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STUDY OF THE CATECHINS AND PROANTHOCYANIDINS OF *Quercus robur**

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More than 20 compounds have been isolated from the bark of Quercus robur. *Monomers: (−)-epicatechin, (−)-epicatechin gallate, (+)-catechin, (+)-catechin gallate, (+)-gallocatechin, (−)-epigallocatechin, and (−)-epigallocatechin gallate; dimeric proanthocyanidins: (+)-catechin-(4α−8)-(+)-catechin, 3-O-galloyl-(+)-catechin-(4α−8)-3-O-galloyl-(+)-catechin, 3-O-galloyl-(+)-gallocatechin-(4β−8)-(+)-gallocatechin, (−)-epicatechin-(4β−8)-3-O-galloyl-(−)-epigallocatechin gallate, 3-O-galloyl-(−)-epicatechin-(4β−8)-3-O-galloyl-(−)-epigallocatechin, 3-O-galloyl-(−)-epigallocatechin-(4β−8)-(+)-catechin; and oligomeric proanthocyanidins: D14−D19.*

The bark of English oak (*Quercus robur*) contains about 13-15% of tanning substances and has long been used for tanning hides. It is used in folk and scientific medicines as an astringent in looseness and bleeding of the gums. There are reports in the literature of a chemical study of the proanthocyanidins of the bark of *Quercus robur* [1-4] and of species with similar chemical compositions, *Q. dentata* and *Q. miyagii* [5, 6]. With the aim of finding new biologically active compounds, we have studied the proanthocyanidins of the bark of *Q. robur* L. fam. Fagaceae, growing on the territory of the CIS.

From a total aqueous alcoholic extract of the oak bark, by partition chromatography on microcrystalline cellulose powder and gel filtration on Sephadex LH-20 we have isolated more than 20 compounds: catechins and low-molecular-mass, oligomeric, and polymeric proanthocyanidins.

From their physicochemical and spectral (UV, IR, PMR) parameters, compounds (**1**)-(**7**) from an ether fraction were identified as (−)-epicatechin (**1**), (−)-epicatechin gallate (**2**), (−)-epigallocatechin (**3**), (−)-epigallocatechin gallate (**4**), (+)-catechin (**5**), (+)-catechin gallate (**6**), and (+)-gallocatechin (**7**). By a comparison of their physicochemical properties and spectral characteristics (alkaline, acid, and thiolytic cleavages; UV, IR, and ^{1}H NMR spectra) compounds (**8**)-(**13**) from an ethyl acetate fraction were identified as dimeric proanthocyanidins: (+)-catechin-(4α−8)-(+)-catechin (**8**), (+)-catechin gallate-(4α−8)-(+)-catechin gallate (**9**), (+)-gallocatechin gallate-(4β−8)-(+)-gallocatechin (**10**), (−)-epicatechin-(4β−8)-(−)-epigallocatechin gallate (**11**), (−)-epicatechin gallate-(4β−8)-(−)-epigallocatechin gallate (**12**), and (−)-epigallocatechin gallate-(4β−8)-(+)-catechin (**13**). Their structures are shown in Fig. 1 [7-11].

From a butanol fraction of the proanthocyanidins we isolated six individual compounds (D14-D19), three of which were of glycosidic nature. Proanthocyanidin D14 consisted of an amorphous cream-colored powder. It had the composition $C_{117}H_{76}O_{44}$ and a molecular mass (M) of 2182, did not melt, and decomposed at 290-300°C. The alkaline fusion of D14 in an atmosphere of nitrogen under conditions excluding oxidation of the cleavage products led to phloroglucinol (**17**) and gallic and protocatechuic acids (**16**) and (**18**). The splitting of D14 with a 5% solution of concentrated hydrochloric acid in butanol in a sealed tube gave cyanidin (**14**) and delphinidin (**15**). These facts showed the mixed nature of the compound, and this was confirmed by the formation of the thioethers (**19**)-(**22**) as a result of thiolytic cleavage and also by the formation of the flavan-3-ols (**1**), (**3**), (**4**), and (**6**) by the catalytic degradation of these thioethers.

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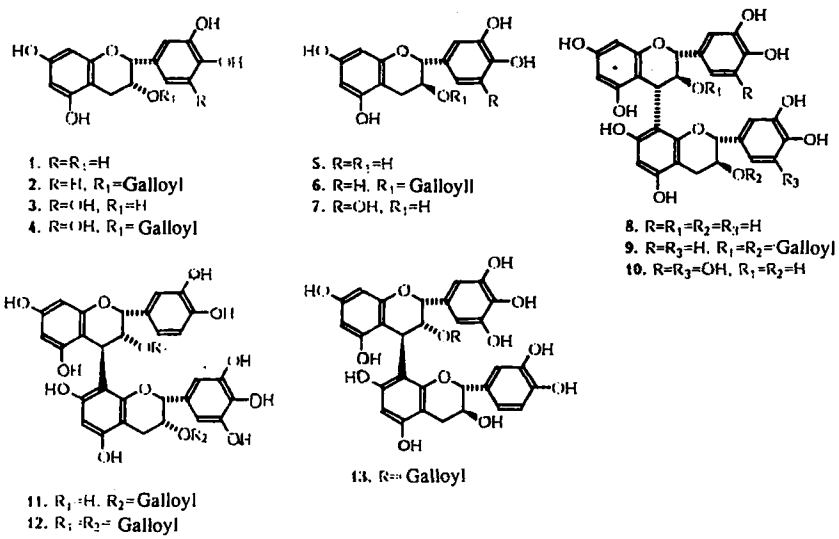

Fig. 1. Flavan-3-ols and dimeric proanthocyanidins.

