

# <sup>13</sup>C-NMR of the Phenolic Compounds

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<sup>13</sup>C-NMR method is applied to determine the structure of phenolic compounds, since the chemical shifts show a systematic change when hydroxy groups are introduced in the aromatic system. In 1961 Lauterbur<sup>1)</sup> studied the shielding effect of substituents in monosubstituted benzenes such as phenol, methoxybenzene, aniline, toluene and so on. It was suggested that the shielding effect follows an additivity trend in para- and meta-positions, but not in ortho-position.

However, in our present study it is found that the phenolic compounds do not always follow the additivity trend as reported. Ternai and Markham<sup>2)</sup> systematically investigated the chemical shifts of flavonoid substances for first time, and found that <sup>13</sup>C-NMR data of acetophenones, cinnamic acids and some simple flavonoids obey the additivity principle. Therefore, this principle was used to interpretate the spectral data of more complicated flavonoids.

Weinges<sup>3)</sup> found out the position of the ether linkage in the acetylated catechin dimer. However, no attention was made to the substituent effects of acetoxy- or hydroxy groups.

In our present study <sup>13</sup>C-parameters such as chemical shift, nuclear overhauser effect (NOE) and signal shape are used to solve the structural problems of some highmolecular phenolics which are isolated from plants.

It was found that the chemical shift data of the simple phenolic compounds such as phenol, catechol, resorcinol, phloroglucinol, pyrogallol and hydroquinone follow the additivity rule<sup>4)</sup>.

A small enhancement of the <sup>13</sup>C-signal, which was observed by decoupling the three bond hydrogen coupling (<sup>3</sup>J c-c-c-H, <sup>3</sup>J c-c-o-H), was very useful for the precise assignment of the catechin signals and for determination of the bond position between the catechin units in catechin polymer.

Furthermore, the result of signal shape analysis could be used to determine the conformation of the catechin dimer and trimer.

## <sup>13</sup>C-NMR Spectra of the Simple Phenolics

Among the phenolic compounds mentioned earlier, only the spectra of the phenol and resorcinol will be here interpreted and the results of the rest are summarized in the Table I.

Levy and coworkers<sup>5)</sup> have calculated the electron density in phenol molecule by using CNDO/2 method. According to the nuclear shielding theory the electron density distribution plays essential role in the chemical shift of aromatic systems.

As shown in table II, the NMR data of phenol show that the shielding effect follows similar trend with the CNDO/2 values.

It is well known that the three bond coupling constant (<sup>3</sup>J c-c-c-H) in benzene molecule is greater than the two bond (<sup>2</sup>J c-c-H) and four bond coupling (<sup>4</sup>J c-c-c-c-H) constant.

Therefore, the three bond coupling constant, together with one bond coupling, is useful

**Table I.** Calculated and measured chemical shifts of phenolic compounds (ppm)

<b>I</b>	C-1 = 157.66(C-1 of I) - 12.34(o-E) = 145.32 (145.58)
	C-3 = 130.48(C-3 of I) - 12.34(o-E) = 118.14 (116.11)
	C-4 = 120.61(C-4 of I) + 1.98(m-E) = 122.59 (119.94)
<b>II</b>	C-1 = 145.58(C-1 of II) - 12.34(o-E) = 133.24 (132.66)
	C-2 = 145.58(C-2 of II) + 1.92(m-E) = 147.56 (145.05)
	C-3 = 116.11(C-3 of II) - 7.89(p-E) = 108.22 (106.88)
	C-4 = 119.94(C-4 of II) + 1.98(m-E) = 121.92 (118.44)
<b>IV</b>	C-1 = 157.66(C-1 of I) + 1.98(m-E) = 159.64 (159.11)
	C-2 = 116.21(C-2 of I) - 12.34(o-E) = 103.87 (103.35)
	C-3 = 130.48(C-3 of I) + 29.16( $\Delta$ -E) = 159.64 (159.11)
	C-4 = 120.61(C-4 of I) - 12.34(o-E) = 108.27 (107.38)
	C-6 = 116.21(C-6 of I) - 7.89(p-E) = 108.32 (107.38)
<b>V</b>	C-1 = 159.11(C-1 of IV) + 1.98(m-E) = 161.09 (158.86)
	C-2 = 103.35(C-2 of IV) - 7.98(p-E) = 95.46 (94.85)
	C-4 = 107.38(C-4 of IV) - 12.34(o-E) = 95.08 (94.85)
<b>VI</b>	C-1 = 157.66(C-1 of I) - 7.89(p-E) = 149.77 (150.66)
	C-2 = 116.21(C-2 of I) + 1.98(m-E) = 118.19 (116.64)

