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OLIGOMERIC PROANTHOCYANIDIN GLYCOSIDES OF *Clementsia semenovii*. II

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The structures of proanthocyanidins CS-3 and CS-4, isolated from the roots of Clementsia semenovii *have been established on the basis of chemical and spectral studies. CS-3 is 7-O-(6-O-galloyl-β-D-Glcp* $\xrightarrow{6}$ *O-β-D-Glcp* $\xrightarrow{6}$ *O-β-D-Glcp* $\xrightarrow{6}$ *O-β-D-Glcp* $\xrightarrow{6}$ *O-β-D-Glcp)-(+)-catechin-(4α—8)-(−)-epigallocatechin-(4β—8)-(+)-catechin-(4α—8)-(−)-epigallocatechin-(4β—8)-(−)-epigallocatechin-(4β—8)-(−)-epigallocatechin, and CS-4 is 3-O-galloyl-7-O-[6-O-galloyl-β-D-Glcp* $\xrightarrow{6}$ *O-β-D-Glcp* $\xrightarrow{6}$ *O-β-D-Glcp-(+)-gallocatechin-(4α—8)-[(+)-catechin-(4α—8)-(3-O-galloyl-(−)-epigallocatechin]$_2$-(4β—8)-(−)-epicatechin.*

There are reports in the literature on a study of the proanthocyanidin composition of *Clementsia semenovii* A. Bor. [1—4]. *Clementsia semenovii* belong to the family Grassulaceae and was originally called *Rhodiola semenovii* [5, 6]. With the aim of finding new physiologically active substances, we have isolated five new proanthocyanidins from the roots of this plant [7]. Isolation was achieved by extracting the raw material with aqueous ethanol, concentration, dilution with water, and successive extraction with diethyl ether, ethyl acetate, and butanol. The butanolic extract was separated on microcrystalline cellulose, followed by rechromatography on Sephadex LH-20. The structures of two of these proanthocyanidins, which we have called CS-3 and CS-4, have now been determined by various physicochemical methods.

According to UV and IR spectroscopy, both compounds (CS-3 and CS-4) were oligomeric proanthocyanidins. Proanthocyanidin CS-3 had the elementary composition $C_{127}H_{128}O_{69}$ and a molecular mass (M) of 2756. To determine its flavan-3-ol unit composition, the proanthocyanidin was cleaved by various chemical methods. The acid hydrolysis of CS-3 (Scheme 1) gave (−)-epigallocatechin (**1**), delphinidin (**2**), cyanidin (**3**), glucose (**4**), and a galloylglucose (**5**). This is proof of the destruction of an interflavan C—C bond: unchanged flavan-3-ol was liberated from the "bottom" part of the compound, while the "upper" flavanol units presumably formed intermediate carbonium ions which underwent spontaneous conversion into anthocyanidins [3, 8].

The decomposition of CS-3 under the action of thiophenol in an acid medium gave (−)-epigallocatechin (**1**) and the thioethers (**6**) and (**7**). Here again, interflavan C—C bonds were broken, and since the reaction took place under acid conditions, carbocations were formed which were captured by the thiophenol and formed thioethers, while the end flavan unit was liberated in unchanged form. Reduction of the thioethers with Raney nickel led to (+)-catechin (**8**) and (−)-epigallocatechin (**1**) [3].

The alkaline hydrolysis of proanthocyanidin CS-3 formed phloroglucinol (**9**), protocatechuic acid (**10**), and gallic acid (**11**). Not only rupture of the interflavan bond but also breakdown of the flavan-3-ol units at the pyran heterocycle had taken place. Phloroglucinol was liberated from rings *A* and the phenolic acids from rings *B*, while the C-3—C-4 atoms of rings *C* were liberated in the form of acetic acid [3, 8].

For analyzing the carbohydrate moiety we used Hakomori methylation [9]. Subsequent methanolysis of the permethyl derivatives led to 2,3,4-tri-O-methyl-*D*-glucopyranose. Enzymatic hydrolysis with β-glucosidase gave β-glucose.

