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Structural Studies on Inclusion Compounds of β-Cyclodextrin with Some Substituted Phenols

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Abstract. A detailed structural study of the inclusion compounds of some substituted phenols such as catechol, guaiacol, protocatechuic aldehyde, vanillin, caffeic acid, ferulic acid and eugenol with β-cyclodextrin (βCD) was carried out by using UV-visible, fluorescence, ^{1}H and solid-state ^{13}C NMR spectroscopic and potentiometric investigations. Based on these studies guaiacol, catechol and eugenol were found to exhibit identical orientations – with the phenyl ring within the cavity and the hydroxyl and methoxyl groups projecting outside; protocatechuic aldehyde, caffeic acid, ferulic acid and vanillin display a different orientation – with the phenol part within the cavity and the aldehyde or carboxyl part projecting outside.

Key words: Substituted phenols, polar and non-polar ends, orientation inside β-cyclodextrin cavity, spectroscopic studies.

1. Introduction

Due to the specific orientation of the guest molecules inside the cyclodextrin cavity selective positional directed attack of the reagent can be favoured resulting in regio- and stereoselective reactions [1,2]. It has been reported that the proportion of *para*-substituted product was high in the presence of βCD when phenol was subjected to hydroxymethylation [3,4] and formylation reactions [5,6] where the phenyl ring was proposed to be present inside the βCD cavity and O^- projecting outwards at the wider end. In order to explain the observed regioselectivity in Reimer-Tiemann, hydroxymethylation and methylation reactions of guaiacol, catechol and 2,4-dihydroxybenzaldehyde respectively [7–9], knowledge of the orientation of the substituted phenol is necessary. The phenols chosen for the structural studies were catechol, guaiacol, protocatechuic aldehyde, vanillin, caffeic acid, ferulic acid and eugenol which possess either hydroxyl or methoxy groups *ortho* to the phenolic hydroxyl while some of them also possess substituents at the *para* position (Figure 1). The orientation of these phenols inside the βCD cavity was examined by detailed spectroscopic, (UV-visible, fluorescence, ^{1}H and solid-state ^{13}C NMR) and potentiometric studies.

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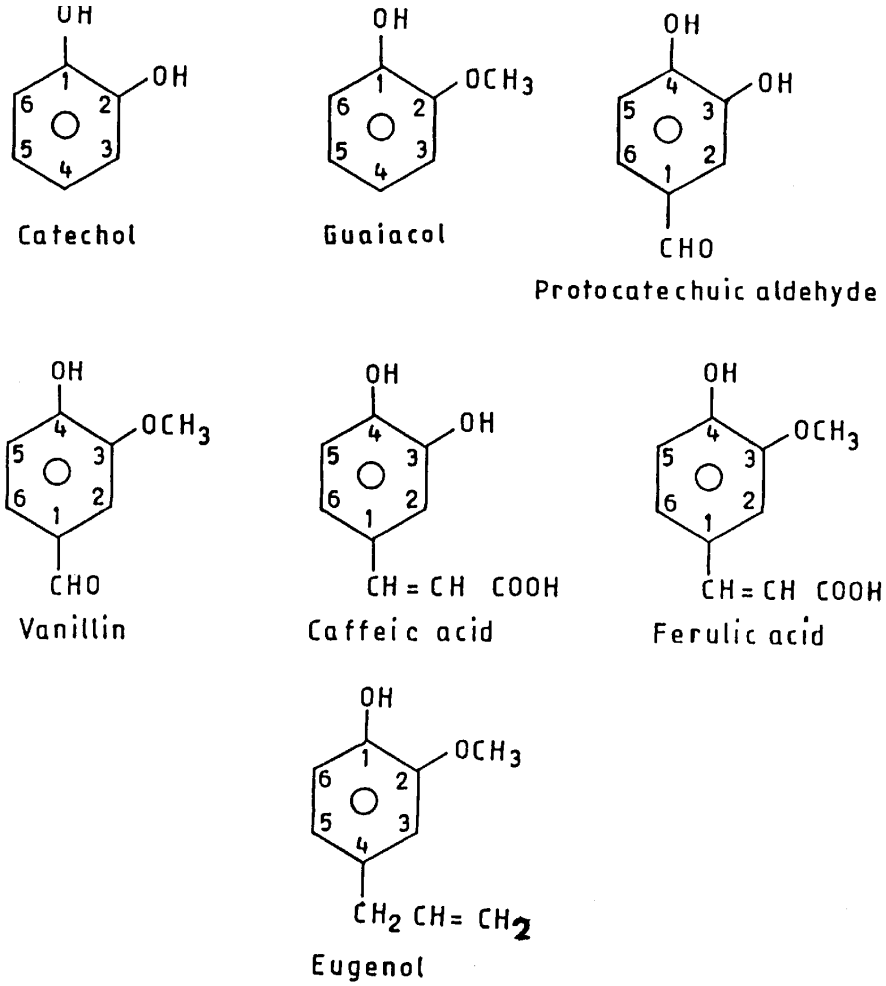