o-E = o-Effect

m-E = m-Effect

p-E = p-Effect

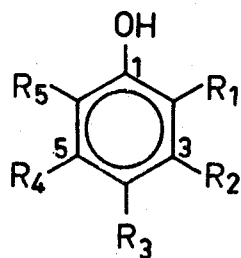
$\Delta$ -E =  $\Delta$ -Effect

The numbers in last parenthesis are measured values.

**Table II.** Effects of hydroxyl radical on the chemical shifts

Effects of OH	Chemical shifts of phenolic- <sup>13</sup> C(ppm)	Chemical shifts of benzene- <sup>13</sup> C(ppm)	Additivity(ppm)	CNDO/2-value
$\Delta$ -Effect*	157.66	128.50	+29.16	+176
o-Effect	116.21	128.50	-12.34	-55
m-Effect	130.48	128.50	+1.98	+31
p-Effect	120.61	128.50	-7.89	-41

\* Increment of HO-bonded carbon atom



R<sub>1-5</sub> = H Phenol (I)

R<sub>1</sub> = OH  
R<sub>2-5</sub> = H Catechol (II)

R<sub>1, R<sub>5</sub></sub> = OH  
R<sub>2-4</sub> = H Pyrogallol (III)

R<sub>2</sub> = OH  
R<sub>1, R<sub>3-5</sub></sub> = H Resorcinol (IV)

R<sub>2, 4</sub> = OH  
R<sub>1, R<sub>3, R<sub>5</sub></sub> = H Phloroglucinol (V)</sub>

R<sub>3</sub> = OH  
R<sub>1, 2, 4, 5</sub> = H Hydroquinone (VI)

**Fig. 1**

parameter in assigning the aromatic carbon atoms. With other word detailed observation of the three bond coupling constant provides supplementary information in the precise assignment of the aromatic carbon atoms.

For example the C-4 atom in phenol molecule couples with two equivalent protons in the