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TABLE 1. Chemical Shifts (ppm) of the Signals of the Carbon Atoms in the ^{13}C NMR Spectrum of Proanthocyanidin CS-3

Carbon atom	Fragment							
	a	b	c	d	e	Glucose	Glucose	Galloyl
2	84.0[x]	77.5	85.5[x]	77.5	78.3			
3	72.6[k]	72.6[k]	73.0[k]	72.6[k]	66.6[t]			
4	38.1	35.6	38.1	35.8	30.5			
6	98.0	98.0	98.0	98.0	98.0			
8	97.6	107.7	107.7	107.7	107.7			
10	100.0[v]	101.3[v]	103.8[v]	101.3[v]	103.8[v]			
5,7,9	155.1[f]	157.5[f]	155.1[f]	157.5[f]	157.5[f]			
1'	131.6	131.6	131.6	131.6	131.6	103.8[v]	101.3[v]	122.5
2'	117.0	111.1	117.0	111.1	111.1	73.0[h]	74.1[h]	111.1
3'	147.0	147.0	147.0	147.0	147.0	78.3	78.3	147.0
4'	147.0	134.1	147.0	134.3	134.3	70.2	70.2	140.3
5'	117.0	147.0	117.0	147.0	147.0	77.5	77.5	147.0
6'	120.0	111.1	120.0	111.1	111.1	65.3[t]	63.7	111.1
COO^-								168.0

Signals labeled with the same letter may be interchanged.

Thus, according the results of chemical methods of analysis, the composition of compound CS-3 included (−)-epigallocatechin, (+)-catechin, glucose, and a galloylglucose.

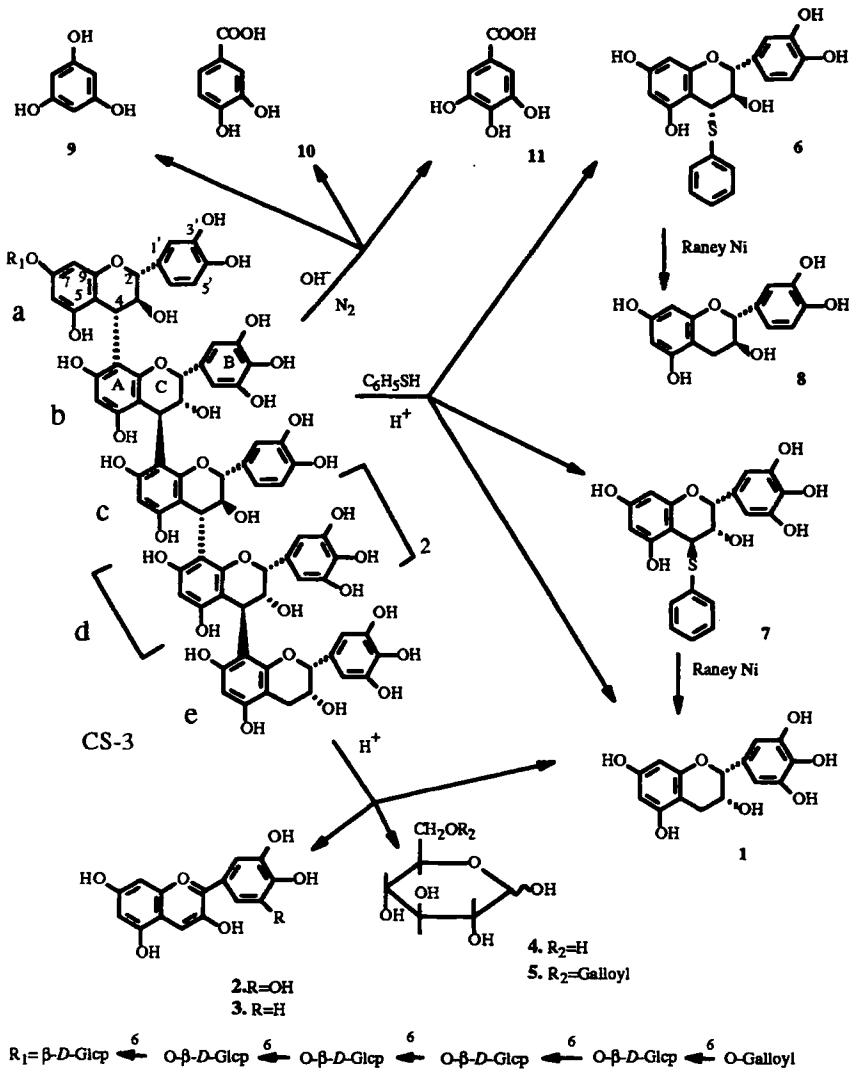
After this preliminary analysis, the structure of CS-3 was studied by ^{13}C NMR spectroscopy. The chemical shifts and their interpretation are given in Table 1. As can be seen from Table 1, signals at 155.1 and 157.5 ppm, related to the oxygen-substituted carbon atoms C-5, -7, and -9 of rings *A*. The signals of the other carbon atoms of rings *A*, C-6 and C-8, when not involved in interflavan bonds, appeared in the 97.6—98.0 ppm region, while the C-8 atoms participating in the formation of interflavan bonds resonated at 107.7 ppm [10].

