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^{1}H AND ^{13}C NMR SPECTROSCOPY IN THE STUDY OF FLAVAN-3-OLS, PROANTHOCYANIDINS, AND THEIR DERIVATIVES

III. ^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF FLAVAN-3-OLS AND PROANTHOCYANIDINS

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This review discusses the possibilities of using carbon-13 nuclear magnetic resonance spectroscopy in the investigation of flavan-3-ols, proanthocyanidins, and their derivatives, and reports differences in the spectral behavior of monomers, oligomers and their derivatives and features of the spectra of high-molecular-mass proanthocyanidins.

^{13}C NMR Spectroscopy of Flavan-3-ols

In preceding publications we have discussed the results of investigations of flavan-3-ols and proanthocyanidins with the aid of proton magnetic resonance spectroscopy [1, 2]. In the present part of the review we shall consider the results of investigations of flavan-3-ols with the use of nuclear magnetic resonance spectroscopy on carbon ^{13}C nuclei (^{13}C NMR). This type of spectral investigation of the flavan-3-ols and proanthocyanidins permits their chemical structures and stereochemistries to be determined. In the case of proanthocyanidins it enables us to establish the position of the interflavan bonds in the aromatic rings, which is one of the important aspects of the study of their structures. The possibility of applying ^{13}C NMR spectroscopy to the study of the high-molecular-mass proanthocyanidins is particularly important, since in this field the use of PMR spectroscopy is limited even when modern equipment with a high working frequency is used.

The main questions in the investigation of the structures of flavan-3-ols with the aid of ^{13}C NMR spectroscopy are the determination of their stereochemistry and the type of oxidation of the aromatic rings. To determine the stereochemistry of the flavan-3-ols at the C-2 and C-3 asymmetric centers it is possible to make effective use of the chemical shift (CS) of the signal of the C-2 carbon atom (Table 1): in the 2,3-*trans*-isomers, the CS of C-2 amounts to 80.7-82.7 ppm [3-12], while in the *cis*-isomers it is 77.9-79.4 ppm [3, 5, 7, 11, 13-19]. In spectra obtained under similar conditions, the signal of the C-3 carbon in (+)-catechin (**1**) is found in a field approximately 1.5 ppm weaker than for its diastereoisomer. Various authors agree about these facts. Diamagnetic effects are observed on passing from a *trans*- to a *cis*-isomer, with respect both to C-2 and to C-3: −3.2 and −1.1 ppm, respectively. (We calculated the values of the induced chemical shifts (ICSs) from the figures given in Table 1.) These differences are, in the main, connected not with the absolute configurations of the asymmetric centers but with the mutual relative configuration of the substituents at the given carbon atoms (Fig. 1).

In the interpretation of the CSs of the signals of aromatic carbons it must be borne in mind, above all, that a magnetic field induces ring currents in the aromatic system. Of all the factors affecting the CSs of aromatic carbons, a considerable contribution is made by the π-electron density [20]. It is well known that this magnitude also correlates with the reactivities of aromatic compounds. A consideration of the phloroglucinol system (ring *A*) shows that the signals of the oxygen-substituted atoms C-5, C-7, and C-9 appear in a fairly weak field (here and below in the text we give the CS ranges from literature figures, and in the tables concrete CS values from the cited references) in the region of 154.6-157.6 ppm. This is connected with the descreening influence of an oxygen atom not only on the carbon directly linked to it but also on a carbon atom in the *meta*-

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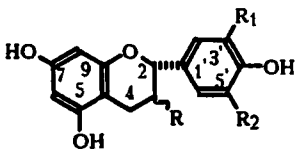
TABLE 1. Chemical Shifts of the ^{13}C Nuclei of Flavan-3-ols, ppm

Compound	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	Literature
1 [1]	82.0	67.9	28.1	156.9	96.7	156.7	95.7	156.3	100.9	131.5	115.6	145.5	145.5	116.6	120.3	3, 7, 6, 11
2 [1]	79.1	66.8	28.6	157.1	96.8	156.7	96.0	156.7	100.3	131.8	115.4	145.0[h]	145.2[h]	116.4	119.6	3, 11
3 [1]	78.6	66.1	28.1	156.0	96.9	155.8	96.2	155.8	100.6	131.4	107.5	145.8	132.7	145.8	107.5	3
4 [2]	79.4	66.3	29.0	157.0	96.4	157.0	95.8	157.0	99.7	131.3	128.9	115.5	157.0	115.5	128.9	64

*The assignments of signals denoted by the same letter may be interchanged.

Solvents: [1]d-Ac-D_2O(1:1); [2]d-Ac.



	R	R_1	R_2
1	βOH	OH	H
2	αOH	OH	H
3	αOH	OH	OH
4	αOH	H	H

Fig. 1. Structures of the flavan-3-ols (**1-4**).

position. And, although this effect is not very great, the simultaneous presence of three substituents in the *meta*-position to one another makes an appreciable paramagnetic contribution to the CSs of these carbon atoms. These structural features of the phloroglucinol system lead to a fairly strong screening influence on the C-6 and C-8 carbon atoms, which are in the *ortho*-position to oxygen substituents. In catechins, the signals of these carbons usually appear in the 95.0-96.8 ppm region, the difference in the chemical shifts of these carbon atoms being small: from 0 to 1.2 ppm. The signal of the C-6 carbon appears in a weaker field than that of the C-8 signal. This feature was first established by L. J. Porter et al. on the basis of a measurement of the times of longitudinal relaxation of C-6 and C-8 [7]. In (+)-catechin the corresponding values of T_1 are 0.235 and 0.30 sec, and in (−)-epicatechin 0.21 and 0.29 sec; i.e., $T_{1C\text{-}6} > T_{1C\text{-}8}$. This is apparently explained by the fact that the mechanism of the spin-lattice relaxation of C-6 is more effective because of the rotational and vibrational degrees of freedom of the OH group at C-5 than the similar mechanisms for C-8 through the ring oxygen atom.

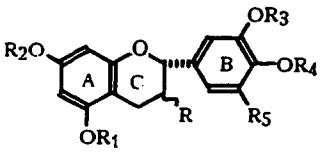
The CSs of the carbons of ring *B* depend on the nature of its oxidation (phenol, pyrocatechol, or pyrogallol). We have isolated characteristic signals for each type of oxidation. Characteristic for afzelechin (**4**) is the coupling of the signals of the carbon atoms C-1′ — 129.5-131.6 ppm, C-2′ 127.8-130.3, and C-3′ — 114.5 ppm [21, 22, 55]. Characteristic for the catechin system is the presence of signals of the unsubstituted carbons: C-2′, 115.0-115.6 ppm; C-5′, 115.4-116.6; and C-6′, 119.6-120.3 ppm [3, 4, 7-9, 11, 16, 18, 23]. In gallocatechins, the signal of the C-4′ carbon is characteristic. It usually appears in the 132.7-133.9 ppm region [3, 18, 24]. For gallic acid, which is present relatively frequently in proanthocyanidins, a characteristic signal is given by the C-4′ carbon at 138.0-139.5 ppm [24,26]. The use of these spectral characteristics permits the types of oxidation of a given aromatic ring to be clearly distinguished.

Thus, the specificity of the chemical shifts of the carbon atoms makes it possible to establish the 2,3-*trans*- or 2,3-*cis*-stereochemistry of the substituents in ring *C*. The presence in the ^{13}C NMR spectrum of a definite combination of the signals of sp^2-hybridized carbon atoms permits the degree and type of oxidation of the aromatic rings to be determined.

Characteristics of the ^{13}C NMR Spectra of Flavan-3-ols with Substituents in the Aromatic Part of the Molecule

Compounds of this group can be isolated from natural materials or be obtained synthetically. In this section we shall consider the influence exerted by the formation of ether and ester bonds at the phenolic hydroxy groups in the compounds given in Fig. 2. We also give the characteristics of the effects of substituents linked directly with aromatic carbon atoms in the compounds the structures of which are shown in Fig. 3.