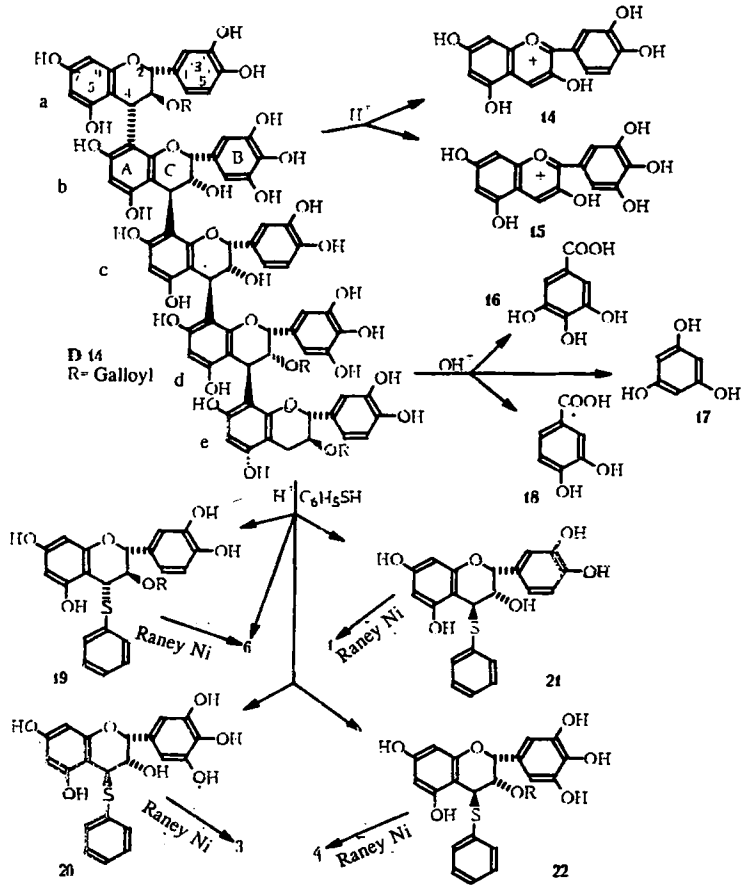

Fig. 2. Structure and scheme of chemical transformations of D14.

In the UV spectrum of the proanthocyanidin, the intensity of absorption fell from a maximum at about 210 nm to a minimum at 258 nm, with a shoulder at 220 and weak absorption at 245 nm. After the minimum a characteristic maximum followed at 278 nm and there was then a gradual decrease in the direction of the visible region of the spectrum, with weak absorption at 305 nm.

TABLE 1. Chemical Shifts (ppm) in the ^{13}C NMR Spectrum of Proanthocyanidin D14

Carbon atom	Fragments of D14						
	a	b	c	d	e	galloyl	
C-2	82.7[f]	77.9[i]	77.5[i]	74.8	83.5[f]		
C-3	73.9	71.3	71.3	73.9	68.7		
C-4	38.8	38.8	38.8	35.5	28.7		
C-6	97.4[x]	97.7[x]	97.7[x]	97.7[x]	98.4[x]		
C-8	96.8[x]	108.2[h]	108.8[h]	108.2[h]	109.1[h]		
C-10	102.0[x]	103.1[x]	102.0[x]	103.1[x]	103.1[x]		
C-5,7,9	155.2[k]	155.5[k]	156.9[k]	155.5[k]	157.3[k]		
C-1'	129.3[y]	132.3[y]	132.3[y]	132.3[y]	132.3[y]	121.3	122.1
C-2'	116.6	108.2[h]	116.6	108.2[h]	116.6	109.6[y]	111.5[y]
C-3'	146.1[r]	146.1[r]	146.1[r]	146.1[r]	146.1[r]	143.7[r]	
C-4'	146.6[r]	134.4	146.6[r]	134.4	146.6[r]	138.9	
C-5'	117.7	147.0[r]	117.7	147.0[r]	117.7	143.7[r]	
C-6'	117.7	108.2[h]	117.7	108.2[h]	117.7	109.6[y]	111.5[y]
-COO-						163.9	
						165.0	
						168.4	

*Signals labeled with the same superscript letters may be interchanged. Underlining indicates galloylation of the hydroxy group at C-3.

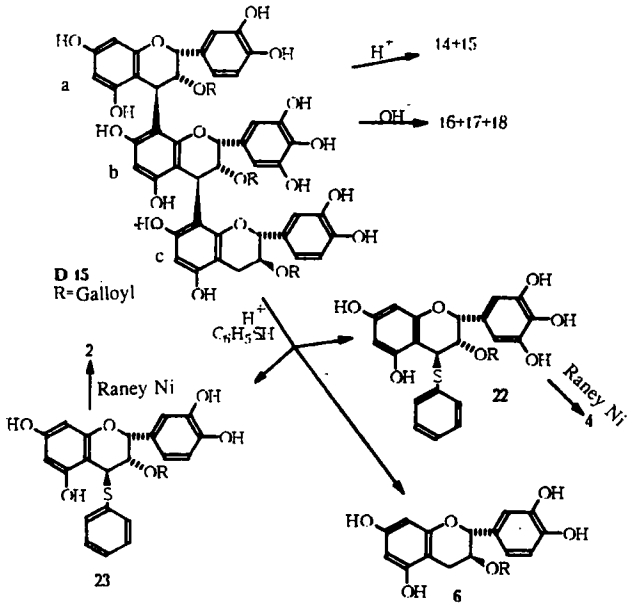

Fig. 3. Structure and scheme of the chemical transformations of D15.

A broad intense absorption band in the IR spectrum of this compound in the 3500-3200 cm^{-1} region was due to the presence of a large number of OH groups. In the 1657 cm^{-1} region there was an intense broadened band of an ester carbonyl group of an aromatic acid. Bands relating to the skeletal vibrations of an aromatic ring appeared in the 1610, 1520, and 1440 cm^{-1} regions. At 1250 and 1040 cm^{-1} there were bands due to vibrations of a =C−O−C group. A band at 1105 cm^{-1} was caused by the C−O−C deformation vibrations of an esterified secondary OH group. A characteristic peak at 1520 cm^{-1} and peaks at 770 and 745 cm^{-1} showed that the content of prodelphinidin units in proanthocyanidin D14 is less than 50%, which was confirmed by a broadening of the peak at 1520 cm^{-1} and the absence of a peak at 1535 cm^{-1}, while the presence of a peak at 805 cm^{-1} showed that its structure includes epicatechins [12].