Figure 1. Structures of the phenols studied.

2. Experimental

2.1. MATERIALS

βCD was obtained from Aldrich Chemical Company, Inc. USA and was used as such. Catechol obtained from Loba Chemie Indoaustranal Co Ltd., Ind, was used after recrystallisation from toluene. Guaiacol was prepared from catechol by methylation using dimethyl sulphate according to the procedure by Bredereck and Hennig [10]. Protocatechuic aldehyde and 6-*p*-toluidinylnaphthalene-1-sulfonic acid (TNS) obtained from Sigma Chemical Company, USA and caffeic acid, ferulic acid and eugenol from Sisco Chemicals Ltd., Ind, were used after recrystallisation or distillation. Vanillin was obtained from Monsantó Chemicals Ltd., London.

2.2. UV-VISIBLE SPECTROSCOPIC STUDIES

UV-visible spectroscopic studies between 600–200 nm were carried out using a Varian Superscan 3 spectrophotometer. The other details are described in Ref. 8. Binding constant values were calculated by the method of Farmoso [11]

$$\frac{1}{\Delta A} = \frac{1}{[\Delta A_{\mathrm{AB}}]K[\beta\mathrm{CD}]} + \frac{1}{[\Delta A_{\mathrm{AB}}]}$$

where ΔA is the observed change in absorbance, $[\Delta A_{\mathrm{AB}}]$ is the difference in absorbance between the free and complexed states, $[\beta\mathrm{CD}]$ is the total βCD concentration and K is the binding constant value obtained from the ratio of intercept to slope from a plot $1/\Delta A$ vs. $1/[\beta\mathrm{CD}]$.

2.3. FLUORESCENCE SPECTROSCOPIC STUDIES

An Aminco-Bowman spectrophotofluorometer was used. Solutions used were similar to those employed for UV studies. To a known volume of the fluorescent probe (TNS), βCD was used in increasing amounts until the maximum fluorescence intensity was attained. The guest compound was then gradually added and the decrease in fluorescence intensity was determined. Excitation was at 364 nm and emission at 464 nm. The values after correcting for dilution were employed for determining binding constant values by the method of Farmoso [11]. Micromolar concentration levels of phenols and TNS and millimolar levels of βCD were employed in these studies like those employed in UV-visible spectroscopic measurements (refer to Figures 2 and 3).

Potentiometric studies were carried out using a Control Dynamics pH meter fitted with an Ingold combination electrode as described in Ref. 8.