Methylation of the phenolic hydroxy groups of the flavan-3-ols (Table 2) has little effect on the CSs of the carbon atoms of ring *C*, regardless of the configurations of the substituents in it [5, 27]. The total acetylation of all the hydroxylic functions of (+)-catechin causes paramagnetic shifts of the signals of all the aromatic carbon atoms [6]. The signals of the carbon atoms bearing ester groups shift downfield by 1-3 ppm. According to the literature [5, 7], for tertiary sp^2-carbons present in the *ortho*-position to acetylated groups the ICSs range from +3 to +12 ppm, the smallest shift being undergone by the signal of the C-6′ carbon, which has no *ortho*-acetyl group. The greatest induced shift is observed for signals of the C-6 tertiary carbon atom, which has two acetylated hydroxy groups in *ortho*-positions. Starting from this, it may be coneluded that for tertiary aromatic carbon atoms present in *ortho*-positions to acetylated hydroxy functions the ICSs have basically an additive character. The formation of ether and ester bonds at phenolic OH groups has an insignificant effect on the chemical shifts of the quaternary carbons. Exceptions are carbons linked with hydroxy groups present in *ortho*-positions. For them, diamagnetic shifts of the



R	R_1	R_2	R_3	R_4	R_5
5 βOH	Me	Me	Me	Me	Me
6 βOAc	Ac	Ac	Ac	Ac	Ac
7 βOβGlc	H	H	H	H	H
8 βOαRha	H	H	H	H	H
9 βOH	βGlc	H	H	H	H
10 βOH	H	βGlc	H	H	H
11 βOH	H	H	βGlc	H	H
12 βOH	H	H	H	βGlc	H
13 βOH	H	Gall	H	H	H
14 βOH	βGlc	H	βGlc	H	H
15 βOH	H	βGlc	βGlc	H	H
16 βOH	H	H	βGlc	βGlc	H
17 αOH	H	H	Me	H	H
18 αOH	Me	H	Me	H	H
19 αOH	Me	Me	Me	H	H
20 αOH	Me	Me	Me	Me	H
21 αOAc	Me	Me	Me	Me	H
22 αOGall	H	H	H	H	H
23 αOβGlc	H	H	H	H	H
24 αO*	H	H	H	H	H
25 αOGall	H	H	H	H	OH
26 αO**	H	H	H	H	OH

*-CO-$(CH_2)_5$-Ar(3,4,5-OH)
**-CO-CH=CH-Ar(4-OH)

Fig. 2. Structures of ethers and esters of flavan-3-ols.

signals appear. Galloylation of the phenolic groups of the flavan-3-ols also occurs. Thus, on the galloylation of the C-7-OH group of (+)-catechin [25], diamagnetic shifts are observed for the signals of the quaternary carbons C-7 and C-10, and paramagnetic shifts for the tertiary aromatic carbon atoms C-6 and C-8.

Among derivatives of the flavan-3-ols are found O- and C-glucosides of (+)-catechin, (+)-afzelechin, and (−)-epicatechin (see Figs. 2 and 3 and Tables 2 and 3). Glycosylation of the phenolic hydroxyls of ring *A* of (−)-epicatechin does not lead to appreciable changes in the CSs of the carbons of ring *C*. Glycosylation induces a paramagnetic shift of 1-2 ppm for the signal of the corresponding carbon atom. The signals of the carbon atoms in the ^{13}C NMR spectrum of the O-glucose residue in the glycosidic moiety appear, respectively, in the following intervals: C-1′ 101.2-103.6; C-2′ 72.9-74.6; C-3′ 76.2-77.6; C-4′ 69.4-71.1; C-5′ 76.5-77.5; C-6′ 60.4-64.7. For C-glycosides it follows from the literature that the CS of a C-6 carbon to which a C-glucose residue is attached is 1.1-1.2 ppm greater than the CS of the corresponding carbon in a C-8-glucoside. CS values of C-glycosides are given in Table 3.

The investigation of the ^{13}C NMR spectra of a group of flavan-3-ol derivatives with substituents in positions 6 or 8 is not only of theoretical but also of practical interest, since they are found in natural materials and may be products of the chemical modification and degradation of proanthocyanidins. Details of the ^{13}C NMR spectra of this group are given in Table 3, and their structures in Fig. 3. In bromo [30] and isoprenyl [29] derivatives of (+)-catechin, likewise, δC-6 > δC-6. The spectral feature that the CS of C-6 is greater than the CS of C-8 in corresponding pairs of derivatives is in harmony with the results of the assignment of the C-6 and C-8 signals in the flavan-3-ols themselves given above. Thus, the δC-6 > δC-8 tendency is retained [4, 7, 8, 16, 31].

Features of the ^{13}C NMR Spectra of Flavan-3-ols with Substituents in Position 3

Information on catechin derivatives acetylated in position 3 (Fig. 2) is frequently found in the literature. The acetylation of the C-3-OH group of (+)-catechin (Table 2) induces the following shifts: $\Delta\delta$ C-2, −3.2; $\Delta\delta$ C-3, +1.1; and $\Delta\delta$ C-4, −3.8 ppm [5, 7]. For (−)-epicatechin the corresponding values are −0.6; −1.3; and +0.2 ppm [5, 23].

TABLE 2. Chemical Shifts of the ^{13}C Nuclei of Ethers and Esters of Flavan-3-ols, ppm

Compound	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	-COO-	Literature
5 [1]	81.7	68.1	27.8														8
6 [2]	77.8	68.4	24.0		108.8		107.7	154.5	110.2	136.2	121.8	142.2	142.2	123.7	124.4		7
6 [2]	76.7	66.7	26.1		108.8		108.0	155.0	109.7	135.9	122.1	142.0	142.1	123.2	124.4		7
7 [1]	79.3	75.6	28.4	156.9	95.3	157.4	96.4	156.0	100.0	131.7	114.6	145.6	145.7	116.0	119.1		9
8 [1]	80.2	74.5	27.6	157.0	95.4	157.6	96.4	156.5	100.2	131.6	114.9	145.6	145.7	115.8	119.6		9
9 [3]	80.9	65.9	27.3	156.4	94.9	154.7	96.0	156.1	100.3	130.3	114.3	144.6	144.6	115.0	118.4		4
10 [1]	80.8	65.8	27.3	155.8	94.8	156.7	96.1	155.7	100.5	130.2	114.2	144.6	144.6	115.0	118.4		4
11 [1]	82.2	67.9	28.5	156.9	95.2	157.3	96.3	156.4	100.5	131.7	117.1	147.3	145.7	116.6	123.3		8
12 [1]	82.1	68.1	28.7	157.1	95.2	157.5	96.2	156.4	100.4	135.8	115.8	145.9	147.7	118.1	119.8		8
13 [4]	82.3	67.3	28.4	156.1[r]	101.0[k]	151.0	103.1[k]	157.1[r]	106.0	131.1	114.9[h]	145.3[m]	145.4[m]	115.6[h]	119.8		25
Gall										119.8	110.0	145.9	139.5	145.9	110.0	165.0	
14 [1]	82.4	67.9	28.8	157.7	97.8	157.5	96.9	156.2	103.1	131.7	116.7	147.7	145.8	117.4	123.4		8
15 [1]	82.0	67.6	27.8	156.8	97.4	157.8	96.5	156.1	103.3	131.6	116.5	147.1	145.7	116.7	123.1		8
16 [1]	82.0	68.4	28.8	157.0	95.2	157.3	96.4	156.3	100.5	135.4	118.4	147.8	147.8	119.0	123.2		8
17 [4]	79.3	66.8	28.9	156.8	95.5[r]	157.3	96.1[r]	157.2	99.6	131.8	111.9	146.6[f]	147.9[f]	115.9	119.4		27
18 [4]	79.4	66.8	29.1	156.7[h]	92.6	157.8[h]	96.5	160.1	100.6	131.9	111.7	146.8[k]	147.8[k]	115.2	120.6		27
19 [4]	79.6	66.6	29.1	156.7[q]	92.6	157.8[q]											27
20 [4]	77.2	67.9	25.8														27
21 [4]	76.6	66.6	26.0														27
22 [1]	77.8	69.7	26.4	156.4[g]	95.3	156.9[g]	96.3	157.0	98.6	130.7	114.6[x]	145.0	145.0	115.4[x]	118.7		28
23 [1]	78.2	73.3	24.2		95.4		96.3										16
24 [1]	78.2	69.8	28.5	155.7[y]	96.5	156.7[y]	95.5	157.5	98.6	130.7	115.2	145.3	145.3	115.9	118.7		11
25 [4]	78.0	69.9	26.4		95.5		96.4		98.7								23
26 [4]	77.8	69.3	26.5		95.5		96.4										23