For further investigations of the spatial and chemical structures we studied the ^{13}C NMR spectra of D14. The spectra were obtained under conditions of total suppression of spin−spin coupling with protons. The interpretation of the series of resonance signals was made by comparison with the chemical shifts (CSs) of structurally close proanthocyanidins and also in light of certain laws that we have deduced as a result of an analysis of results from the ^{13}C NMR spectroscopy of monomeric, dimeric, and polymeric proanthocyanidins. Table 1 gives the results of an assignment of the CSs of the resonance signals of

TABLE 2. Chemical Shifts (ppm) in the ^{13}C NMR Spectrum of Proanthocyanidin D16

Carbon atom	D16 fragment				
	a	b	c	d	galloyl
C-2	75.5	80.3–82.7	80.3–82.7	80.3–82.7	
C-3	75.5	71.3–73.1	71.3–73.1	68.2	
C-4	34.6	35.5	35.5	-*	
C-6	96.1	96.1	96.1	96.1	
C-8	96.1	107.6	107.6	107.6	
C-10	100.8	100.8	100.8	100.8	
C-5,7,9	155.0	155.0	155.0	155.0	
C-1′	130.1	130.1	130.1	130.1	123.8
C-2′	109.6	113.3–114.5	113.3–114.5	113.3–114.5	109.6
C-3′	143.7[p]	143.7[p]	143.7[p]	143.7[p]	143.7[p]
C-4′	132.5	144.5[p]	144.5[p]	144.5[p]	138.9
					135.9
C-5′	143.7[p]	113.3–114.5	113.3–114.5	113.3–114.5	143.7[p]
C-6′	109.6	119.7	119.7	119.7	109.6
-COO-					163.9
					165.2
					168.4

*Signal masked by a signal of the solvent.

Signals labeled with the same superscript letters may be interchanged. Underlining indicates galloylation of the hydroxy group at C-3.

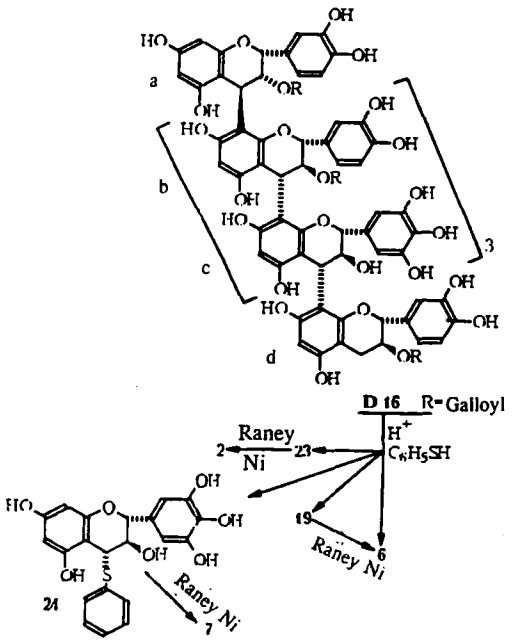

Fig. 4. Structure and scheme of the chemical transformations of D16.

the carbon atoms in the ^{13}C NMR spectra of proanthocyanidin D14 — (+)-catechin gallate, (−)-epicatechin, (−)-epigallocatechin gallate, and gallic acid.

Resonance signals in the 155.2-157.3 ppm region related to the C-5, C-7, and C-9 carbon atoms of the phloroglucinol nuclei — rings *A*. A consideration of the CSs of the carbon atoms of rings *B* of this compound permitted the identification of the presence of catechin and gallocatechin systems in it. Intense signals at 143.7-147.0 ppm related to the C-3′ and C-4′ atoms of ring *B* of (+)-catechin (**5**) and also to C-3′ and C-5′ of ring *B* of epigallocatechin. The C-4′ atom of (−)-epigallocatechin resonated at 134.4 ppm. Signals at 129.3-132.3 ppm related to the C-1′ atoms of rings *B* of the catechins. Intense signals at 108.2-109.1 ppm were given by the C-2′ and C-6′ atoms of ring *B* of gallocatechin and of gallic acid and also by the C-8 atoms of substituted phloroglucinol rings *A*. Signals at 102.0 and 96.8-98.4 ppm belonged, respectively, to C-10, C-8, and C-6 of

TABLE 3. Chemical Shifts (ppm) in the ^{13}C NMR Spectrum of Proanthocyanidin D17

Carbon atom	D17 fragment					
	a	b	c	d	galloyl	glucose
C-2	83.2	81.8	83.2	81.8		
C-3	73.5[s]	73.8[s]	70.5[s]	67.8		
C-4	38.8	36.7	38.8	-*		
C-6	95.3	95.3	95.3	95.3		
C-8	95.3	107.4	107.4	107.4		
C-10	101.2	101.2	101.2	101.2		
C-5,7,9	152.0[f]	153.5[f]	153.5[f]	154.6[f]		
C-1′	130.6	130.6	130.6	130.6	119.9	101.2
C-2′	115.6	109.4	115.6	115.6	109.4	72.9[s]
C-3′	145.1	145.1	145.1	145.1	145.1	76.9
C-4′	145.1	133.2	145.1	145.1	138.8	70.5[s]
					140.1	
C-5′	115.6	145.1	115.6	115.6	145.1	76.9
C-6′	118.1[h]	109.4	118.1[h]	119.9[h]	109.4	64.9
						61.5
-COO-					168.4	

*Signal masked by a signal of the solvent.
Signals labeled with the same superscript letters may be interchanged.
Underlining indicates galloylation of the hydroxy group at C-3.