An analysis of the chemical shifts of the carbon atoms of rings *B* led to the conclusion that CS-3 consisted of catechin and gallocatechin flavan units. We assigned resonance signals at 117.0 ppm to the C-2′ and C-5′, and that at 120.0 ppm to the C-6′, unsubstituted atoms of rings *B* of the catechins, and an intense signal at 147.0 ppm to the C-3′ and C-4′ substituted carbon atoms of rings *B* in the catechins and to the C-3′ and C-5′ atoms of rings *B* of the epigallocatechins; resonance signals at 111.1 ppm were assigned to the C-2′ and C-6′ atoms of rings *B* of the (−)-epigallocatechins, while the screened C-4′ carbon atoms resonated at 134.1—134.3 ppm. A signal at 131.6 ppm was assigned to the C-1′ atoms of rings *B* of the catechins [11, 12].

The C-3 and C-4 signals of the upper units appeared in weaker fields (72.6 and 38.1 ppm) than the signals of the same carbon atoms of the bottom flavan unit (66.6 and 30.5 ppm).

A further consideration of the spectrum permitted some additional conclusions to be drawn. In the first place, the position of the C-10 signals in the fragments, at 103.8, 101.3, and 100.0 ppm, showed that the interflavan bonds in CS-3 were of the C-4—C-8 type. In the alternative case — i.e., with the formation of C4—C-6 interflavan bonds — we would have observed the signals of the C-10 atoms in a stronger field, at 98.6—99.4 ppm [12]. In the second place, the appearance of signals of the C-2 atoms at 85.5, 84.0, 78.3, and 77.5 ppm showed that the proanthocyanidin molecule included flavanols with both the 2,3-*trans*- and 2,3-*cis*- configurations of the asymmetric centers [11, 14].

Analysis of the ^{13}C NMR spectrum of compound CS-3 showed that it was glycosylated. A consideration of the chemical shifts of the carbon atoms of the carbohydrate fragment led to the conclusion that it consisted of *D*-glucopyranose, as was shown by the signals of C-1 (101.3 and 103.8 ppm), C-3 (78.3 ppm), and C-5 (77.5 ppm) of glucose. Because of their participation in the formation of the glycosidic bond, the signals of the glucose anomeric centers, C-1, were shifted downfield and appeared at 101.3 and 103.8 ppm. The position of the C-6 signal of one glucose residue at 63.7 ppm, and also the presence of signals characteristic for gallic acid (signal of the carbonyl group at 168.0 ppm and of the C-4 carbon atom at 140.3 ppm) showed the acylation of the terminal glucose in the sixth position by gallic acid [15—17].



To determine the position of attachment of the carbohydrate fragment, we started from the assumption that in catechin glycosides the sugar fragment is attached in the C-5 or C-7 position of the molecule. When CS-3 was decomposed under the action of thiophenol in an acid medium, no glucose was found in the terminal flavan unit and it was most likely that the sugar residue was attached at C-7 of the "top" flavan unit, since its addition in the "middle" blocks is sterically hindered.

The combination of the facts obtained by various methods led to the conclusion that CS-3 is 7-O-(6-O-galloyl-β-*D*-Glc*p* $\xrightarrow{6}$ O-β-*D*-Glc*p* $\xrightarrow{6}$ O-β-*D*-Glc*p* $\xrightarrow{6}$ O-β-*D*-Glc*p* $\xrightarrow{6}$ O-β-*D*-Glc*p*)-(+)-catechin-(4α—8)-(−)-epigallocatechin-(4β—8)-(+)-catechin-(4α—8)-(−)-epigallocatechin-(4β—8)-(−)-epigallocatechin-(4β—8)-(−)-epigallocatechin.