*The assignments of the signals marked with the same superscript letters may be interchanged

Underlining indicates the presence of a substituent. Gall — gallic acid residue.

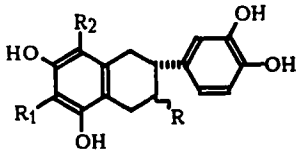
Solvents: [1]d-Ac+D_2O; [2]$CDCl_3$; [3]DMSO-d_6+D_2O; [4]d-Ac.

TABLE 3. Chemical Shifts of the ^{13}C Nuclei of Flavan-3-ols with Substituents at C-6 and C-8, ppm

Compound	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	Literature
27 [1]	81.7	67.9	28.4	155.5[h]	105.0	155.7[h]	96.2	155.3	101.9	131.7	115.1	145.6	145.6	119.3	119.3	8
Glcp										76.8	74.5	79.0	70.2	82.2	61.4	
28 [1]	81.1	67.5	27.6	156.0[i]	96.8	156.4[i]	103.9	154.4	100.6	131.8	114.9	145.2	145.3	115.8	119.3	8
Glcp										75.6	72.8	78.9	70.8	81.4	61.9	
29 [2]	82.4	68.4	29.1	154.4[k]	108.5	155.2[k]	95.8	155.2[k]	100.9	130.2	115.2[s]	145.7	145.7	115.9[s]	120.2	29
										22.8	124.2	130.9	17.9	25.9		
30 [2]	82.4	68.3	29.1	154.2[m]	96.0	154.6[m]	107.2	154.6[m]	100.4	129.9	115.2[n]	145.5	145.5	115.6[n]	119.7	29
										22.6	125.0	132.1	17.8	25.9		
31 [2]	82.6	68.0	29.1	156.0	66.6	156.6	96.0	157.3	101.5							30
32 [2]	83.1	68.1	27.9	157.6	96.0	156.7	63.7	155.2	101.7							30
33 [1]	79.2	66.3	28.1		105.0		96.5		Glc	76.8	74.5	79.0	70.2	82.2	61.4	16
34 [1]	79.2	66.7	29.2		97.2		103.8		Glc	76.0	73.2	78.9	70.5	81.2	61.6	16
35 [1]	79.9	66.4	29.7	153.8	89.5	156.4	96.5	153.6	101.4	131.6	115.0[r]	145.3[p]	145.4[p]	115.4r	119.0	16

*The assignments of the signals marked with the same superscript letters may be interchanged.

Solvents: [1]d-Ac + D_2O; [2]d-Ac.



R	R_1	R_2
27 βOH	Glc	H
28 βOH	H	Glc
29 βOH	CH_2-CH=C(CH_3)$_2$	H
30 βOH	H	CH_2-CH=C(CH_3)$_2$
31 βOH	Br	H
32 βOH	H	Br
33 αOH	Glc	H
34 αOH	H	Glc
35 αOH	Br	H

Fig. 3. Structures of flavan-3-ols with substituents in the aromatic part of the molecule.

The ICSs of (+)-catechin roughly reproduce the usual pattern that is observed on the acetylation of a hydroxy group in a six-membered ring [32]. In (−)-epicatechin an anomalous negative ICS is observed for C-3. Analysis of literature results on spin-spin coupling constants in the PMR spectra of these derivatives [33, 34] showed that when (−)-epicatechin was acetylated it did not change its conformation. This means that the observed phenomenon is apparently not connected with conformational isomerism in ring *C*.

The effects of acetylation in the *trans*-isomer are close to those observed in cyclohexanol: −3.3, +2.3, and −3.3 ppm (calculated by ourselves from figures given in the literature [5, 23, 32]). A feature of the acetylation of the *cis*-isomer is the negative effect observed for the C-3 carbon, which bears the acetylated group. Analysis of literature information on the spectra of analogous derivatives of other flavan-3-ols [5, 7] showed that with the given configuration of the substituents in the dihydropyran ring the effect under consideration may have either a positive or a negative sign. Literature figures for the spin-spin coupling constants in the PMR spectra [35-37] show that all analogous derivatives have the half-chair conformation. Consequently, the phenomenon observed in the ^{13}C NMR spectrum is not connected with conformational isomerism in ring *C*. It is obviously due to hindered rotation around the sp^3-sp^2 bond.

The structures of flavan-3-ols glycosylated in the C-3 position are given in Fig. 2, and the results of the assignment of the signals of their carbon atoms in Table 2. The ^{13}C NMR chemical shifts of a glycosylated (+)-catechin have a fairly wide scatter [7, 9, 12]. The CSs given by L. J. Porter [7] for (+)-catechin 3-O-glucoside are very close to those for the corresponding derivative of (−)-epicatechin, the structure of which has been confirmed by PMR spectra [16]. The CSs induced by sugars on the glycosylation of the C-3-OH group are given in Table 4. They show that inversion of the asymmetric center at C-3 of flavan-3-ol leads to appreciable changes in the glycosylation effect observed for C-4. As follows from the SSCCs in the proton magnetic resonance spectra [8, 23, 38], this is possibly due to inversion of ring *C* of (+)-catechin from the pentacoplanar conformation into the half-boat conformation with the formation of the C-3-O-glycoside [12]. In addition, the configuration of the asymmetric center influences the β-effect of glycosylation.

In spite of the fact that galloylated derivatives of the flavan-3-ols are found fairly frequently in Nature, we have discovered no information on the ^{13}C NMR spectrum of (+)-catechin 3-O-gallate. The effects of the galloylation of (−)-epigallocatechin calculated from literature figures [23, 28] — C-2, −1.4; C-3, +2.8; C-4, −2.2 — do not have the anomalous character that is observed on the acetylation of the C-3-OH group which we have discussed above.

Features of the Carbon Spectra of Flavan-3-ols Having a Substituent at C-4

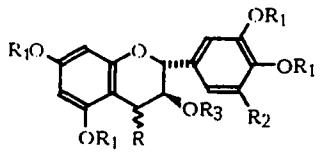
As already mentioned in the section devoted to the PMR spectra of flavan-3-ols [1], compounds of this group can be obtained in the degradation of proanthocyanidins. In addition, just as in PMR spectroscopy, 4-phloroglucinol derivatives are model compounds in the study of the ^{13}C NMR spectra of the proanthocyanidins [3, 5, 7, 39]. This is leading to an increased interest in them by researchers occupied with the study of the properties of polyphenols of this series.