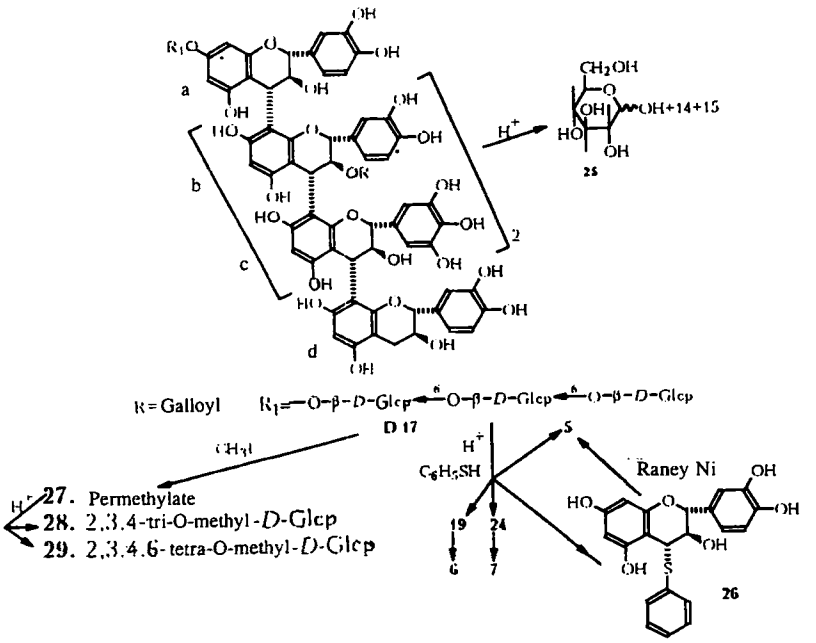

Fig. 5. Structure and scheme of the chemical transformations of D17.

rings *A* of the terminal blocks free from interflavan bonds. The chemical shifts of the C-10 carbon atoms in proanthocyanidin D14 were characteristic for proanthocyanidins with C-4−C-8 interflavan bonds [5, 13, 14].

Signals of the C-2 carbon atoms of the heterocyclic rings *C* of the catechins appeared at 74.8, 77.5, 77.9, 82.7, and 83.5 ppm, and those of C-3 at 73.9, 71.3, and 68.7 ppm. The substituted C-4 carbon atoms gave signals at 36.5 and 38.8 ppm, while the signal of an unsubstituted C-4 was observed at 28.7 ppm. Analysis of the spectra of the dimeric proanthocyanidins in the light of the influence of substituents at C-4 and the effects of the galloylation of the hydroxy groups at C-3 of the heterocyclic nuclei [5, 15-17] permitted the assignment of all the signals of the C-2, C-3, and C-4 carbons and the determination of the stereochemistry of the substituents of rings *C* of the catechin residues. The C-3 and C-4 signals of flavan-3-

TABLE 4. Chemical Shifts (ppm) in the ^{13}C NMR Spectrum of Proanthocyanidin D18

Carbon atom	D18 fragment					
	a	b	c	d	galloyl	glucose
C-2	83.2–84.9	83.2–84.9	83.2–84.9	80.6		
C-3	72.3	73.7	72.3	68.3		
C-4	38.2	35.2	38.2	–*		
C-6	96.4	96.4	96.4	96.4		
C-8	96.4	106.7	106.7	106.7		
C-10	103.6[f]	102.5[f]	103.6[f]	100.8[f]		
C-5,7,9	153.6	153.6	153.6	153.6		
C-1′	130.9	130.9	130.9	130.9	120.1	102.5[f]
C-2′	108.9	108.9	114.9	114.9	110.4	74.2
C-3′	144.3	144.3	144.3	144.3	144.3	78.7
C-4′	132.6	132.6	144.3	144.3	137–139	72.3
C-5′	144.3	144.3	114.9	114.9	144.3	78.7
C-6′	108.9	108.9	114.9	119.0h	110.4	66.1
						63.9
						61.5
–COO–					165.5–168.5	
					170.9	

*Signal masked by a signal of the solvent.

Signals labeled with the same superscript letters may be interchanged. Underlining indicates galloylation of the hydroxy group at C-3.

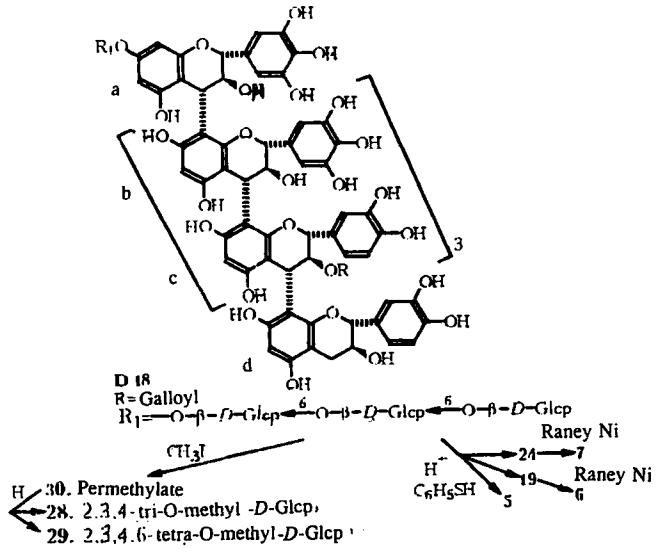

Fig. 6. Structure and scheme of the chemical transformations of D18.

ols in the upper position were shifted downfield in comparison with the signals of the corresponding carbon atoms of the blocks occupying the lower position in the proanthocyanidins.

The spectrum of D14 also contained the signals of three gallic acid residues: signals of carbonyl carbons at 163.9, 165.0, and 168.6 ppm; of C-1′ at 122.0 ppm and of C-2′ and C-6′ at 111.5 ppm. A contribution to the intense signals in the 143-147 ppm region was made by the C-3′ and C-5′ atoms. The signal of the C-4′ carbon atom is characteristic for gallic acid. It undergoes a paramagnetic shift of approximately 4-8 ppm in comparison with the corresponding resonance of the gallo system of flavan-3-ols. This is obviously due to the *para*-effect of the carbonyl group.

Thus, we give the chemical constitution and spatial structure of proanthocyanidin D14 on the basis of physicochemical and spectral characteristics in Fig. 2.