Compound CS-4 had the elementary composition $C_{122}H_{112}O_{52}$, with the molecular mass (M) 2568. To establish its structure we used chemical methods for the fragmentation of proanthocyanidins (Scheme 2). The acid hydrolysis of CS-4 gave (−)-epicatechin (**12**), delphinidin (**2**), cyanidin (**3**), glucose (**4**), and a galloylglucose (**5**). The action of thiophenol in an acid medium on CS-4 led to (−)-epicatechin (**12**) and the thioethers (**6**, **13**, and **14**). After the decomposition of the mixture of thioethers under the action of Raney Ni, (+)-catechin (**8**), (−)-epigallocatechin 3-O-gallate (**15**), and gallocatechin 3-O-gallate (**16**) were obtained. On alkaline hydrolysis, CS-4 formed phloroglucinol (**9**), protocatechuic acid (**10**) and gallic acid (**11**). According to the results of enzymatic hydrolysis with β-glucosidase, proanthocyanidin CS-4 contained β-*D*-glucopyranose residues [6, 8]. Methylation followed by methanolysis of the permethyl derivatives led to 2,3,4-tri-O-methyl-*D*-glucopyranose [9].

As a result, it was established that proanthocyanidin CS-4 was glycosylated and consisted of gallocatechin 3-O-gallate, (+)-catechin, (−)-epicatechin, and (−)-epigallocatechin 3-O-gallate.

TABLE 2. Chemical Shifts (ppm) of the Signals of the Carbon Atoms in the ^{13}C NMR Spectrum of Proanthocyanidin CS-4

Carbon atom	Fragment						
	a	b	c	d	Glucose	Glucose	Galloyl
2	80.5	83.2	77.8[x]	78.9			
3	74.1[f]	72.2[k]	72.2[k]	67.8[k]			
4	36.5	38.5	36.5	30.8			
6	97.8	97.0	97.8	97.8			
8	97.8	107.7	107.1	107.7			
10	101.0[v]	104.1[v]	101.0[v]	104.1[v]			
5,7,9		153.8-158.8					
1'	132.3	132.3	132.3	132.3	101.0[v]	104.1[v]	121.8
2'	111.5	116.2	111.5	116.2	75.9[f]	74.1[f]	111.5
3'	147. 1	147.1	147. 1	147.1	78.9[x]	78.9[x]	147.1
4'	134.4	147.1	134.4	147.1	70.1[k]	70.1[k]	140.7
5'	147.1	117.7	147.1	117.7	77.8[x]	77.8[x]	147.1
6'	111.5	120.6	111.5	120.6	67.8[z]	63.7	111.5
					65.3[z]		
COO							169.2

Signals labeled with the same letter may be interchanged.