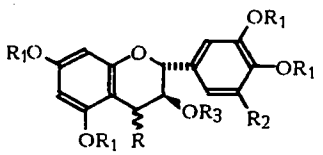
Let us consider the influence that phloroglucinol and other substituents occupying position 4 exert on the chemical shifts of the carbons of ring *C*. There is information in the literature on the chemical shifts in the ^{13}C NMR spectra of some flavan-3-ol derivatives having substituents at C-4 (Fig. 4 and Tables 5 and 6) [3, 5, 7, 23, 39-42]. It may be concluded from a comparison of the figures given in the tables that, regardless of the relative configurations of the substituents, the α-effect of each of the 4-α-derivatives given is relatively greater than that of the β-analogs. Since this effect does not depend on the rela-

TABLE 4. Effects of the Glycosylation of the C-3-OH Group of Flavan-3-ols (ppm)*

Compound	C-2	C-3	C-4	Literature
(+)-Catechin 3-O-α-rhamnoside	−2.1	+6.5	−0.1	9
(+)-Catechin 3-O-β-glucoside	−3.0	+7.6	−0.9	9
	−2.9	+5.0	+0.5	12
(−)-Epicatechin 3-O-β-glucoside	−1.1	+6.4	−4.2	16

*The calculations were based on CS values from the literature sources given in tables.





	R	R_1	R_2	R_3
36	αPhl	H	H	H
37	αPhl	Ac	H	Ac
38	βPhl	Me	H	H
39	αOH	H	H	H
40	βSCH_2Ar	Ac	H	Ac
41	αPhl	H	OH	H
42	αPhl	Ac	OAc	Ac

	R	R_1	R_2	R_3
43	βPhl	H	H	H
44	βPhc	Ac	H	Ac
45	βPhl	H	H	H
46	βSCH_2Ar	H	H	H
47	αPhl	Ac	Ac	Ac

*Phl – phloroglucinol

Fig. 4. Structures of flavan-3-ols with substituents in position 4.

tive configurations of the substituents it can be used to establish the absolute configuration of the asymmetric center at C-4 in these groups of derivatives

It also follows from Table 6 that the β- and γ-effects are connected with the relative configurations of substituents at C-3 and C-4: in the case of a 3,4-*cis*-configuration these effects are relatively smaller than in the *trans*-isomers, the γ-effect being more pronounced.

The study of the spectral features of flavan-3-ols having a substituent at C-4 has shown that the methods of ^{13}C NMR spectroscopy can be used successfully to establish the configuration of substituents in ring *C*.

Characteristics of the ^{13}C NMR Spectra of Dimeric Proanthocyanidins

The use of ^{13}C NMR spectroscopy in the investigation of the structures of proanthocyanidins permits the following problems to be solved: identification of the stereochemistry of substituents in ring *C*; determination of the type of interflavan bond (C-4−C-6 or C-4−C-8); establishment of the number and position of hydroxy groups in the aromatic rings *A* and *B*; and determination of the presence of ether and ester groupings in ring *C*.

Let us consider literature information on the low-molecular-mass proanthocyanidins — dimers of the first and second groups. To the first group are assigned proanthocyanidins in which the "upper" position is occupied by a flavan-3-ol with the 2,3-*trans*-configuration, and to the second group proanthocyanidins in which the "upper" position is occupied by a flavan-3-ol with the 2,3-*cis*-configuration. Details of the ^{13}C NMR spectra of dimers and their derivatives are given in Table 7, and their structures in Fig. 5.

On the formation of an interflavan bond in ring *C* of the "upper" block of a proanthocyanidin of the first group (Table 7), the signals of the C-2, C-3, and C-4 atoms appear, respectively, in the intervals of (ppm) 83.2-83.9; 73.2-73.9 (70.5 for fisetinidol), and 37.6-38.3 (41.2 ppm for fisetinidol) [5, 6, 9, 24, 25, 43], the following shifts being induced: C-2, +1.2; C-3, +5.0; and C-4 +9.6 [5, 39]. They are analogous to those observed in 4-α-phloroglucinol derivatives of (+)-catechin [3, 5, 7]. The values that we have calculated from the results of various authors agree well [5, 6, 24, 43]. The positive value of the

TABLE 5. Chemical Shifts of the ^{13}C Nuclei of Flavon-3-ols with Substituents in Position 4, ppm

Compound	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	Literature
36 [1]	83.7	72.9	37.9		97.6		96.4		106.8	131.7	116.3	145.3	145.7	116.2	121.0	7
37 [2]	82.4	73.3	38.0													5
38 [3]	78.2	71.8	32.2	160.0[b]	92.8	158.2[b]	91.4	156.3[b]	105.5	132.1	110.5	149.0	149.0	111.1	120.3	41
39 [4]	81.0	74.0	72.6		97.3		95.5			130.6	115.7	145.6	146.1	115.8	120.6	42
40 [3]	72.6	70.3	38.2		109.8		107.8	155.2	108.7	135.8	122.2	142.0	142.1	123.2	124.5	7
41 [4]	83.8	73.0	37.9	157.0	97.8	157.0	96.4	157.0	107.0	131.1	108.7	146.2	133.9	146.2	108.7	3, 7
42 [3]	79.3	71.6	36.8		110.4		108.7	157.0	114.9	134.3	119.9	143.3	135.2	143.3	119.9	7
43 [4]	76.6	72.6	36.5	157	96.4	157	95.7	157	102.0	132.2	115.5	144.9	145.2	116	119.6	3
44 [5]	72.5	70.4	36.4													5
45 [4]	76.6	72.6	36.5	157	96.4	157	95.7	157	102.0	132.2	115.5	144.9	145.2	116	119.6	3
46 [4]	74.4	70.3	42.9		96.4		95.2		99.4	131.0	114.8	144.4	144.4	115.7	118.8	7
47 [5]	73.8	70.7	33.8		108.9		107.7	154.8	110.5	135.5	119.2	136.6	143.3	119.2	135.5	23

*The assignments of the signals denoted by the same letters may be interchanged.

Solvents: [1]DMSO-d_6; [2]$C_6D_6-NO_2$; [3]$CDCl_3$; [4]d–Ac+D_2O; [5]d–Ac.

TABLE 6. Induced Chemical Shifts of the Signals of the ^{13}C Nuclei for the Atoms of Rings *C* of Flavan-3-ols with Substituents in the C-4 Position ($\Delta\delta$, ppm)

Substituent	C-2	C-3	C-4	Literature
(+)-Catechin	γ-effect	β-effect	α-effect	
Ph α*	+1.5	+5.1	+9.6	5
	+1.7	+5.3	+10.1	39
β**	−3.5	+3.7	+4.4	5, 41
OH α	−1.6	+5.6	+42.0	5
	−0.8	+4.3	+45.8	6
β**	−5.0	+2.5	+33.7	5
β-S-CH_2-Ar***	−5.2	+1.9	+14.2	7
(−)-Epicatechin				
Ph α	−4.2	+3.9	+10.5	5
β	−2.5	+5.8	+7.9	3, 7
β-S-CH_2-Ar	−4.7	+4.5	+14.3	7

*Close values are observed for the corresponding derivative of (+)-gallocatechin calculated from literature figures [3, 7].

**Calculated from the CSs for methyl ethers.

***Calculated from figures for the peracetates.

γ-effect and the relatively large α-effect show the α-orientation of the substituent at C-4. This conclusion follows from a comparison with the figures for the 4-phloroglucinol derivatives discussed above.

The signals of the sp^3-hybridized carbon atoms of the "upper" block for the proanthocyanidins of the second group are also given in Table 7 [5, 7, 12, 13, 16, 23, 24, 28, 29, 40, 44-52]. The chemical shifts of the C-2 and C-4 carbons may be singled out as characteristic for the proanthocyanidins of the first and second groups. They can be used for determining the relative configurations of substituents in ring *C* of the "upper" block.

The formation of the interflavan bond in the proanthocyanidins of the second group induces the following shifts, which we have calculated from literature figures [5, 16, 23, 89 [sic]] (ppm): $\Delta\delta$ C-2 from −2.8 to −1.1; $\Delta\delta$ C-3 from +5.2 to +6.5; $\Delta\delta$ C-4 from +8.0 to +8.6. A feature of the ICSs for this group is the negative sign for the C-2 signal, which is analogous to what is observed in 4β-phloroglucinol derivatives.