Proanthocyanidin D15 was an optically active amorphous powder. Its molecular mass was approximately 1338. Its spectral characteristics were close to those of D14, but D15 differed from D14 by the fact that, as followed from its ^{13}C NMR spectrum, its molecule included galloylated (–)-epicatechin and (–)-epigallocatechin blocks. The ratio of the C-2 signals of *cis*- and *trans*-substituted flavan-3-ols in the spectrum of D15 showed that the latter compound contained less (+)-catechin (5)

TABLE 5. Chemical Shifts (ppm) in the ^{13}C NMR Spectrum of Proanthocyanidin D19

Carbon atom	D19 fragment					
	a	b	c	d	galloyl	glucose
C-2	78.1	78.1	75.8	81.0		
C-3	71.6	71.6	74.2	65.0		
C-4	37.5	37.5	33.6	–*		
C-6	95.9	95.9	95.9	95.9		
C-8	92.0	107.7	107.7	107.7		
C-10	101.5	101.5	101.5	101.5		
C-5,7,9	152.5[i]	154.0[i]	154.0[f]	156.6[f]		
C-1′	130.6	130.6	130.6	130.6	120.1	101.5
C-2′	115.9	115.9	109.5	115.9	109.5	74.2
C-3′	144.5[h]	144.5[h]	144.5[h]	144.5[h]	144.9[h]	75.8
C-4′	144.9[h]	144.9[h]	132.2 135.7	144.9	138.8	69.6
C-5′	117.4	118.0[k]	109.5	117.4	144.9[h]	75.8
C-6′	118.7[k]	119.5	118.7[k]	118.7[k]	109.5	63.1 60.8
					166–169	

*Signal masked by a signal of the solvent.

Signals labeled with the same superscript letters may be interchanged. Underlining indicates galloylation of the hydroxy group at C-3.

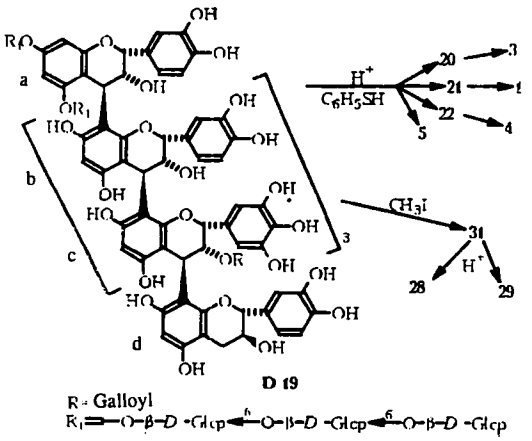

Fig. 7. Structure and scheme of the chemical transformations of D19.

than D14. Alkaline fusion of D15 likewise led to phloroglucinol (**17**) and gallic and protocatechuic acids (**16**) and (**18**). The splitting of D15 with hydrochloric acid gave cyanidin (**14**) and delphinidin (**15**).

A study of the products (**2**), (**4**), and (**6**) of the thiolytic cleavage of D15 after the reductive decomposition of the resulting thioethers (**22**) and (**23**) with Raney nickel confirmed what has been said above. Consequently, its physicochemical parameters showed that this compound consisted of (+)-catechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate.

Starting from the structure and configuration of proanthocyanidin D14 in view of the closeness of the chemical compositions of D14 and D15 and the biogenetic synthesis of proanthocyanidins in plants, for the latter we propose the most probable structure and configuration of D15 shown in Fig. 3.

The polymeric proanthocyanidin D16 had a molecular mass of ~2550. Alkaline fusion of D16 in an atmosphere of nitrogen again led to the formation of three cleavage products: phloroglucinol and gallic and protocatechuic acids.

The UV and IR spectra of proanthocyanidins D16 and D14 were close. An investigation of the structure of D16 with the aid of ^{13}C NMR spectroscopy showed that its spectrum contained signals of (−)-epicatechin gallate, (+)-catechin (**5**), (+)-gallocatechin, and gallic acid (see Table 2).

The C-2, C-3, and C-4 chemical shifts (80-83, 71-73, and 35.5 ppm, respectively) were close to the corresponding values for galloylated catechins having the *trans-trans* configuration [13]. In D16, the bond between the catechin blocks was

of the C-4−C-8 type, as was shown by the C-10 CS (101 ppm) [14]. The signals of the C-8 carbon atoms through which interflavan bonds were realized appeared at 107.6 ppm. C-8 carbon atoms not involved in interflavan bonds give signals at about 96 ppm. Signals of the C-5, C-7, and C-9 carbon atoms of the phloroglucinol systems appeared at about 155 ppm. The CSs of the C-1′ carbons atoms of rings *B* of the catechins amounted to 130 ppm. The C-2′ and C-5′ signals appeared in the 113-115 ppm region. We assigned overlapping broad intense signals at about 144 ppm to the C-3′ and C-4′ atoms of the catechins and the C-3′ and C-5′ atoms of the gallocatechins. The CSs of the C-6′ carbon atoms of the catechin blocks were close to 120 ppm. For the C-4′ atoms of rings *B* of gallocatechin systems a characteristic signal appears at 133 ppm. In addition, the spectrum contained signals of gallic acid, characteristic for which are those of the carbonyl carbon at 164-169 ppm and also that of C-4 at 139 ppm. In the spectrum of D16 the signal of the C-3 carbon atom of ring C of the galloylated catechin occupying the bottom position in the polymer was found at 68 ppm.

An investigation of the products (**2**), (**6**), and (**7**) of the thiolysis of D16 after the reductive degradation of its thioethers (**19**), (**23**), and (**24**) with Raney nickel confirmed what has been said above. Thus, proanthocyanidin D16 has the structure and configuration shown in Fig. 4.

The polymeric proanthocyanidin D17 formed an optically active amorphous brown powder. After hydrolysis of this compound with hydrochloric acid, *D*-glucose (**25**), cyanidin (**14**), and delphinidin (**15**) were detected.