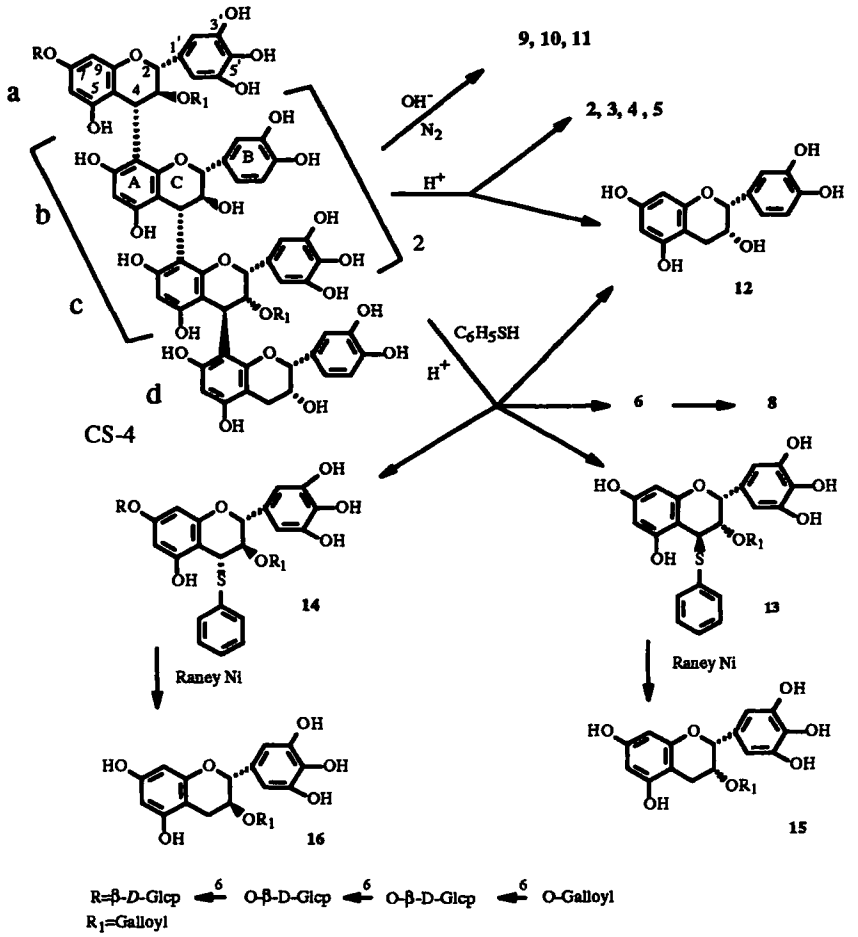
These preliminary chemical results were confirmed by the characteristics of the ^{13}C NMR spectrum. Analysis of the ^{13}C NMR spectrum of CS-4, the assignment of the signals in which was made in the same way as for CS-3, permitted the identification of the signals of gallocatechin 3-O-gallate, catechin, epicatechin, and epigallocatechin 3-O-gallate (Table 2).

The resonance signals of the C-5, C-7, and C-9 oxygen-substituted carbon atoms of rings *A* appeared in the form of a broadened signal between 153.8 and 158.8 ppm, while we assigned resonance signals at 97.8 and 107.7ppm to the C-6 and C-8 carbon atoms of rings *A*. The spectrum contained a combination of signals of the unsubstituted carbon atoms of ring *B* that is characteristic for the catechin system: C-2′ — 116.2 ppm; C-5′ — 117.7, and C-6′ — 120.0 ppm [9, 10]. The most characteristic indication of the gallic type of oxidation of ring *B* is the presence in the spectrum of a signal of C-4′ atoms at 134.4 ppm. Resonance signals at 147.1 ppm belonged to the atoms C-3′ and C-5′ of rings *B* of the gallocatechins and C-3′ and C-4′ of rings *B* of the catechins. The C-2′ and C-6′ signals of the gallocatechins appeared at 111.5 ppm, while signals at 132.3 ppm related to the C-1′ atoms of rings *B* of the flavan-3-ols [10—12].

We established the stereochemistries of the asymmetric centers of the flavan units of CS-4 on the basis of the values of the signals of the C-2 carbon atoms of the pyranose rings *C*, appearing at 80.5, 83.2, 77.8, and 78.9 ppm [10, 13]. The positions of the C-10 signals at 101.0 and 104.1 ppm showed that the interflavan bonds in CS-4 were of the C-4—C-8 type [13].

The ^{13}C NMR spectrum of CS-4 showed the signals of galloylated catechins. This followed from the positions of the C-2 and C-4 signals at 80.5 and 36.5 ppm, respectively, their upfield shift being due to the substitution of C-3 in rings *C* by gallic acid, while in nongalloylated catechins the C-2 and C-4 carbon atoms appear at 83.2 and 38.5 ppm [16, 17]. Furthermore, the spectrum included signals for gallic acid: of carbonyl carbon at 169.2 ppm and of C-1 at 121.8, C-2 and C-6 at 111.5, and C-3 and C-5 at 147.1 ppm and the signal of the C-4 atom at 140.7 ppm that is characteristic for this acid.

In the ^{13}C NMR spectrum of proanthocyanidin CS-4 there were signals of the carbon atoms of a glucan. As a result of an analysis of the carbohydrate moiety we came to the conclusion that it consisted of three β-*D*-glucopyranose residues linked to one another by (1—6)-β-glycosidic bonds. There are six of the combinations signals of the carbon atoms C-1 at 101.0 and 104.1 ppm, C-3 at 78.9, and C-5 at 77.8 ppm that are characteristic for β-*D*-glucopyranose. The substituted C-6 carbon atoms of the first two glucose residues appeared at 67.8 and 65.3 ppm, while the appearance of C-6 at 63.7 ppm showed acylation of the terminal glucose by gallic acid in the sixth position [15]. The site of attachment of the sugar residue was established on the same principles as in the investigation of CS-3. The most probable position of its attachment is the C-7 carbon atom of the top flavan unit.