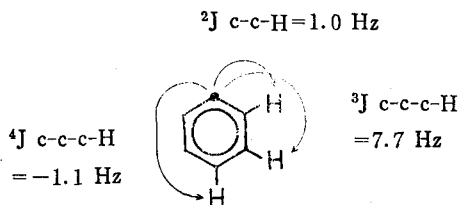


Fig. 2. Long range coupling of  ${}^{13}\text{C}$ -atom in benzene molecule

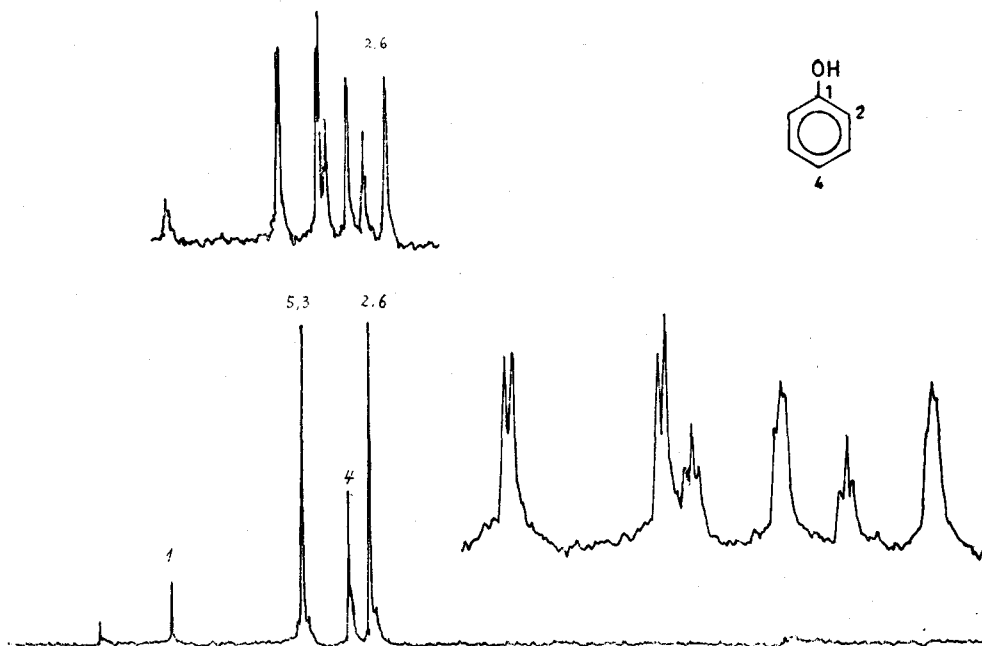


Fig. 3. Proton-coupled  ${}^{13}\text{C}$  spectra of phenol

three bond position, namely with protons on C-2 and C-6, so that it gives a symmetric triplet in  ${}^1\text{H}$ -coupled spectrum (C-4 atom: 120.61 ppm).

Since C-3(or C-5) has only one proton in the three  $\beta$  bond position a doublet appears at 130.48 ppm.

C-2(or C-6) which couples with two nonequivalent protons in the three bond position shows an unsymmetrical triplet.

The chemical shift data of phenol are used as starting reference in the calculation of the resorcinol chemical shifts.

The calculated values are found to be the

sum of the chemical shift of the corresponding phenol carbon atoms and its additivity constant.

As shown in fig. 4, one hydroxy group is introduced to the metaposition of the phenol molecule, making resorcinol molecule.

To ortho-, meta- and para-carbon atom from the new hydroxy group are added the corresponding additivity constants.

From this addition the calculated chemical shifts of resorcinol result.

The result shows an excellent agreement between the calculated and measured values in all positions.

The C-2 atom which stands in ortho-posi-

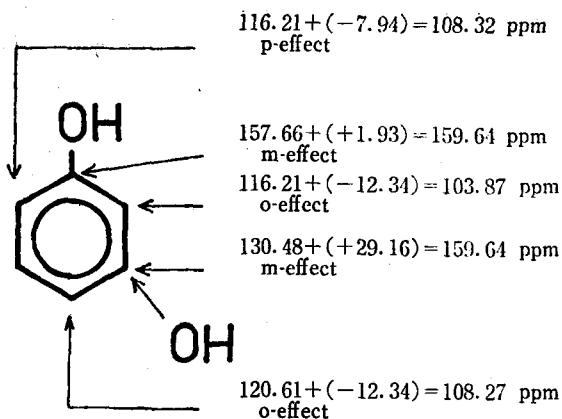


Fig. 4. Resorcinol from phenol and calculation of its chemical shifts

tion with respect to two hydroxy groups has twofold ortho-shielding effects [ $128.50(\text{benzene}) + 2 \times (-12.34) = 103.82 \text{ ppm}$ ]. As a result its peak appears in highest field.