2.4. NMR SPECTROSCOPIC STUDIES

^{1}H NMR spectra were recorded on Bruker WH 270 and AMX 400 NMR instruments fitted with a Spectrospin magnet operating at 270 and 400 MHz respectively and at 20 °C and an Aspect 2000 computer. About 200 scans were collected for each spectrum. βCD in DMSO-d_6 (0.6 M) was added in gradual amounts to a solution of the guest molecule in DMSO-d_6 (0.1 M) and spectra were recorded. ^{1}H NMR spectra were also recordeed in D_2O. ^{13}C CP/MAS NMR spectra were recorded at 75 MHz on a Bruker MSL-300 NMR spectrometer operating at the same temperature. About 500 mg of complex sample was packed in the rotor and spun at 3–3.5 KHz. A Hartmam-Hahn contact time of 1 ms was employed with a total recycle time of 6 sec. Typically 7500 scans were measured and all signals were referenced relative to glycine at 42.1 ppm to within $\pm$ 0.2 ppm. A 1:1 complex for solid-state ^{13} C-NMR was prepared by adding equimolar amounts of protocatechuic aldehyde to βCD in water followed by concentration and filtration.

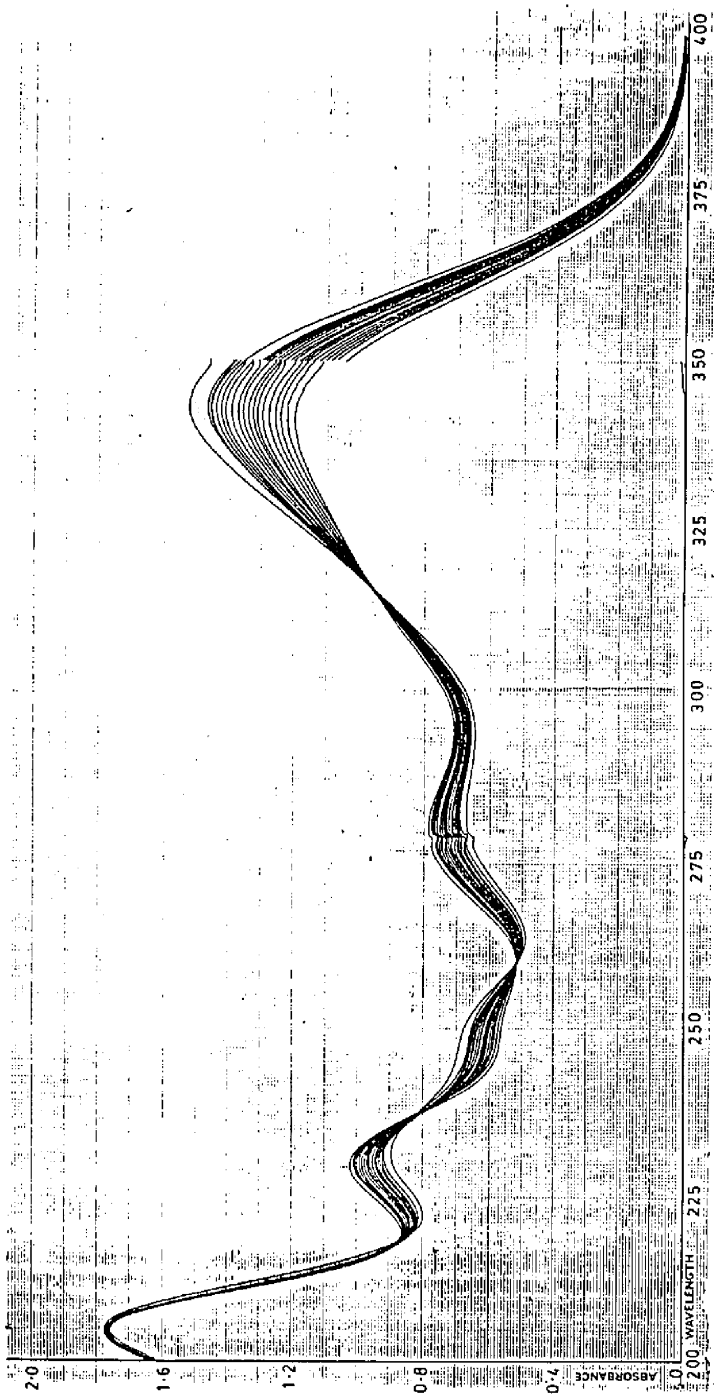


Figure 2. UV-visible spectra showing the effect of gradual addition of βCD to protocatechuic aldehyde in water. [Protocatechuic aldehyde] = 1.16×10^{-4} M; [βCD] = 0–3.02×10^{-4} M. A jump in the spectra due to the change in the light source at 350 nm and a dent at 278 nm are instrumental artifacts.