On the basis of the chemical and spectral characteristics presented above, we came to the conclusion that proanthocyanidin CS-4 is an oligomeric proanthocyanidin glycoside and has the structure of 3-O-galloyl-7-O-[6-O-galloyl-β-Glcp$\xrightarrow{6}$ O-β-Glcp$\xrightarrow{6}$ O-β-Glcp]-(+)-gallocatechin-(4α—8)-[(+)-catechin-(4α—8)-(3-O-galloyl-(−)-epigallocatechin)]$_2$-(4β—8)-(−)-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 2000 FT IR spectrometer in tablets with potassium bromide. ^{13}C NMR spectra were obtained on a Tesla BS 567 A instrument (25 MHz for ^{13}C nuclei) in CD_3OD solution, internal standard TMS, δ-scale. The concentrations of the substances ranged around 15—20%. Carbon spectra were obtained under conditions of complete suppression of spin-spin interaction with protons. Molecular masses were determined on a MOM 3170 ultracentrifuge and by gel filtration on a calibrated column of Sephadex LH-20. PC and TLC on Silufol UV-254 plates were employed to check the homogeneity of the substances. The following solvent systems were used: 1) chloroform—butan-1-ol—acetone—formic acid—water (3.5:13:10:10:8); 2) butan-1-ol—acetic acid—water (4:1:5); 3) chloroform—butan-1-ol—acetone—formic acid—water (3.5:12:20:10:8); 4) isoamyl alcohol—36% HCl—water (5:11:1); and 5) butan-1-ol—acetic acid—water (40:12:18).

The analyses of all the compounds corresponded to the calculated values.

Extraction of the Proanthocyanidins. Air-dry roots of *Clementsia semenovii* (6 kg) were comminuted and extracted six times with 35% aqueous ethanol. The extracts were evaporated in vacuum at 40°C (to a volume of 3 liters), diluted with water (in a ratio of 1:3), and extracted successively with diethyl ether (to eliminate catechins and low-molecular-mass weakly

polar compounds), ethyl acetate (to extract the catechins and the proanthocyanidins with a low degree of polymerization), and butanol (to isolate the oligomeric proanthocyanidins). This gave 157.5, 52.5, and 222 g of the corresponding fractions. The aqueous residue yielded a total of 1660 g of high-molecular-mass proanthocyanidins.

Isolation of the Proanthocyanidins. The butanolic fraction (100 g) was mixed with cellulose (100 g) and transferred to a column of microcrystalline cellulose (5 × 170 cm, 1100 g). Methanol—chloroform (1:9—9:1), pure methanol, and methanol—water (9:1—7:3) were used for elution, with the collection of 50-ml fractions. Homogeneous fractions were combined. The eluates containing the homogeneous fractions were rechromatographed on a column of Sephadex LH-20 (5 × 160 cm). Elution was conducted with water—methanol (1:4—2:3), 20-ml fractions being collected. The homogeneity of the fractions was checked by TLC.

Proanthocyanidin CS-3. 1.145 g, $C_{127}H_{128}O_{69}$, M 2754. UV spectrum (nm): λ_{min} 255, λ_{max} 220, 243, 274, 340. IR-spectrum: (ν_{max}, cm^{-1}): 3341, 1691, 1612, 1536, 1449, 1342, 1230, 1148, 1099, 1035, 819, 765, 735.

Proanthocyanidin CS-4. 0.880 g, $C_{122}H_{112}O_{62}$, M 2568. UV spectrum (nm): λ_{min} 260, λ_{max} 207, 243, 274, 305. IR-spectrum: (ν_{max}, cm^{-1}): 3243, 1695, 1614, 1539, 1448, 1338, 1208, 1147, 1100, 1033, 825, 734.