In the section devoted to the PMR spectroscopy of the proanthocyanidins, we discussed the influence exerted by galloylation on the chemical shifts of the protons [2]. Below we shall consider the influence of galloylation on the parameters of ^{13}C NMR spectra. Table 8 gives the chemical shifts observed in galloylated and nongalloylated blocks occupying the upper position in proanthocyanidins of the first and second groups. The effects of the galloylation of (+)-catechin occupying the "upper" position in a proanthocyanidin, calculated from literature figures [5, 25], are as follows (ppm): $\Delta\delta$ C-2, −1.6; $\Delta\delta$ C-3, −0.3; and $\Delta\delta$ C-4, −2.0. In this case, there is an anomaly in the C-3 ICS. It is possible that this phenomenon is connected with configurational isomerism. For gallic acid itself as a component of proanthocyanidins the signal of the C-4 carbon, δ = 138.0-139.3 ppm, and that of the carbonyl carbon, δ = 165.0-166.8 ppm, are characteristic.

The chemical shifts of the aromatic carbon atoms of the "upper block" [24, 43] are close to the corresponding values observed in the flavan-3-ols. In a block occupying the "lower" position in a proanthocyanidin, the formation of the interflavan bond induces a paramagnetic shift of the signal of the carbon of ring *A* (C-6 or C-8) through which the interflavan bond is realized. The ICS ranges between +10.4 and +12.9 ppm [6, 7, 12, 23]. The signals of these carbons appear in the interval of chemical shifts with δ = 106.5-109.0 ppm [5-7, 12, 16, 23, 24, 28, 40, 43-46, 49, 51-53].

For proanthocyanidins containing flavan-3-ols with the gallic type of oxidation of ring *B* it is possible to observe overlapping of the C-2′ and C-6′ signals of the gallic system at δ = 106.2-111.1 ppm and of the signals of the C-6 and C-8 carbon atoms connected with the "upper" block that we have discussed above [24, 26]. We consider that the most characteristic signal for the gallic type of oxidation of ring *B* is that of the C-4′ carbon, which appears in the interval of chemical shifts from 132.7 to 133.7 ppm [24, 26]. On analyzing the spectral details given in the literature for dimers, it is also possible to state that in the spectra of the proanthocyanidins of the first group the signal of the C-2′ and C-6′ carbons of the "upper" block appears in a weaker field — δ = 108.1-108.7 ppm — than that of the "lower" block — δ = 107.2 ppm [24]. However, this rule is not followed for proanthocyanidins of the second group [26]. For the pyrocatechol type of oxidation, we single out as characteristic

TABLE 7. Chemical Shifts of the Signals of the ^{13}C Nuclei of Dimeric Proanthocyanidin Derivatives, ppm

Compound	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	-COO-	Literature
48 [1] u	83.7	73.9	38.3	155.0–158.7	96.9–98.1	155.0–155.7	96.9–98.1	155.0–158.7	102.4	131.9[h]	115.9–116.9	145.4[i]	145.9[i]	115.9–116.9	120.3[k]		43
l	81.9	68.6	28.6	155.0–158.7	96.9–98.1	155.0–158.7	109.0–107.5	155.0–158.7	102.4	132.5[h]	114.8	145.4[i]	145.9[i]	115.9–116.9	121.2[k]		
49 [1] u	81.9	72.9	36.0		96.7[f]		96.5[f]		101.5	131.3[g]							25
l	81.0	68.1	28.3		97.2[f]		–			130.5[g]							
Gall										121.6	109.7	145.3	138.0	145.3	109.7	165.0	
50 [1] u	83.5	73.3	37.9		96.7		96.3			131.3	108.7	146.0	133.7	146.2	108.7		24
l	80.9	67.8	–		97.3		107.4			131.6	115.0	144.6	144.8	116.3	119.6		
51 [1] u	83.5	70.5	41.2	129.7	109.2		103.4										6
l	81.2	68.2	28.0	154.6	96.3		107.9										
52 [1] u	83.6	73.0	37.6		97.4		96.3			131.1	108.3	146.2	133.5	146.2	108.3		24
l	81.6	67.7	–		111.1		96.3			131.1	115.4	145.4	145.5	116.5	120.3		
53 [2] u	83.6	73.2	38.3														5
l	79.5	66.9	29.7														
54 [3] u	76.7	72.4	36.7	153.6–157.9	95.6[l]	153.6–157.9	96.7[l]	153.6–157.9	100.7	131.8[m]	114.4[n]	144.8–145.1	144.8–145.1	115.4[p]	119.1		28
l	81.2	68.2	28.0	153.6–157.9	97.0[l]	153.6–157.9	107.2	153.6–157.9	100.7	132.0[m]	115.4[n]	144.8–145.1	144.8–145.1	115.0[p]	119.1		
55 [3] u	75.2	74.5	34.0	153.9[r]	95.1[s]	153.9[r]	96.7	153.9[r]	100.7	131.1[t]	114.5[x]	144.8–	144.8–	115.4[x]	119.1		28
l	81.6	67.3	–	156.9[r]	95.7[s]	156.9[r]	106.5	156.9[r]	101.4	131.4[t]	114.5[x]	145.1	145.1	115.4[x]	119.6		
Gall										120.1	109.9	145.4	–	145.3	109.7		
										121.2							
56 [3] u	76.2	72.6	36.6	157.8	95.5	153.6	96.0	155.4	102.5	132.2	115.3	145.1	145.3	115.8	119.1		6
l	79.0	71.7	38.7	158.0	97.1	155.1	107.4	157.4	102.5	132.2	116.2	145.6	145.6	116.2	120.5		
-S-CH_2-	45.1									140.0	130.0	129.1	127.5	129.1	130.0		
57 [3] u	76.7	71.8	37.1						101.0								44
l	82.2	68.0	–						98.6								
58 [2] u	76.5	72.7	38.3		95.8[y]		96.2[y]										46
l	78.8	66.2	–		96.9[y]		106.7										

TABLE 7 (continued)

Compound	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	-COO-	Literature
59 [1] u	77.8	72.9	36.5		95.6		96.2										23
l	76.9	69.3	26.6		97.1		107.1										
60 [3] u	75.5	74.8	33.6	154.5[w]	95.3[v]	156.6[w]	96.9[v]	155.4[w]	99.3	130.4[u]	114.4[t]	144.5[t]	145.0[t]	115.3[h]	118.6[g]		28
l	77.8	69.1	–	156.9[w]	95.0[v]	155.4[w]	106.8	155.4[w]	101.9	131.0[u]	114.7[t]	144.5[t]	145.0[t]	115.5[h]	119.3[g]		
Gall										120.1	109.9	145.4	138.7	145.4	109.9	166.2	
										120.2	109.9	145.4	138.7	145.4	109.9	166.5	
61 [1] u	76.6	72.1	37.0		96.9		96.0										7
l	78.9	66.6	28.9		108.6		96.9										
62 [3] u	73.0	73.0	34.4	155.0[j]	95.0[k]	155.5[j]	96.1[k]	156.6[j]	99.8	130.0[v]	106.3	145.7[m]	132.7[m]	145.7[m]	106.3		26
l	77.8	69.7	–	158.1[j]	106.9	157.4[j]	96.8[k]	158.1[j]	99.8	130.1[v]	106.3	145.7[m]	132.7[n]	145.7[m]	106.3		
Gall										121.1	109.7	145.5[m]	139.2	145.5[m]	109.7	166.3	
63 [2] u	77.1	64.9	28.7	157.0[p]	95.5	157.0[p]	96.2	157.0[p]	99.2	129.1	111.9	144.9[r]	133.0	145.8[r]	108.0		23
l	77.1	64.9	28.7	157.1[p]	95.5	157.1[p]	96.2	157.1[p]	99.2	129.1	111.9	144.9[r]	133.0	145.8[r]	108.0		
64 [2] u	76.1	68.6	26.8		95.7[s]		96.5[s]									166.2	23
l	77.2	64.9	28.6		95.9[s]		96.1[s]										
65 [2] u	76.1	68.6	26.8		96.1		98.3									167.0	23
l	76.1	68.9	26.8		96.4		98.3									167.0	

*The assignments of the signals denoted by the same superscript letters may be interchanged. Gall — gallic acid residue.
Solvents: [1]d–Ac+D_2O (1:1); [2]d–Ac; [3]d–Ac+D_2O.