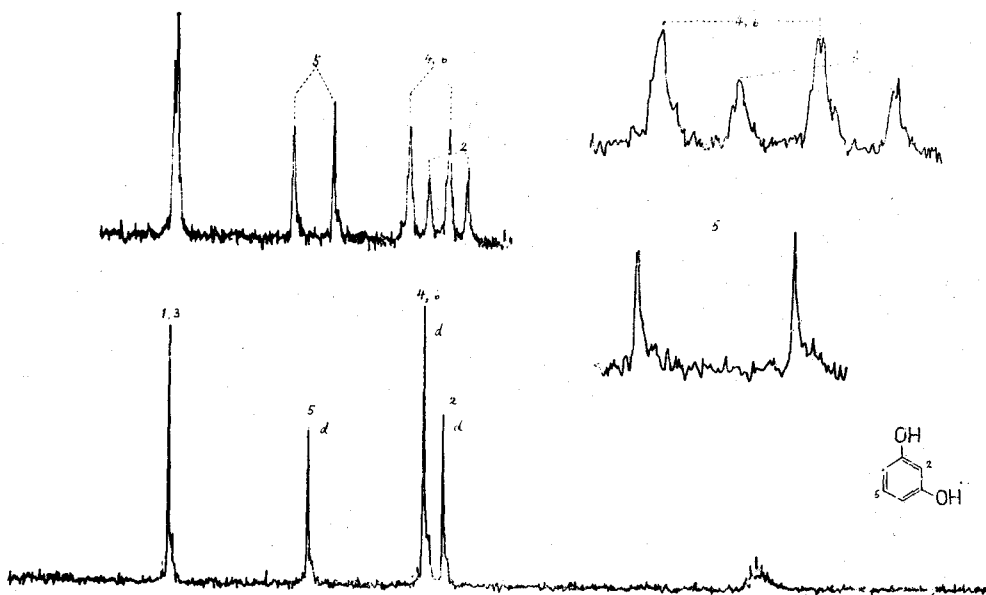


Fig. 5.  $^{13}\text{C}$ -NMR spectrum of resorcin

The same result is observed for the peak height of the C-1 and C-3, and of the C-4 and C-6. The latter carbon atoms have three protons for such a NOE.

The NOE caused by decoupling three bond coupling protons is much smaller than the one

The C-4 atom(or C-6) has ortho-and para-effects. Therefore, it appears in the second highest field.

The three bond coupling constants and the signal shape in the  $^1\text{H}$ -coupled spectrum can be used for assignment of the chemical shifts as well. Thus the doublet at  $140.66 \text{ ppm}$  corresponds to the C-5 atom, because it has no three bond coupling partner on the meta-position.

As described in the phenol spectrum, the symmetric and unsymmetric triplets correspond to C-2 and C-4(or C-6) respectively.

The C-2 atom has two three bond coupling protons, while the C-5 atom has no such protons. Therefore the NOE is greater on the C-2 atom than the C-5, so that the peak of the C-2 atom higher than the C-5 in the decoupled spectrum(Fig. 5).

bond case( $^1\text{J c-H}$ ), because the enhancement of the signal height of NOE is dependent on  $r^{-6}$ ( $r$ : distance between the coupling partners).

This idea was applied to analyse the spectra of the catechol, pyrogallol, phloroglucinol, hydroquinone, catechins and hydroxy-

coumarins<sup>4)</sup>.

From these results the chemical shift of the phenolic compounds can be grouped into three types:

- Catechol-type: C-1 and C-2 145 ppm  
C-3 and C-6 116-107 ppm  
Pyrogallol-type: C-1 132 ppm  
C-2 and C-6 145 ppm  
C-3 and C-5 106 ppm  
Resorcinol-type: C-1 and C-3 159 ppm  
C-2 103-95 ppm  
C-4 and C-6 107 ppm

### <sup>13</sup>C-NMR of Catechins

The results of the <sup>13</sup>C-NMR of the simple

phenolic compounds can be applied to analyse the catechin spectrum (Fig. 6).

(+)-Catechin molecule consists of two phenolic parts, one of which is phloroglucinol and the other catechol. Thus we can assign its chemical shift readily by using the chemical shift of phloroglucinol and catechol. Two signals at 93.83 and 95.11 ppm correspond to C-6 and C-8, three signals at 156.05, 155.23 and 156.33 ppm to C-5, C-7 and C-9, two signals at 114.42 and 115.03 ppm to C-2' and C-5', and the overlapped signal at 144.72 ppm to C-3' and C-4'. The other signals which are from alkylsubstituted aromatic carbon and alkyl-carbon, can be readily assigned by general method.