Alkaline Cleavage of CS-3. With the passage of a slow current of nitrogen, a 20-ml four-necked flask was charged with 60 mg of the substance and then 5 ml of 50% KOH solution was added. With continuous stirring the bottom part of the flask was immersed in a bath of low-melting metal alloy at 155—164°C. The bath temperature was raised over 5 min to 230°C, and then the reaction mixture was rapidly cooled by immersing the flask in ice water, acidified with 20% sulfuric acid. The contents of the flask were diluted with water and extracted with ethyl acetate. The combined ethyl acetate extract was dried with anhydrous sodium sulfate. After drying and elimination of the solvent, the residue was chromatographed on a column of polyamide. Phloroglucinol (**9**) and protocatechuic and gallic acids (**10** and **11**) were detected among the hydrolysis products.

The Alkaline Cleavage of CS-4 was carried out by the procedure described above. Again, phloroglucinol (**9**) and protocatechuic and gallic acids (**10** and **11**) were detected.

Acid Cleavage of CS-3. A solution of 50 mg of the substance in 2 ml of ethanol was treated with 1 ml of 2 N hydrochloric acid and heated on the water bath under reflux in a current of nitrogen for 2 h. The reaction mixture (colored crimson) was diluted with water and extracted with ethyl acetate (2 ml × 3). The extract was washed and dried, and the solvent was distilled off. The residue was chromatographed on a column of Sephadex LH-20, with elution by 60% ethanol. This gave (−)-epigallocatechin, $C_{15}H_{14}O_7$, M 306, mp 216—218°C, $[\alpha]^{24}$ −55° (*c* 0.33; acetone) (**6**); delphinidin, R_f 0.36 (2 N HCl), λ_{max} 554 nm (0.1% HCl in ethanol) (**2**); cyanidin, R_f 0.8 (2 N HCl), λ_{max} 518 nm (0.1% HCl in ethanol) (**3**); *D*-glucose, R_f 0.50 (butanol—pyridine—water, 6:4:3), spot reagent aniline phthalate (**4**); and glucose 6-O-gallate, $C_{13}H_{16}O_{10}$, mp 137—138°C, $[\alpha]^{23}$ +22° (*c* 0.032; acetone) (**5**).

The Acid Cleavage of CS-4 was carried out by the method described above. In the hydrolysate we detected (−)-epicatechin, $C_{15}H_{14}O_6$, M 290, mp 241—243°C, $[\alpha]^{24}$ −69° (*c* 0.48; acetone—water, 1:1) (**12**), delphinidin (**2**), cyanidin (**3**), glucose (**4**), and glucose 6-O-gallate (**5**).

Thiolytic Cleavage of CS-3. A mixture of 400 mg of CS-3 and 4 ml of thiophenol was treated with 2 ml of acetic acid and 10 ml of ethanol and left at room temperature for 48 h. The reaction mixture was concentrated, giving an oily residue, which was chromatographed on Sephadex LH-20 (1 × 20 cm) with elution by ethanol. This gave 33 mg of (−)-epigallocatechin (**1**) and 225 g of an amorphous substance consisting of a mixture of two thioethers (**6** and **7**).

Cleavage of Thioethers (6) and (7). The thioethers (225 mg) were mixed with 4 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 3 h. It was then filtered and the filtrate was concentrated and chromatographed on a column of Sephadex LH-20, with elution by 80% ethanol. This led to the isolation of two compounds: 32 mg of (+)-catechin, $C_{15}H_{14}O_6$, mp 178-180°C. $[\alpha]^{24}$ +183°C (*c* 0.9; acetone—water.1:1), λ_{max} 282 nm (log ε 3.51) R_f 0.64 (system 1) (**8**) and 41 mg of (−)-epigallocatechin, $C_{15}H_{14}O_7$, M 306, mp 216—218°C, $[\alpha]^{24}$ −55° (*c* 0.33; methanol) λ_{max} 272 nm (log ε 3.10), R_f 0.42 (system 1).

Thiolytic Cleavage of CS-4. Compound CS-4 was cleaved with thiophenol and acetic acid, and the reaction product was purified by the method described above. This gave (−)-epicatechin (**12**) and the thioethers (**6**), (**13**) and (**14**), the catalytic decomposition of which led to (−)-epicatechin 3-O-gallate with the composition $C_{22}H_{18}O_{11}$, mp 210—211°C, $[\alpha]^{22}$ −132° (*c* 0.38; methanol—water), λ_{max} 278 nm (log ε 3.91), R_f 0.64 (system 1) (**15**), and gallocatechin 3-O-gallate, mp 205—207°C; λ_{max} 278 (log ε 3.93. R_f 0.68 (BAW); acetyl derivative, mp 168°C (**16**).