Table 3 gives the assignments of the signals in its ^{13}C NMR spectrum. Resonance signals at 152.0-154.6 ppm related to the C-5, C-7, and C-9 carbons of the phloroglucinol nuclei. Intense signals at 145.1 ppm belonged to the C-3′ and C-4′ atoms of rings *B* of the catechins and also to the C-3′ and C-5′ atom of rings *B* of the gallocatechins. In the gallo systems the C-4′ carbon atoms resonated at 133.2 ppm, and signals at 130.6 ppm related to the C-1′ atoms of rings *B* of the gallocatechins. Signals of the C-2′ and C-5′ atoms of the gallocatechins appeared at 115.6 ppm and of the C-2′ and C-6′ atoms of the gallocatechins at 109.4 ppm.

Resonance signals at 168.4, 119.9, 109.4, 145.1, and 140.1 ppm related to the carbon atoms of gallic acid residues. Their assignments are also shown in Table 3. Intense resonance at 107.4 ppm was given by the C-8 carbons of the substituted rings *A*, and a signal at 101.2 ppm by the C-10 atoms of the catechin blocks.

Signals appearing at 95.3 ppm related to the C-6 and C-8 unsubstituted carbon atoms of the phloroglucinol nuclei. The substituted C-4 carbon atoms appeared at 36.7 (galloylated catechins) and 38.8 ppm (nongalloylated blocks). The signals of the unsubstituted C-4 atom of the terminal catechin was masked by a signal of the solvent. The presence of signals of the C-2 carbons at 81.8 and 83.2 ppm showed that in compound D17 the substituents at C-2 and C3 of the heterocycle had the *trans*-configuration (2R,3S).

In the ^{13}C NMR spectrum of proanthocyanidin D17, in addition, there were signals from the carbon atoms of carbohydrate residues. A study of these groups of signals made it possible to assign them to the carbon atoms of three glucose residues bound in succession by 1-6 bonds. The carbon atoms of the anomeric centers resonated at 101.2 ppm, the signals of the C-2 carbons were observed at 72.9 ppm, and those of the C-3 and C-5 carbons at 76.9 ppm, while the C-4 atoms resonated at 70.5 ppm. The substituted C-6 carbon atoms of the first and second glucose residues gave a signal at 64.9 ppm, while the signals from the terminal glucose block appeared at 61.5 ppm. According to the results of thiolysis, it is most likely that the three-glucose chain was attached to the C-7 atom of the top catechin block.

On alkaline fusion in an atmosphere of nitrogen excluding oxidation of the cleavage products, three cleavage products were formed from D17: phloroglucinol (**17**) and gallic and protocatechuic acids (**16**) and (**18**) in approximately equal amounts. Thiolysis led to the thioethers (**19**), (**24**), and (**26**), which, on catalytic cleavage with Raney Ni, gave the flavan-3-ols (**5**), (**6**), and (**7**). The presence of the three sugar residues, the sequence of their linkage, and their position in the proanthocyanidin were established by a study of the permethylate (**27**) and the product of its hydrolysis (**28**) and (**29**).

Starting from the facts given above, for proanthocyanidin D17 we propose the structure and configuration shown in Fig. 5 as the most probable.

A comparison showed the close similarity of the UV, IR, and ^{13}C NMR spectra of proanthocyanidins D17 and D18. An interpretation of the chemical shifts of the signals in the ^{13}C NMR spectrum of proanthocyanidin D18 is given in Table 4. The presence in the spectrum of D18 of signals close in characteristics to those of D17 but broader showed its higher molecular mass. In actual fact, the alkaline fusion of D18 formed phloroglucinol and gallic and protocatechuic acids (**16**)-(**18**), while acid hydrolysis gave *D*-glucose (**25**), cyanidin (**14**), and delphinidin (**15**). The thiolysis of this compound led to the following compounds: the thioethers (**19**) and (**24**) and the flavan-3-ol (**5**). Degradation of the thioether (**19**) and (**24**) gave the flavan-3-ols (**6**) and (**7**). The presence of three sugar residues, the sequence of their linkage, and their position in the proanthocyanidin were established on the same bases (**28**)-(**30**) as in the investigation of D17 (Fig. 6).

On alkaline fusion, proanthocyanidin D19, just like the compounds described above, gave phloroglucinol and gallic and protocatechuic acids, and, on acid hydrolysis, *D*-glucose, cyanidin, and delphinidin. A comparison of its spectral characteristics with those of the compounds described above showed some difference of the IR and ^{13}C NMR spectra of D19 from those of the preceding two proanthocyanidins.

In the IR spectrum of D19, in addition to the absorption bands present in the spectra of compounds D17 and D18, there was a weak absorption band at 805 cm^{-1}. This showed that the substituents at C-2 and C-3 had the *cis*-orientation (2R,3R) and the substituents at C-3 and C-4 the *trans*- orientation (3R,4S). At the same time, the presence of a weak signal of the C-2 carbon at 81.0 ppm in the ^{13}C NMR spectrum showed the presence of *trans*-oriented substituent at C-2 and C-3 of the heterocycle of the bottom block.

A comparison of the ^{13}C NMR spectra of the proanthocyanidins that we had studied showed that in the D19 molecule (^{13}C NMR results, see Table 5) there were glucose residues linked with one another by 1-6 bonds. This was shown by the fact that cleavage of the permethylate (**31**) yielded the methylated sugars (**28**) and (**29**). The thiolytic cleavage of proanthocyanidin D19 gave the thioethers (**20**), (**21**), and (**22**) and the flavan-3-ol (**5**). Catalytic degradation of the thioethers led to the flavan-3-ols (**1**), (**3**), and (**4**).

On the basis of the facts given above and in the light of the biogenesis of polymeric proanthocyanidins, we propose as the most probable structure and configuration of D19 those shown in Fig. 7.

It must be mentioned that in the bark of English oak the amounts of (+)-catechin (**5**) and (+)-gallocatechin (**7**) are greater than those of the epimeric forms both in the free state and as constituents of polymeric proanthocyanidins.

EXPERIMENTAL

General Information. The UV spectra of the proanthocyanidins and their derivatives were taken in alcoholic solution on a Hitachi EPS-3T instrument and IR spectra on a Carl Zeiss, Jena, UR-20 instrument in tablets with potassium bromide.