A precise localization of the chemical shift

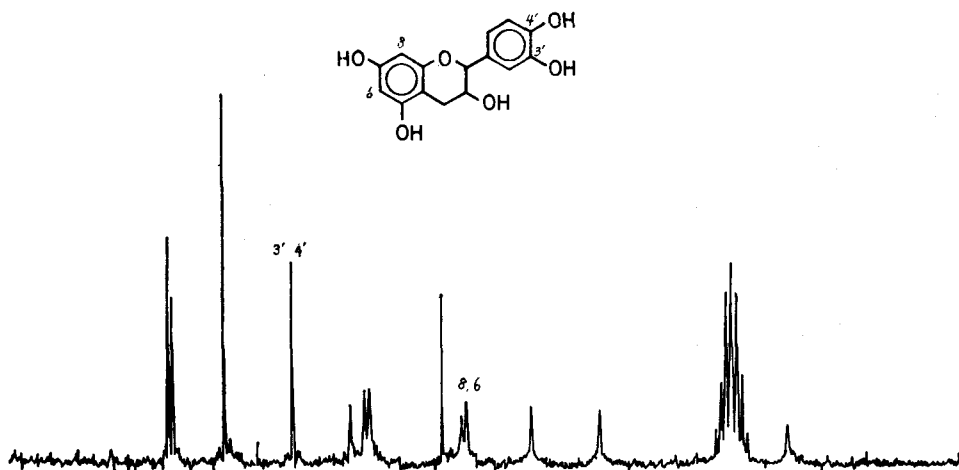


Fig. 6. <sup>13</sup>C-NMR spectrum of catechin

of C-6 and C-8 can be done by applying the three bond NOE. Since it is possible to assume that three bond coupling can be made through C-C-O-H system, the C-6 atom, which has two such systems, namely C<sub>6</sub>-C<sub>5</sub>-O-H and C<sub>6</sub>-C<sub>7</sub>-O-H, will show higher signal than the C-8, which possess only one such system, C<sub>8</sub>-C<sub>7</sub>-O-H.

The validity of this assumption can be proved when we compare the spectrum of tetramethyl-

(+)-catechin with the (+)-catechin spectrum.

In the former spectrum (Fig. 7) two peaks at 91.27 and 93.14 ppm, which correspond to the C-6 and C-8 of the (+)-catechin, have almost same signal height, because the possibility for the three bond coupling disappears in the methylated substance.

The NOE in this absorption region is also observed in the spectra of (-)-epicatechin and