TABLE 8. Limits of the Chemical Shifts of the ^{13}C Nuclei of Rings *C* of Galloylated and Nongalloylated Blocks Occupying the "Upper" Position in Proanthocyanidins

"Upper block"	C-2	C-3	C-4
(+)-Catechin	83.2–83.9	73.2–73.9	37.6–38.3
(+)-Catechin 3-O-gallate	81.9	72.9	36.0
(−)-Epicatechin	76.4–77.8	71.8–73.0	36.3–37.4
(−)-Epicatechin 3-O-gallate	73.0–77.0	71.2–75.2	33.6–34.5

*The table was compiled from results published in [5, 6, 12, 13, 16, 24-26, 28, 43-53].

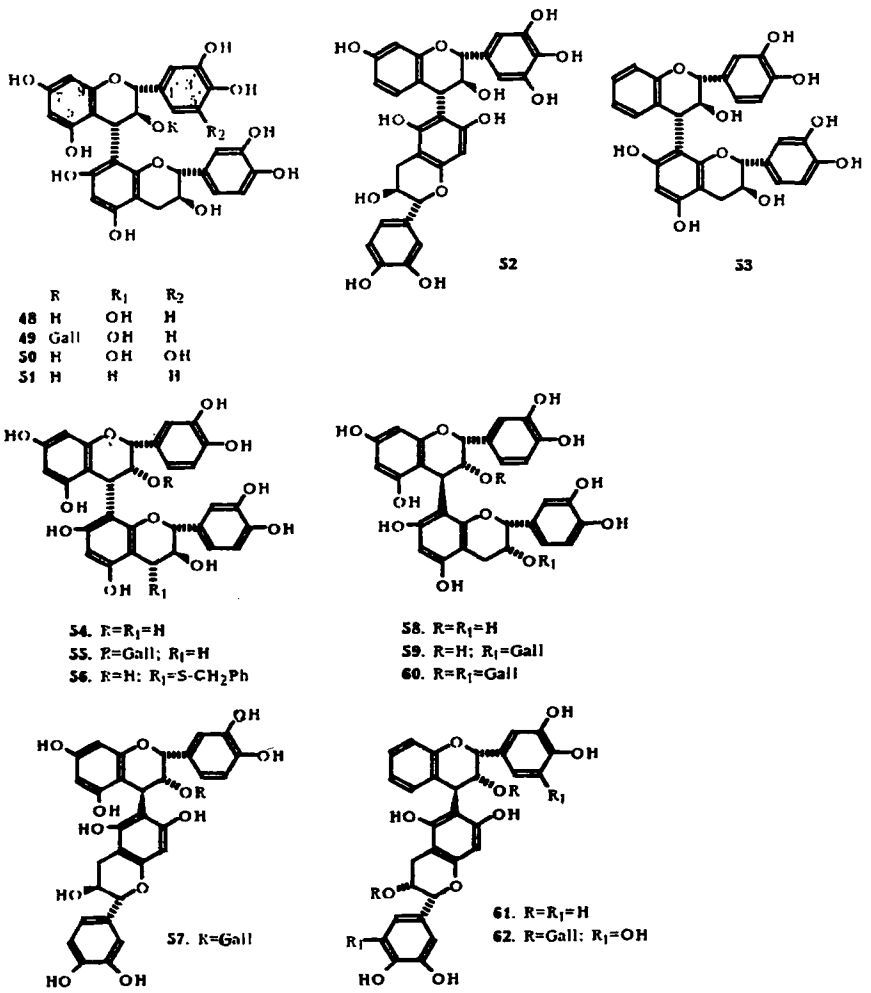
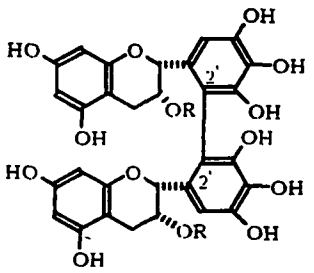


Fig. 5. Structures of dimeric proanthocyanidins and their derivatives.

the simultaneous appearance of signals from the following carbons (ppm): C-2′, δ = 114.4-116.9; C-5′, δ = 115.3-116.9; and C-6′, δ = 118.6-121.1 [12, 23, 24, 28, 29, 43, 45, 52]. For afzelechin, in which a hydroxy group is present in the *para*-position to the C-2 — C-1′ bond we may consider as characteristic the signals of the C-2′ and C-6′ carbon atoms with δ = 128-130 ppm, and of C-3′ and C-5′ with δ= 114-115 ppm. The signals that we have singled out as characteristic for various types of oxidation of ring *B* can be used fairly effectively for its identification in oligomeric and polymeric proanthocyanidins.

The most important aspect of the structure of proanthocyanidins is the revelation of the type of interflavan bond: C-4 — C-6 or C-4 — C-8. G. Nonaka et al. have proposed a method for solving this problem with the aid of ^{13}C NMR spectroscopy [44]. Investigating dimers, trimers, and proanthocyanidins with a large number of flavan blocks, they showed that in the spectra of proanthocyanidins with a C-4 — C-8 interflavan bond the signal of the C-10 carbon is shifted upfield:



	R	R_1
63.	H	H
64.	H	Gall
65.	Gall	Gall

Fig. 6. Structures of dimeric proanthocyanidins with a C-2′−C-2′ interflavan bond and their derivatives.

δ = 98.6-99.4 ppm (in a tetramer — 100.0 ppm) as compared with proanthocyanidins in which the interflavan bond is of the C-4 — C-8 type: for C-10, δ = 100.4-101.5 ppm. The CS figures of some other authors [12, 23, 28, 45, 52] agree with this rule; however, on galloylation it is sometimes possible to observe screening of the C-10 atom, as well [26, 50]. This phenomenon observed in some cases may be connected with conformational isomerism in ring *C* or with hindered rotation around the interflavan bond. The substituted atoms of the aromatic rings have the same spectral characteristics as in the spectra of the flavan-3-ols.

Among the dimeric proanthocyanidins there are compounds in which the bond between the flavan blocks is of the C-2′ — C-2′ type [23]. Their structures are shown in Fig. 6 and details of their ^{13}C NMR spectra are given in Table 7. On the formation of the interflavan bond in these substances the signal of the corresponding C-2′ carbon shifts downfield by approximately 3-4 ppm. The spectral characteristics of the other atoms change insignificantly. The compounds are convenient models for revealing the effects of acylation of a C-3-OH group.

Features of the ^{13}C NMR Spectra of Oligomeric and Polymeric Proanthocyanidins

^{13}C NMR spectroscopy plays an important role in the investigation of the structures and stereochemistries of high-molecular-mass compounds [54]. It can also be used effectively for the study of such complex structural forms as the polymeric proanthocyanidins. A necessary condition for this is sufficiently high solubility in water, organic solvents, or mixtures of them, giving solution not having excessively high viscosities.