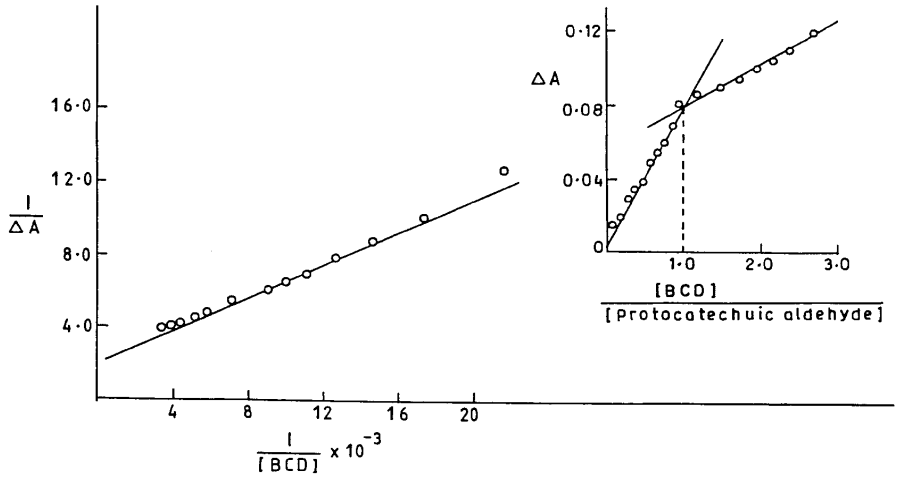


Figure 3. Double reciprocal plot for the calculation of the binding constant value of the protocatechuic aldehyde–βCD complex from UV-visible spectroscopic studies in 0.1M $NaHCO_3$ buffer at pH = 10.5. [Protocatechuic aldehyde] = 7.21×10^{-4} M; A 1.82×10^{-3} M stock solution of βCD was prepared from which additions to protocatechuic aldehyde were made. *Inset*: Plot of the ratio of βCD to protocatechuic aldehyde concentration vs. change in absorbance at 346 nm.

3. Results and Discussion

3.1. UV-VISIBLE SPECTROSCOPY

UV-visible spectroscopy is an important tool to study the complexation of substituted phenols with βCD and the phenols studied include protocatechuic aldehyde, catechol, guaiacol and caffeic acid. In water, protocatechuic aldehyde gave three λ_{max} values at 346 nm ($n - \pi^*$ transition of the carbonyl group), 276 nm ($\pi - \pi^*$ transition of the phenolic group) and 229nm ($\pi - \pi^*$ transition of the phenyl ring) with well defined isosbestic points at 315.5 nm, 259.5 nm and 237 nm, when addition of βCD to protocatechuic aldehyde was studied (Figure 2). During the addition of βCD, the absorbance at 346 nm was found to increase in intensity whereas those at 276 nm and 229 nm were found to decrease along with a red shift for the 229 nm band (246 nm). The binding constant values were calculated by monitoring the change in absorbance at λ_{max} (Table I) with a typical plot shown in Figure 3. In alkaline medium protocatechuic aldehyde showed hyperchromic absorption at λ_{max} 346 nm and 248 nm with βCD along with isosbestic points at 364 nm, 318 nm, 258 nm and 235 nm. Catechol showed two absorption maxima in alkaline medium, a hyperchromic one at 280 nm ($\pi - \pi^*$ transition of the phenolic group) and a hypochromic one at 222 nm ($\pi - \pi^*$ transition of the phenyl ring) on adding βCD with a well defined isosbestic point at 248 nm. In water, catechol did not show any change in the spectra when βCD was added. Caffeic acid showed three absorptions at λ_{max} values of 312.5 nm ($\pi - \pi^*$ transition of the double bond,

Table I. UV-visible spectroscopic studies of the complexation of substituted phenols with β-CD.