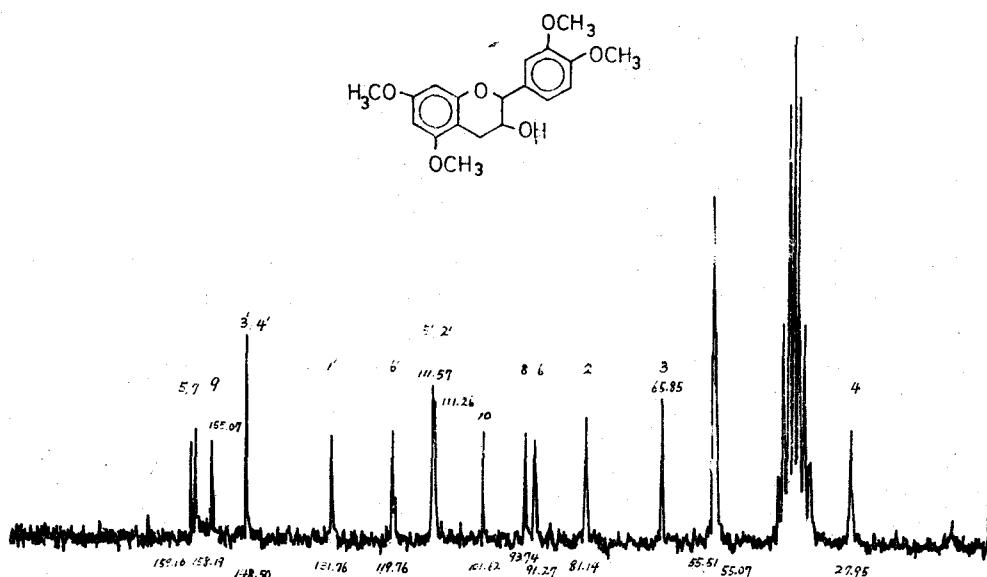


Fig. 7.  $^{13}\text{C}$ -NMR spectrum of tetramethyl catechin

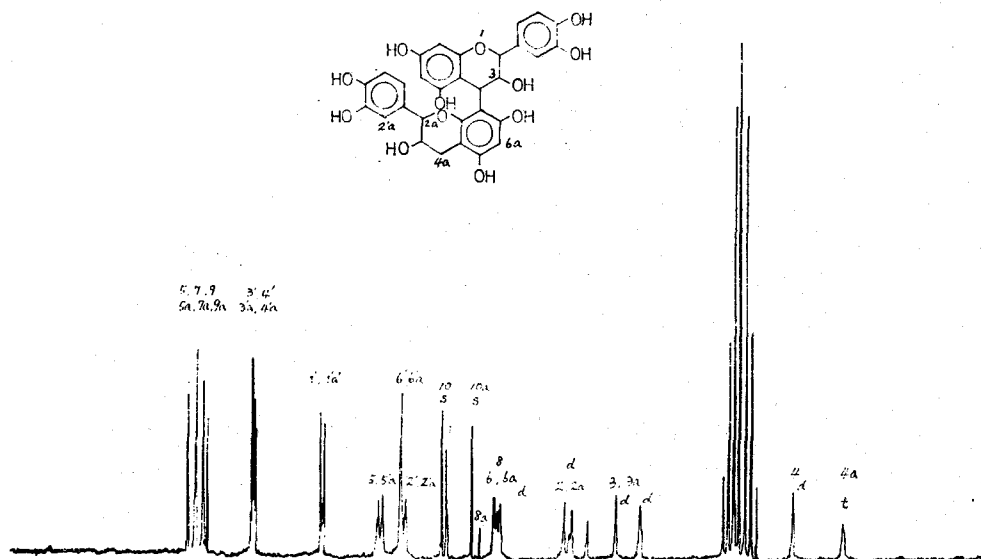


Fig. 8.  $^{13}\text{C}$ -NMR spectrum of catechin dimer

(+)-dihydroquercetin.

Therefore, it is concluded that the C-8 signal appears in the lower field with lower intensity than C-6 signal (C-8 : 95.11 and C-6 : 93.83 ppm).

This result is very useful in determining the bond positions of the catechin units in catechin

polymer.

### $^{13}\text{C}$ -NMR of Catechin Dimer

In our early study isolation and structure elucidation of several catechin dimers and trimers were reported<sup>6)</sup>.

One of the dimers, which is main tannin component in cortex *Quercus* and tormentillae root, consists of two (+)-catechin units.

Two catechin units are bonded through C-4 of the first catechin unit and C-6 or C-8 of the second unit.

In the phloroglucinol region of the dimer spectrum (Fig. 8), there appear three signals (97.43, 96.94 and 96.32 ppm), one of which has apparently lower intensity than other two.

According to the result of (+)-catechin analysis, the higher peaks must belong to C-6 and C-6a, and the lower one in the middle to C-8.

This unequality of the peak heights in the phloroglucinol region can not be observed in the spectrum of the peracetylated dimer, because the three carbon atoms have no chance of the

three bond NOE.

From this reason it is claimed that the bond position is between C-4 of the first catechin unit and C-8a of the second unit.

### <sup>13</sup>C-NMR of Catechin Trimer

The trimer was isolated from tormentillae root.

HCl-hydrolysis of the trimer gives a dimer, (+)-catechin, cyanidin chloride and some unidentified products (Fig. 8)

The dimer was identified as the same dimer as for mentioned. It is resulted, according to hydrolysis mechanism<sup>8)</sup>, from rupturing of the bond between the first and second catechin units, and the (+)-catechin from the third catechin unit.

The result of the hydrolysis justifies that the

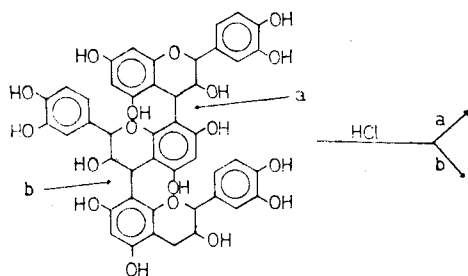


Fig. 9. HCl hydrolysis of catechin trimer

C-8 atom of the third catechin unit is the bonding position.

Only the bond position of the second catechin unit with the first unit still has to be determined.