Before proceeding to an analysis of the spectra of the high-polymeric proanthocyanidins, let us briefly consider features of the ^{13}C NMR spectra of oligomers (see Table 9 and Fig. 7). In the main, the spectral characteristics of trimers, tetramers, pentamers, and hexamers are close to those observed for dimers. However, in the spectra of oligomers both with the 2,3-*trans*- [24, 25] and with the 2,3-*cis*- [5, 7, 16, 44, 45, 55-58] configurations, a small — 1-2 ppm — diamagnetic shift of the signal of the C-3 carbon is found fairly frequently. In the *trans*-isomers there is also a paramagnetic shift of the signals of the C-2 carbon by 1-2 ppm [45]. On analyzing the same authors' figures for the PMR spectra of oligomers with the 2,3-*cis*-configuration [45], we came to the conclusion that this phenomenon may not be connected with conformational isomerism in ring *C*. It sometimes appears even in dimers [44]. It is apparently a consequence of rotational isomerism around sp^2-sp^3 bonds. Otherwise, the parameters of the spectra of trimers, tetramers, and pentamers are close to the corresponding values observed for dimers [5, 7, 16, 24, 51, 52, 55, 57]. However, in their identification the existence of various forms of stereochemistry in ring *C* and of various types of oxidation of the aromatic systems must be borne in mind.

In generalizing information on the spectra of dimers and oligomers we have singled out groups of signals that are characteristic for establishing the stereochemistry, the types of oxidation of the aromatic rings, and the positions of bonds. All these rules are valid for the polymeric proanthocyanidins and enable the main parameters of their structures to be established.

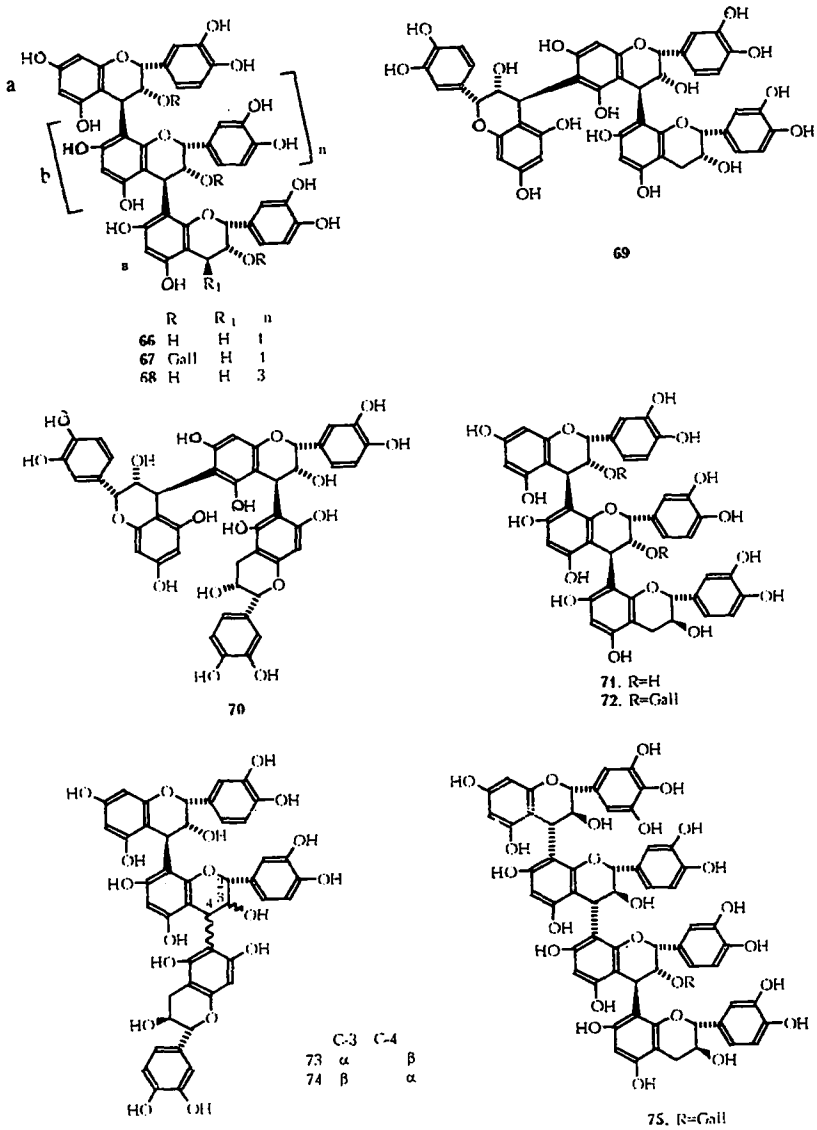


Fig. 7. Structures of oligomeric proanthocyanidins and their derivatives.

The ^{13}C NMR spectra of the polymeric proanthocyanidins (Fig. 8) have a number of features that are characteristic for the spectra of the majority of polymers [3, 7, 59-61]. Their signals are broadened in connection with hindered rotation around sp^2-sp^3 bonds. This factor can be used to evaluate any rotational isomerism. In the study of polymeric proanthocyanidins by the ^{13}C NMR method (Table 10) it is also possible to solve the following important structural problems: identification of the stereochemistry of substituents in ring *C*, determination of the ratio of 2,3-*cis*- and 2,3-*trans*-isomers, establishment of the types of interflavan bonds, determination of the ratio of procyanidin (PC) and prodelphinidin (PD) blocks, evaluation of the molecular mass or length of the polymer chain, and also other features of the chemical and spatial structures of the proanthocyanidins.

For stereospecific polymers the most important question is the solution of stereochemical problems. In the proanthocyanidins, this is the determination of the stereochemistry of the substituents in ring *C*. The chemical shift of the C-2 carbon is stereospecific. In blocks with the 2,3-*trans*-configuration the CS interval for C-2 is 83-85 ppm, while for blocks with the 2,3-*cis*-configuration it is 76-77 ppm [3, 59, 60]. In this connection we must note the influence of the relative configuration on the signal of the C-4 carbon.

It appears in the range of 36-37 ppm for proanthocyanidins with the 2,3-*cis*-configuration, and at about 38 ppm for polyflavans with the 2,3-*trans*-configuration. These values are retained with a change in the solvent [61]. However, it is better to use the C-2 chemical shift for determining relative configurations since its difference in corresponding pairs of diastereomers is greater. In polymers with mixed stereochemistries the C-2 CSs are also used for determining the quantitative ratios of the

TABLE 9. Chemical Shifts of the Signals of the ^{13}C Nuclei of Oligomeric Proanthocyanidins, ppm

Compound	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	-COO-	Literature
66 [1]	76.5	73.0	36.8		96.1		96.0										7
	76.5	71.9	36.8		97.1		107.1										
	78.9	66.2	29.1		97.5		107.6										
67 [2]	75.7	72.9	34.0[f]	153.9–	95.7[g]	153.9–	97.0[g]	153.9–	101.5	130.9	114.6–	144.5–145.1		115.5[h]	119.3		52
	76.1	73.0	33.6[f]	157.0	96.3[g]	157.0	106.4	157.0	102.4	131.2	114.9	144.5–145.1		115.7[h]	119.3		
	77.6	69.1	30.7.		97.0[g]		106.9		99.2	130.9		144.5–145.1		115.5[h]	119.3		
68 [2]	76.2	71.5	36.9														16
	76.2	71.5	36.9														
	76.2	72.0	36.9														
	76.2	72.2	36.9														
	78.6	66.2	–														
69 [2]	76.6	72.7[h]	37.2		95.9[l]		96.5[l]		100.5								51
	76.6	72.3[h]	37.2		96.5[k]		95.9[l]		99.9								
	79.0	66.1	29.2		96.5[l]		107.0[k]		100.5								
70 [2]	77.2	72.7[l]	37.5[v]		96.5[m]		95.5[m]		101.3								51
	77.2	72.3[l]	37.2[v]		107.2[n]		96.5[m]		98.5								
	79.0	66.1	29.4		107.7[n]		96.0[m]		98.5								
71 [2]	76.7	72.9	36.9		96.9		96.1										58
	76.7	71.4	36.9		97.2		106.9										
	81.4	67.5	28.2		97.2		108.0										
72 [2]	75.8	74.9	34.5[p]	153.3–	96.9[q]	153.3–	95.7[q]	153.3–	100.3[r]	130.8[s]	114.3–	144.7–145.2		114.3	119.1[t]		52
	75.8	72.8	33.6[p]	157.1	96.3[q]	157.1	106.4	157.1	101.2[r]	132.0[s]	115.8	144.7–145.2		115.8	119.3[t]		
	80.9	67.0	30.8		96.9[q]		106.4		102.4[r]	131.2[s]		144.7–145.2			119.1[t]		
Gall										121.2	110.2	145.5	138.8	145.5	110.2	165.6	
										121.5		145.7	139.9	145.7		167.0	