Phenol	Water			$NaHCO_3$ buffer (pH 10.5)		
	λ_{max} nm	Binding constant value M^{-1}	βCD: phenol	λ_{max} nm	Binding constant value M^{-1}	βCD: phenol
Catechol	–	NC[b]	–	280,222	1665[a] ± 192	1 : 1
Protocatecuic aldehyde	346 276 229	6700[a] ± 777	1 : 1	346,248	5100 ± 586	1 : 1
Caffeic acid	312.5 285 214.5	516 ± 59.3	1 : 1		NC	–
Guaiacol		NC	–	–	NC	–

[a] Average binding constant values using wavelengths indicated.
[b] Not much change in UV spectra.

hyperchromic), 285 nm ($\pi - \pi^*$ transition of phenolic group, hypochromic) and 214.5 ($\pi - \pi^*$ transition of the phenyl ring, hypochromic) with isosbestic points at 291 nm, 254 nm and 238 nm. The magnitude of the change was found to be less indicating that the binding is weak. In alkaline medium caffeic acid did not show any change in the spectra. Guaiacol did not show any change in the spectra both in ethanol-water (ethanol was used due to insolubility in water at neutral pH) mixture as well as sodium bicarbonate buffer at pH 10.5.

The existence of isosbestic points indicate that two chemically different species namely free and complexed states are present in the system, the composition of which is defined by a single parameter, namely absorbance [12] and λ indicating the affected groups in the vicinity of the UV absorbing chromophore. The stoichiometry of the complex was found to be 1 : 1 in all cases (Figure 3, inset).

In all these cases, inclusion of phenols results in transfer from a polar to a non-polar environment as the interior of βCD is apolar. Depending on the groups included the corresponding absorbances are affected. A decrease of polarity increases the intensity of the $n - \pi^*$ transition but decreases the intensity of the $\pi - \pi^*$ transition [13]. While protocatechuic aldehyde and caffeic acid exhibit the above mentioned trend catechol shows an increase in the intensity of the $\pi - \pi^*$ transition indicating that the phenolic hydroxyl groups may be projecting outwards towards the solvent. Hence, based on the same argument, the phenolic hydroxyl groups of protocatechuic aldehyde should be present inside the βCD cavity with the aldehyde groups partly projecting outwards.

3.2. Fluorescence Spectroscopy

Cyclodextrins (α and β) are known to enhance the fluorescence of 8-anilinonaphthalene–1-sulfonic acid (ANS) and TNS when included within the cyclodextrin

cavity [14]. The increase in fluorescence is due to the change in polarity due to inclusion within an apolar environment which stabilises the excited state of the fluorophore [15]. In the presence of competitive guest molecules displacement of ANS and TNS from the cavity results in a decrease in the fluorescence as the fluorophore is transferred from a non-polar to a polar environment.

While addition of protocatechuic aldehyde and caffeic acid to TNS-βCD (1 : 16) showed a very rapid decrease in the TNS fluorescence intensity both in water and at pH 10.5, catechol showed a decrease in intensity only in water. Guaiacol did not exhibit any change in ethanol : water and bicarbonate buffer at pH 10.5. Quantitative determination of the binding constant values using the fluorescence data based on the method described above gave values different from those measured from UV studies. However, the fluorescence data showed that phenols displaced TNS from the βCD cavity.