The (+)-configuration of the first catechin unit was determined by means of <sup>1</sup>H-NMR.

There are 5 peaks in the phloroglucinol region of the trimer spectrum (102.14, 100.00, 98.53, 97.64 and 96.80) (Fig. 10).

Three of them (102.14, 100.00 and 98.53 ppm) appear in the lower field with lower intensity, and the other two in higher field

with higher intensity.

The two signal in lower field (102.14 and 100.00 ppm) belong to the bonded C-8a and C-8b, while the one in the middle (98.53 ppm) comes from the unbonded C-8 atom of the first catechin unit.

Applying the NOE the last two signals (97.64 and 96.80 ppm) with higher intensity in the region are from C-6, C-6a and C-6b.

As in the dimer it can be concluded that the bonding position of the second catechin unit in the trimer is C-8a.

From this result the structure of the trimer

is proposed as the structure in Fig. 10.

### The Conformation of the Catechin Polymer

The bonding position of the catechin units in the catechin dimer and trimer described so far gives us some clues for the understanding of the basic skeleton of the polymer structure.

However, an analysis of the conformation is required in order to determine the precise polymer structure.

The two catechol moieties in the catechin dimer may be positioned either in opposite or in the same side (trans-or cis-form). Meanwhile  $^1\text{H-NMR}$  found out that the second catechin unit in the dimer is equatorial on the C-4 of the first catechin unit, that is, the planes of two catechin units lie each other with an angle of about  $120^\circ$ <sup>7,8)</sup>.

Therefore, if the catechol moieties in such a

catechin polymer stand in the same side, every 6 catechin units form a cycle, and a spiral structure is formed by repeating such a cycle (Fig. 11).

But if the catechol moieties exist in opposite side, then the polymer has a planar-zigzag form (Fig. 11).

Such a spatial arrangement of a catechin polymer plays certainly an important role in its binding with the protein molecules.

The signal shape analysis of  $^{13}\text{C-NMR}$  of the polymer gives us some argumentation on the conformation.

In the spectrum of (+)-catechin the signal of C-3' and C-4' (144.72ppm) collapse into one peak (Fig. 6) while there are three peaks in the corresponding region of the dimer spectrum (145.93~145.31ppm), one sharp and two short ones. The sharp peak corresponds to a collapsed peak, which means that one catechol moiety in the dimer stands in same fashion as the

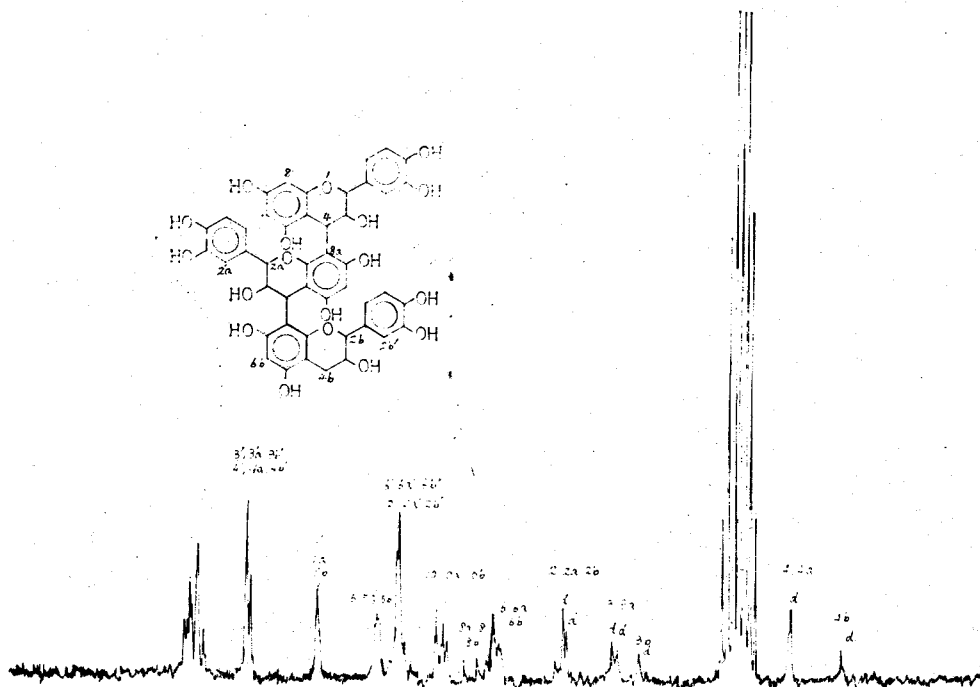


Fig. 10.  $^{13}\text{C-NMR}$  spectrum of catechin trimer



monomer catechin (Fig. 8).