TABLE 9 (continued)

Compound	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	-COO-	Literature
73 [2]	76.5	72.9	36.9						101.2								44
	76.5	71.7	36.9						101.5								
	82.1	68.0	–						99.4								
74 [2]	76.1	72.8	36.4														45
	83.4	71.0	36.9														
	82.4	67.9	–														
75 [2]	83.0	72.5	37.0	155.0[p]	97.5	155.0[p]	97.5	155.0[p]	102.0	131.5	108.5[r]	146.0	–	146.0	108.5[r]		24
	83.0	72.5	37.0	156.0[p]	97.5	156.0[p]	107.0[r]	156.0[p]	102.0	131.5	116.0	146.0	146.0	116.0	119.0		
	76.5	76.5	34.0	155.0[p]	97.5	155.0[p]	108.5[r]	155.0[p]	102.0	131.5	108.1[r]	146.0	–	146.0	108.1[r]		
	83.0	67.5	–	156.0[p]	97.5	156.0[p]	108.5[r]	156.0[p]	102.0	131.5	116.0	146.0	146.0	146.0	119.0		
Gall										119.0	110.1	146.0	139.0	146.0	110.1		

*The assignments of the signals denoted by the same superscript letters may be interchanged
The CS intervals for C-5, C-7, and C-9 relate to all the blocks. Gall — gallic acid residue.
Solvents: [1]d-Ac+D_2O (1:1); [2]d-Ac+D_2O.

TABLE 10. Chemical Shifts of the ^{13}C Nuclei of Polymeric Proanthocyanidins, ppm

Compound	2	3	4	5	6	7	8	9	10	1′	2′	3′	4′	5′	6′	Literature
76 [1] PD	84	73	38	159	98	157	107	157	107	133	108	146	134	146	108	3
77 [1] PD	77	73	37	156	97	156	107	156	102	132	108	146	133	146	108	3
78 [1] PC	77	72	37	156	98	156	107	156	102	132	115	145	145	116	119	60
79 [2] PC																
cis	76.9	71.8	38.0	157.4	97.8	157.1	105.9	156.8	103.0	131.6	115.6	146.1	146.1	117.5	119.2	61
trans	84.9															

*Solvents: [1]d–Ac+D_2O (1:1); [2]DMCO-d_6.

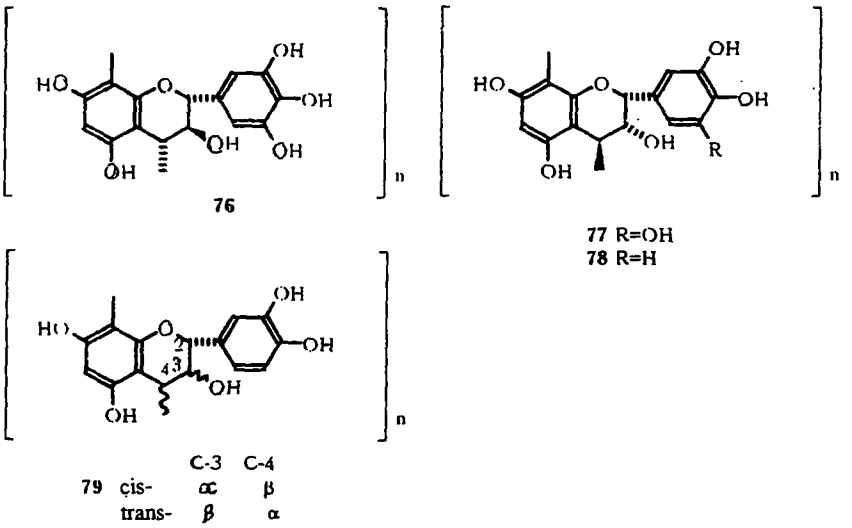


Fig. 8. Structures of polymeric proanthocyanidins.

blocks with the 2,3-*cis*- and 2,3-*trans*-configurations [61]. Quantitative measurements have also been made by other authors. The results of these investigations will be given below.

In the field of aromatic carbons, the pattern of distribution of the chemical shifts is close to that observed for low-molecular-mass proanthocyanidins. Signals of unsubstituted carbon atoms of ring *A* appear in the 95-98 ppm region. A carbon atom involved in the interflavan bond gives a signal in the region of 106-107.5 ppm. In all the polymeric proanthocyanidins for which we have found information on their ^{13}C NMR spectra the interflavan bond is of the C-4 — C-8 type, and, for the most part, the signal of the C-10 carbon has a chemical shift of 102-103 ppm [3, 59-61]. This phenomenon agrees well with the conclusion drawn in an analysis of the spectral characteristics of proanthocyanidin oligomers. We assume that the value of the C-10 chemical shift can be used to determine the type of interflavan bond both in oligomers and in polymers for proanthocyanidins with ring *A* having the phloroglucinol type of oxidation.

L. Y. Foo and L. J. Porter were the first to make quantitative measurements of the ratios of procyanidin (PC) and prodelphinidin (PD) blocks in polymeric proanthocyanidins with the aid of ^{13}C NMR spectroscopy [3, 62]. They used the integral intensities of the C-3′ signals in the ^{13}C NMR spectra. In the fragments under consideration, these nuclei have similar times of longitudinal (spin-lattice) relaxation (T_1) and values of the nuclear Overhauser effect (NOE), which permits direct integration to be used, and although the chemical shifts of these signals are fairly close — 143-145 ppm for PC and 146 ppm for PD — this method can be used on spectrometers with a high working frequency [3, 59, 60].

The signals are highly broadened in the ^{13}C NMR spectra of all polymers. This broadening is caused, in part, by the high viscosity of polymer solutions [54] and also by hindered rotation around chemical bonds. As we have already mentioned, in proanthocyanidins there is hindered rotation about bonds formed by sp^3 and sp^2 carbon atoms. Z. Czohanska et al. [3, 63] have proposed to calculate rotational energy as the mean value of the energies of rotation of each of the blocks, which permits an estimate of the mobility of the molecules in solution and the possibilities of their interaction with other substances.

$$E_{tot}=\Sigma N_i [E]_i,$$

where $[E]_i = [E]^T \times M_i/100$; $E = 174 \times N_i - 32 \times (1 - N_i)$; M_1 is the mass of a block; and N_i is the proportion of the block in the polymer. 174 and 32 are coefficients found by the method of least squares.

They also used the ratio of the integral intensities of the C-3 atoms of the "lower" terminal block and of the other units of the chain for determining the mean molecular mass of the polymer or, more accurately, its upper limit.

Thus, we have generalized the large amount of information that can be provided by NMR spectroscopy on the structure of the flavan-3-ols and proanthocyanidins, have noted its main advantages, and have revealed the main features of the spectral characteristics and the link between the spectral parameters and the structure of this biologically important class of natural compounds and possibilities in the study of features of their structure. Nuclear magnetic resonance spectroscopy is one of the leading methods in the investigation of the structure and stereochemistry of the flavan-3-ols and proanthocyanidins and in this

connection has concrete prospects when modern spectrometers with high sensitivity, high working frequency, and broad methodological possibilities are used.

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