^1H NMR spectra were obtained on Bruker WM-200 SY/200 MHz and Tesla BS-657 A/100 MHz instruments in Me_2CO-d_6 solution with HMDS as internal standard, δ-scale.

^{13}C NMR spectra were recorded on Tesla BS-567 A/25 MHz and Bruker WM-200 SY/50.3 MHz spectrometers in the mixed solvent Me_2CO-d_6–D_2O (1:1) with TMS as internal standard. The concentrations of the substances ranged from 15 to 20%. Molecular masses were determined on a MOM-3170 ultracentrifuge (speed 8000 rpm, t = 30 °C, angle = 30°, time 30 min) and by gel filtration on a calibrated column of Sephadex LH-20. To check the homogeneity of the substances we used PC and TLC on Silufol UV-254 plates. Solvent systems: 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); 5) butan-1-ol–acetic acid–water (40:12:18); 6) 6 N HCl.

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

Extraction and Isolation of the Catechins and Proanthocyanidins. Oak bark (10 kg) was extracted six times with 80% ethanol, and the extracts were evaporated in vacuum at 40°C to 3 liters. The concentrated extract was treated successively with diethyl ether, ethyl acetate, and *n*-butanol. The extracts obtained amounted to 39.4, 46.1, and 267.3 g, respectively. The aqueous residue yielded 1.36 kg of highly polymeric proanthocyanidins.

Separation of the Catechins. The ether extract (39.4 g) was chromatographed on a column of silica gel (6 × 180 cm). For elution we used an ethyl acetate–hexane (1:2-4:1) system. As a result, the following compounds were isolated and were identified by their physicochemical characteristics: (−)-epicatechin (**1**) (0.062 g); (−)-epicatechin gallate (**2**) (0.047 g); (−)-epigallocatechin (**3**) (0.089 g); (−)-epigallocatechin gallate (**4**) (0.053 g); (+)-catechin (**5**) (0.317 g); (+)-catechin gallate (**6**) (0.210 g); and (+)-gallocatechin (**7**) (0.293 g).

Separation of the Dimers. The ethyl acetate fraction (46.1 g) was chromatographed on a column of Sephadex LH-20 (5 × 160 cm). For elution we used a methanol–water (8:2-6:4) system. Resulting fractions of similar composition were combined and rechromatographed. Five dimeric proanthocyanidins were isolated: (+)-catechin-(4α–8)-(+)-catechin (**8**) (0.339 g) with the composition $C_{30}H_{26}O_{12}$, $[\alpha]_D^{22}$ −241° (*c* 0.44; ethanol); 3-O-galloyl-(+)-catechin-(4α–8)-3-O-galloyl-(+)-catechin (**9**) (0.170 g), composition $C_{44}H_{34}O_{20}$, $[\alpha]_D^{25}$ −169° (*c* 0.31; acetone); (+)-gallocatechin-(4α–8)-(+)-gallocatechin (**10**) (0.163 g), composition $C_{30}H_{26}O_{14}$ $[\alpha]_D^{25}$ −141° (*c* 0.38; ethanol); (−)-epicatechin-(4β–8)-(−)-epigallocatechin (**11**) (0.113 g), composition $C_{30}H_{26}O_{13}$ $[\alpha]_D^{23}$ +38° (*c* 0.29; acetone); 3-O-galloyl-(−)-epicatechin-(4β–8)-3-O-galloyl-(−)-epigal

locatechin (**12**) (0.129 g), composition $C_{44}H_{34}O_{21}$, $[\alpha]_D^{23}$ +95.8° (*c* 0.42; acetone) and 3-O-galloyl-(−)-epigallocatechin-(4β−8)-(+)-catechin (**13**) (0.117 g), composition $C_{37}H_{30}O_{18}$, $[\alpha]_D^{23}$ +11.5° (*c* 0.34; acetone).

Separation of the Proanthocyanidins. The butanol extract (100 g) was mixed with 100 g of cellulose and transferred to a column of microcrystalline cellulose (100 g), and elution was performed with chloroform−ethyl acetate (1:10-1:20), ethyl acetate, and acetone, with the collection of 100-ml fractions. Monitoring was carried out by TLC. Eluates containing identical components were combined, evaporated, and rechromatographed on a column of Sephadex LH-20 (5 × 180 cm), with elution by ethanol−water (8:2-6:4).

Proanthocyanidin D14. 0.891 g, $C_{117}H_{74}O_{44}$, M 2182, $[\alpha]_D^{25}$ +69.5° (*c* 0.81; acetone−water (1:1)). UV spectrum: λ_{max} 210, 220, 245, 278, 305 nm, λ_{min} 258 nm. IR spectrum: ν_{max} 3500-3200, 2935, 1657, 1610, 1520, 1495, 1440, 1320, 1250, 1200, 1150, 1105, 1040, 825, 805, 770, 745 cm^{-1}. For ^{13}C NMR, see Table 1.

Proanthocyanidin D15. 0.940 g, $C_{66}H_{50}O_{31}$, M 1338, $[\alpha]_D^{23}$ +147° (*c* 1.1; acetone−water (1:1)). UV spectrum: λ_{max} 210, 222, 245, 278, 305, nm, λ_{min} 258 nm. IR spectrum: ν_{max} 3500-3300, 2938, 1658, 1610, 1533, 1515, 1495, 1445, 1320, 1250, 1200, 1148, 1105, 1038, 828, 805, 771, 745 cm^{-1}.

Proanthocyanidin D16. 2.138 g, M-2550, $[\alpha]_D^{25}$ −113° (*c* 1.0; acetone−water (1:1)). UV spectrum: λ_{max} 210, 220, 245, 278, 310 nm, λ_{min} 258 nm. IR spectrum: ν_{max} 3500-3200, 2936, 1656, 1610, 1535, 1520, 1493, 1440, 1320, 1250, 1200, 1150, 1105, 1040, 826, 804, 775, 744 cm^{-1}. For ^{13}C NMR, see Table 2.