3.3. POTENTIOMETRY

The pK values in free and complexed states of the different substituted phenols are shown in Table II. With the exception of protocatechuic aldehyde which exhibited an increase in pK values for the phenolic hydroxyl, all the other substituted phenols studied, namely, guaiacol, caffeic acid, ferulic acid, eugenol and vanillin showed a decrease in pK values for the same group. Catechol did not exhibit any change in pK value. The carboxyl groups of ferulic and caffeic acids showed higher pK values similar to that observed for carboxylic acids in the presence of βCD [2]. Similarly the pK values of phenols in the presence of cyclodextrin were also found to be lesser or equal to those in the absence of cyclodextrins [2]. Specific disposition of ionisable groups of the guest molecule results in increase or decrease of their pK values. An ionisable phenolic group in an apolar environment as in protocatechuic aldehyde results in lesser dissociation and hence an increase in pK value. Orientation of the phenolic hydroxyl away from the cavity projecting outwards results in facile dissociation facilitated by hydrogen bonding of the phenoxide oxygen to the secondary hydroxyl groups of βCD and hence a lower pK value than in the free state. However, in ferulic and caffeic acids the carboxyl groups are probably hydrogen bonded to the secondary hydroxyl which prevents degree of dissociation of the same resulting in a higher pK value than in the free state. However, the hyperchromicity observed at 312.5 nm for caffeic acid indicates that the trans double bond is not completely buried in the cavity in which case there would have been hypochromicity instead of hyperchromicity. While the phenyl rings containing the OH groups may be present inside the cavity (lowering of pK values and hypochromicity of absorption) the trans double bond may be partly present inside the cavity, the carboxyl groups may be projecting outwards through the wider end and hydrogen bonded to the secondary hydroxyl on the rim (higher pK and hyperchromicity).

Table II. Potentiometric studies of some substituted phenols in the presence of βCD.

Phenol	pK values[a]	
	Free	Phenol + βCD
Protocatechuic aldehyde	7.29	7.80
Catechol	9.40	9.40
Guaiacol	10.28	10.02
Caffeic acid		
COOH	4.70	4.95
OH	9.46	9.28
Ferulic acid		
COOH	4.96	5.15
OH	9.68	9.52
Eugenol	10.47	10.32
Vanillin	7.66	7.43

[a] Error in pK value is $\pm$ 0.1.

3.4. NMR SPECTROSCOPY

NMR studies further confirmed the orientations inferred from the above mentioned studies. DMSO-d_6 and D_2O were used as the solvent for NMR measurements.

^{1}H-NMR spectra of catechol and protocatechuic aldehyde in the free state and in the presence of βCD in D_2O are shown in Figures 4 and 5. NMR data for the inclusion complexes of guaiacol, catechol and protocatechuic aldehyde are shown in Table III (D_2O). In all the cases (in DMSO-d_6) signals from ionisable protons, namely, hydroxyl and carboxyl protons were found to broaden out completely as the βCD concentration was increased. The chemical shift values of aromatic protons in general were not much affected in all the phenols studied. However, the signals broadened as evidenced from the observed reduced splitting.

In DMSO-d_6 the 2-OH and 3-OH protons of βCD from the wider end and 6-OH from the narrower end showed slight upfield (0.02–0.05 ppm) and downfield shifts (0.03–0.07 ppm) respectively. The splitting of H–1, found as a doublet at lower concentrations of βCD, was lost at higher concentrations. Other βCD protons such as H–2, H–3, H–4, H–5 and H–6a,b also were not much affected. The observed effects on the OH groups indicated that complexation does affect the rims of the wider and narrower ends much more than the interior of βCD. While the observed upfield shifts may be due to a ring current effect, the downfield shifts may be due to hydrogen bonding interaction.