Methylation of CS-3. A solution of 0.4 g of CS-3 in 10 ml of dimethyl sulfoxide was treated with 0.1 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 stirring

was continued for another 4 h, after which the reaction mixture was poured into ice water (30 ml) and extracted with chloroform. The extract was treated with sodium thiosulfate, washed with water, and dried with anhydrous sodium sulfate. After evaporation, the residue was methylated similarly five more times, the reaction product was separated by column chromatography, and 0.206 g of amorphous permethylate was obtained.

Hydrolysis of the Permethylate of CS-3. To 5 ml of aqueous methanol (1:1) was added 5% H_2SO_4; then 0.206 g of the permethylate was dissolved in this mixture and it was heated on the water bath for 8 h, after which the reaction mixture was neutralized, filtered, and evaporated to dryness. The residue was purified on a chromatographic column. This gave 0.059 g of a methylated carbohydrate, which was identified by GLC and TLC as 2,3,4-tri-O-methyl-*D*-glucopyranose.

Methylation of CS-4. The reaction was carried out by the method described above. The compound obtained was identified as 2,3,4-tri-O-methyl-*D*-glucopyranose.

Enzymatic Hydrolysis of CS-3. The enzyme β-glucosidase was added to a solution of 0.02 g of CS-3 in 10 ml of water. The reaction mixture was kept in a thermostat at 30°C for 6 h. Polyphenols were precipitated with a solution of lead acetate, and β-glucose was detected in the filtrate by paper chromatography.

The enzymatic hydrolysis of CS-4 was conducted by the method described above. β-Glucose was again detected in the hydrolysis products.

REFERENCES

1. E. A. Krasnov, *Khim. Prir. Soedin.*, 545 (1976).
2. Kim Kvan Khi, Z. A. Kuliev, A. D. Vdovin, M. R. Yagudaev, and V. M. Malikov, *Khim. Prir. Soedin.*, 723 (1989).
3. Kim Kvan Khi, Z. A. Kuliev, A. D. Vdovin, M. R. Yagudaev, and V. M. Malikov, *Khim. Prir. Soedin.*, 771 (1991).
4. V. M. Malikov, A. G. Kurmukov, É. Kh. Batyrov, M. I. Aizikov, and Z. A. Kuliev, *USSR Inventors' Certificate 1155601, Otkrytia, Izobreteniya*, No. 18, 90 (1985).
5. A. G. Borisova, *Flora of the USSR* [in Russian], Moscow—Leningrad, Vol. 9 (1939), p. 8.
6. *Plant Resources of the USSR. Flowering Plants, their Chemical Composition and Use. Caprifolaceae—Plantaginaceae Family,* [in Russian] Nauka, Leningrad (1989), p. 193.
7. K. N. Matamarova, Z. A. Kuliev, A. D. Vdovin, N. D. Abdullaev, and M. B. Murzubraimov, *Khim. Prir. Soedin.*, 756 (1998).
8. M. I. Zaprometov, *Fundamentals of Polyphenolic Compounds* [in Russian], Nauka, Moscow (1974), p. 63.
9. S. Hakomori, *J. Biochem. (Tokyo)*, **55**, 205 (1964).
10. A. D. Vdovin, Z. A. Kuliev, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 545 (1997).
11. D. Sun, H. Wong, and Y. Foo, *Phytochemistry*, **26**, 1825 (1987).
12. Z. Czohanska, L. Y. Foo, R. H. Newman, and L. J. Porter, *J. Chem. Soc., Perkin Trans. I*, 2278 (1980).
13. G. Nonaka, F. Hsu, and I. Nishioka, *J. Chem. Soc., Chem. Commun.*, 781 (1981).
14. F. Hsu. G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **33**, 3293 (1985).
15. A. S. Shashkov and O. S. Chizhov, *Bioorg. Khim.*, **2**, 437 (1976).
16. G. Nonaka, M. Muta, and I. Nishioka, *Phytochemistry*, **22**, 237 (1983).
17. Y. Kashidawa, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **34**. 4083 (1986).