Proanthocyanidin D17. 0.911 g, M-2600, $[\alpha]_D^{23}$ −140° (*c* 1.0; ethanol−water (1:1)). UV spectrum: λ_{max} 210, 221, 245, 278, 308 nm. IR spectrum: ν_{max} 3500-3300, 2935, 1658, 1610, 1535, 1516, 1495, 1442, 1320, 1250, 1140, 1105, 1038, 829, 805, 773, 745 cm^{-1}. For ^{13}C NMR, see Table 3.

Proanthocyanidin D18. 1.101 g, M-3250, $[\alpha]_D^{23}$ −178° (*c* 0.95; ethanol−water (1:1)). UV spectrum: λ_{max} 210, 220, 243, 278, 310 nm, λ_{min} 258 nm. IR spectrum: ν_{max} 3500-3200, 2936, 1657, 1612, 1533, 1519, 1493, 1445, 1320, 1250, 1140, 1105, 1040, 825, 805, 775, 743 cm^{-1}. For ^{13}C NMR, see Table 4.

Proanthocyanidin D19. 6.207 g, M-3800, $[\alpha]_D^{23}$ +98° (*c* 1.0; ethanol−water (1:1)). UV spectrum: λ_{max} 210, 220, 245, 278, 307 nm, λ_{min} 258 nm. IR spectrum: ν_{max} 3500-3200, 2930, 1655, 1611, 1535, 1520, 1492, 1445, 1320, 1250, 1140, 1104, 1040, 830, 805, 775, 740 cm^{-1}. For ^{13}C NMR, see Table 5.

Alkaline Cleavage D14-D19. In a slow current of nitrogen, a mixture of 50-70 mg of substance and 5 ml of 50% KOH was immersed in a bath with a temperature of 155-160°C, and then the temperature was raised to 230°C in 5 min. The reaction mixture was rapidly cooled, acidified, diluted with water, and extracted with ethyl acetate. The extract was dried, the solvent was distilled off, and the residue was chromatographed on polyamide. Three compounds were obtained, which were identified as phloroglucinol (**17**) and gallic and protocatechuic acids (**16**) and (**18**).

Acid Cleavage of D14-D16. A solution of the substance (100-150 mg) in 4 ml of ethanol was treated with 3 ml of 2 N HCl, and the reaction mixture was heated in the water bath under reflux in a current of nitrogen for 2 h. Then it 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 Sephadex LH-20, with elution by 80% ethanol. This gave (+)-catechin gallate (**6**), mp 196-197°C, $[\alpha]_D^{23}$ +3.2° (*c* 0.52; acetone−water (1:1)), λ_{max} 280 nm (log ε 4.11), R_f 0.69 (system 2).

In the hydrolysate, delphinidin (**15**) and cyanidin (**14**), R_f 0.35 and 0.69, respectively, were detected by PC (system 6).

The acid cleavage of D17-D19 was conducted by the method described above. We detected (+)-catechin (**5**), mp 179-180°C $[\alpha]_D^{24}$ +19° (*c* 0.29; methanol−water (1:1)), λ_{max} 280 nm (log ε 3.91), R_f 0.65 (system 5), cyanidin, delphinidin, and *D*-glucose (R_f 0.51; butan-1-ol−pyridine−water (6:4:3)).

Thiolytic Cleavage of D15. A mixture of 0.390 g of substance and 4 ml of phenylmercaptan was treated with 2 ml of acetic acid in 10 ml of ethanol and the resulting reaction mixture was kept at room temperature for 48 h. The course of the reaction during the first 8 h was monitored by TLC every hour. The reaction mixture was concentrated to give an oily residue, which was chromatographed on Sephadex LH-20, with elution by 80% ethanol. This gave 0.027 g of (+)-catechin gallate (**6**) and 0.262 g of an amorphous substance — a mixture of the two thioethers (**22**) and (**23**).

Cleavage of the Thioethers from D15. The thioethers (0.262 g) were mixed with 3 ml of ethanol−acetic acid (9:1), Raney nickel catalyst was added, and the reaction mixture was kept at 50°C for 2 h. Then it was filtered, and the filtrate was concentrated and chromatographed on Sephadex LH-20, with elution by 80% ethanol. Two compounds were obtained: 0.033 g of (−)-epicatechin gallate and 0.039 g of (−)-epigallocatechin gallate, mp 211-212°C, $[\alpha]_D^{22}$ −186° (*c* 0.19; acetone−water (1:5)), λ_{max} 278 (log ε 4.01), R_f 0.64 (system 2).

Thiols D14 and D16; Catalytic Cleavage of Their Thioethers (19)-(24). The reactions were carried out by the method described above. The compounds obtained were identified as follows: for D14 — (+)-catechin gallate, (−)-epicatechin, (−)-epigallocatechin (**3**), and (−)-epigallocatechin gallate (**4**); for D16 — (+)-catechin gallate and (+)-gallocatechin.

Thiols D17-D19; Catalytic Cleavage of Their Thioethers (19)-(22), (24), (26). The reactions were carried out by the method described above. The compounds obtained were identified as follows: for D17 and D18 — (+)-catechin (**5**), (+)-catechin gallate (**6**), and (+)-gallocatechin; for D19 — (+)-catechin (**5**) and (−)-epicatechin (**1**), mp 242-243°C $[\alpha]_D^{22}$ −71° (*c* 0.21; acetone−water (1:1)), λ_{max} 282 (log ε 3.30), R_f 0.51 (system 2), (−)-epigallocatechin gallate (**4**).

Preparation of the Permethylates of D17-D19 (27), (30), (31). The substances (0.45-0.5 g) were subjected to Hakomori methylation [18]. The resulting products were transferred to a column and were washed with the benzene−acetone (5:1) system. The compositions of the methylated sugars and the permethylates were determined after their acid hydrolysis: 2,3,4-tri-O-methyl-*D*-glucopyranose (**28**) and 2,3,4,6-tetra-O-methyl-*D*-glucopyranose (**29**) were detected by GLC.

The TLC of the hydrolysate of the permethylate showed the presence of the same methylated sugars (chloroform−methanol (12:1) system).

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