_D^{20}$ −135° (c 0.06; methanol−water (1:1)), cyanidin (**5**), and delphinidin (**13**) and the detection of D-glucose (**14**) (butan-1-ol-pyridine−water (6:4:3) system, R_f 0.50; revealing agent aniline phthalate).

Thiolytic Cleavage of the Proanthocyanidins. Cleavage of RP-1. A mixture of 360 mg of RP-1 and 5 ml of phenyl merçaptan was treated with 3 ml of acetic acid in 10 ml of ethanol, and the resulting reaction mixture was left at room temperature for 48 h, the course of the reaction during the first 10 h being monitored by TLC every hour. After concentration, the oily residue was chromatographed on Sephadex LH-20, with elution by ethanol. In this way, 14 mg of (+)-catechin and 132 mg of an amorphous substance — a mixture of three thioethers [10] — were obtained.

Cleavage of the Thioethers. The thioethers (132 mg) were mixed with 4 ml of ethanol–acetic acid (9:1), and then the catalyst (Raney nickel) was added to the reaction mixture, and it was kept at 50°C for 1 h. After filtration, the filtrate was concentrated and chromatographed on Sephadex LH-20, with elution by 80% ethanol. Three compounds were obtained: (−)-epicatechin (**11**), (+)-catechin (**4**), and (+)-gallocatechin (**12**).

Cleavage of RP-2. The compound (345 mg) was cleaved and the reaction products were purified by the methods described above. The reaction mixture was chromatographed on Sephadex LH-20 (60% ethanol), to give 18 mg of (−)-epicatechin 3-gallate and 161 mg of a mixture of thioethers.

Cleavage of the Thioethers. The thioethers (161 mg) were mixed with 2.5 ml of ethanol–acetic acid (9:1), the catalyst (Raney nickel) was added to the reaction mixture, and it was kept at 50°C for 1 h. Then it was filtered, and the filtrate was concentrated and chromatographed on Sephadex LH-20, with elution by 80% ethanol. This gave two compounds: (−)-epigallocatechin 3-gallate and (−)-epicatechin.

Enzymatic Hydrolysis of RP-1 and RP-2. A glycoside (RP-1 or EP-2; 0.130 g) was dissolved in 10 ml of water, and the enzyme β-glucosidase (0.1 g of an enzyme flour from *Amygdalus communis* [12]) was added. The reaction mixture was placed in a thermostat and kept at 30°C for 6 h. The polyphenols were precipitated with a solution of lead acetate, and glucose was detected in the filtrate by paper chromatography.

Methylation of RP-1. A solution of 0.181 g of the glycoside in 20 ml of dimethyl sulfoxide was treated with 0.2 g of sodium hydride, and the mixture was stirred at room temperature for 1 h. Then 5 ml of methyl iodide was added dropwise and the reaction mixture was stirred for another 4 h, after which it was poured into ice water (50 ml) and extracted with a mixture of ethyl acetate and chloroform. The extract was treated with sodium thiosulfate, washed with water, and dried with anhydrous sodium sulfate. After the solvent had been distilled off, the residue was methylated similarly another five times. The reaction products were separated by column chromatography, and 0.06 g of amorphous permethylate was obtained.

Hydrolysis of the Permethylate of RP-1. A solution of 0.06 g of the permethylate in 10 ml of aqueous methanol (1:1) containing 5% of sulfuric acid was heated on the water bath for 8 h and then the reaction mixture was neutralized with barium acetate, the precipitate was filtered off, and the filtrate was evaporated to dryness. The residue was purified on a chromatographic column, giving 0.010 g of methylated carbohydrates. The methylated sugars were identified by TLC in comparison with authentic specimens. 2,3,4-Tri-O-methyl-D-glucopyranose [10] was detected.

Methylation of RP-2. Methylation followed by acid hydrolysis of the permethylate and the detection of methylated sugars were carried out by the method described above. 2,3,4-Tri-O-methyl-D-glucopyranose and 2,3,4,6-tetra-O-methyl-D-glucopyranose were obtained.

REFERENCES

1. Plant Resources of the USSR. Flowering Plants, Their Chemical Composition and Use. Capifoliaceae–Plantaginaceae Family [in Russian], Nauka, Leningrad (1990), p. 197.
2. Flora of the Kirgiz SSR [in Russian], Vol. 6, Frunze (1957), p. 8.
3. A. D. Vdovin, Z. A. Kuliev, and N. D. Abdullaev, Khim. Prir. Soedin., 545 (1997).
4. D. Sun, H. Wong, and Y. Foo, Phytochemistry, **26**, 1825 (1987).
5. G. Nonaka, O. Kawahara, and I. Nishioka, Chem. Pharm. Bull., **31**, 3906 (1983).
6. Y. Kashiwada, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., **34**, 4083 (1986).
7. G. Nonaka, F. Hsu, and I. Nishioka, J. Chem. Soc., Chem. Commun., 781 (1981).
8. A. S. Shashkov and O. S. Chizhov, Bioorg. Khim., 437 (1976).
9. B. M. Keneshov, Z. A. Kuliev, A. D. Vdovin, N. D. Abdullaev, A. B. Makhmatkulov, and A. A. Nishanov, Khim. Prir. Soedin., 588 (1997).
10. A. B. Makhmatkulov, Z. A. Kuliev, A. D. Vdovin, M. R. Yagudaev, and V. M. Malikov, Khim. Prir. Soedin., 59 (1992).

11. Kim Kvan Khi, Z. A. Kuliev, A. D. Vdovin, M. R. Yagudaev, and V. M. Malikov, Khim. Prir. Soedin., 771 (1991).
12. A. N. Belozerskii and N. I. Proskuryakov, Practical Handbook on Plant Biochemistry [in Russian], Sov. Nauka, Moscow (1951), p. 338.