The another catechin moiety in the dimer interacts with other structural moieties, thus this interaction gives two peaks in the slightly highly field.

If two catechol moieties stand in the same side, one expects a steric interaction between themselves, and thus a group of complicated peaks will appear in the corresponding region. Since such complicated peaks do not appear in the region (145.93–145.31ppm) of the dimer spectrum, the catechol moieties must stand in the opposite side.

In the catechin trimer spectrum the shape of the signals in the corresponding region (Fig. 10, 145.91 and 145.29ppm) is simple, one sharp and one short peaks, although 6 carbon atoms participate in producing two such signals.

The higher peak in the lower field (145.91 ppm) comes from the carbon atoms of two catechol moieties which have almost same steric

circumstance as the catechol moiety of the monomer catechin molecule.

The small signal at 145.29ppm belongs to another catechol moiety, which is sterically different from the aforementioned catechol moieties.

This means that two of the three catechol moieties lie in a similar steric situation.

This observation lead us to suggest that the catechin trimer looks as the structure in Fig.9.

Accordingly the molecular shape of the catechin trimer is rather a zigzag planar form than a spiral form (Fig. 11).

In order to verify NMR results we have tried to modify the trimer structure chemically, for example to prepare acetonide or an ester linkage with malonic and succinic dichlorides from the closely standing hydroxy groups.

Because of the unstability of the catechin polymer on chemical treatment, it is still not successful.

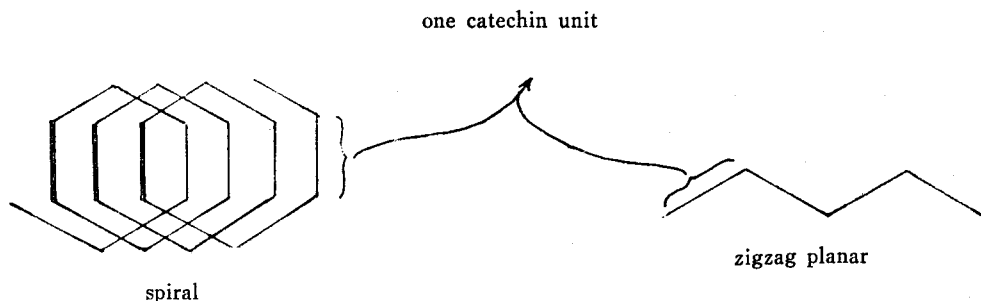


Fig. 11. Alternative shapes of (+)-catechin polymer

### Summary

- 1) The hydroxy-substitution in the simple phenolic compounds follows an additivity rule in the chemical shifts of their aromatic carbon atoms.

In para-and ortho-effects there is a good agreement between calculated and measured

values, but the meta-effect is not certain.

- 2) The additivity rule was applied to assign the chemical shifts of catechins.
- 3) The nuclear overhauser effect was applied to assign the chemical shifts of C-8 and C-6 atoms of catechins and their polymer.

The signal of C-8 is lower in intensity and appear in lower field than C-6.

- 4) The results of the NOE were applied to

determine the bonding positions of catechin units in the catechin dimer and trimer.

The bonding positions are C-8a and C-8b atoms of the second and third catechin units.

- 5) It was tried to determine the conformation of the catechin dimer and trimer by analyzing the signal shapes of C-3' and C-4' atoms in the catechol moieties. The catechol moieties lie in opposite side in the dimer and trimer structure.

A combined analysis of  $^{13}\text{C}$ - and  $^1\text{H}$ -NMR results lead to the suggestion that such a catechin polymer is a zigzag planar form.

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