^{1}H-NMR spectra in D_2O were more informative (Table III). Protocatechuic aldehyde H–2 and H–5 protons showed 0.06 ppm and 0.04 ppm downfield shifts respectively. The aldehyde protons showed a 0.08 ppm downfield shift. Among the βCD protons, H–3, H–5 and H–1 showed 0.06, 0.13 and 0.01 ppm upfield

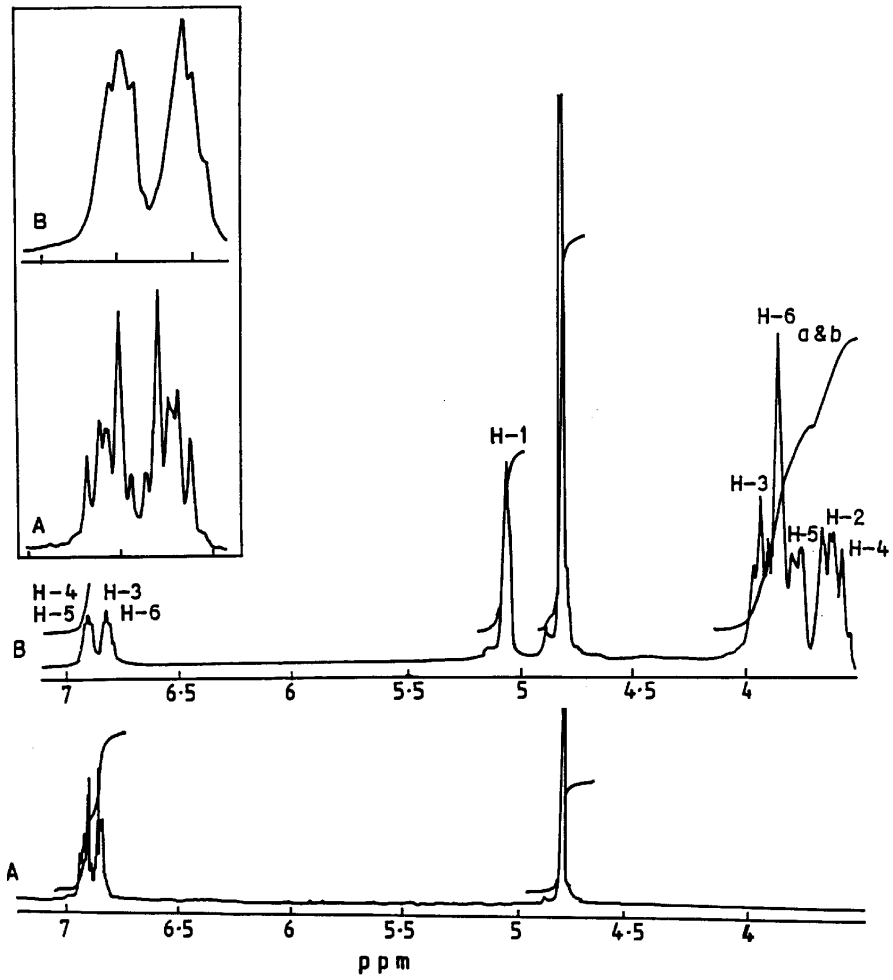


Figure 4. ^{1}H-NMR spectra of catechol (A) and its 1 : 1 complex with βCD(B) in D_2O at 270 MHz. [Catechol] = 0.016 M. A molar equivalent of βCD was added. Inset: Expanded region of aromatic portion; A-free and B-complex.

shifts and H–2 showed a 0.04 ppm downfield shift respectively. Catechol protons showed upfield shifts: H–4 and H–5 showing 0.02 ppm and H–3 and H–6 showing 0.04 ppm respectively. βCD H–3 and H–5 showed 0.02 and 0.09 ppm upfield shifts respectively. Other protons were not much affected. A similar trend was also observed in the case of guaiacol [8].

Solid-state ^{13}C-NMR spectra of the protocatechuic aldehyde-βCD complex are shown in Figure 6 and the data are given in Table IV. The assignment of the βCD chemical shift values were based on those of Veregin *et al.* [17]. While the carbon atom bearing the phenolic hydroxyl groups C–4 and C–3 showed a 1 ppm downfield shift, C–5 and C–2 which are next to the OH groups showed the

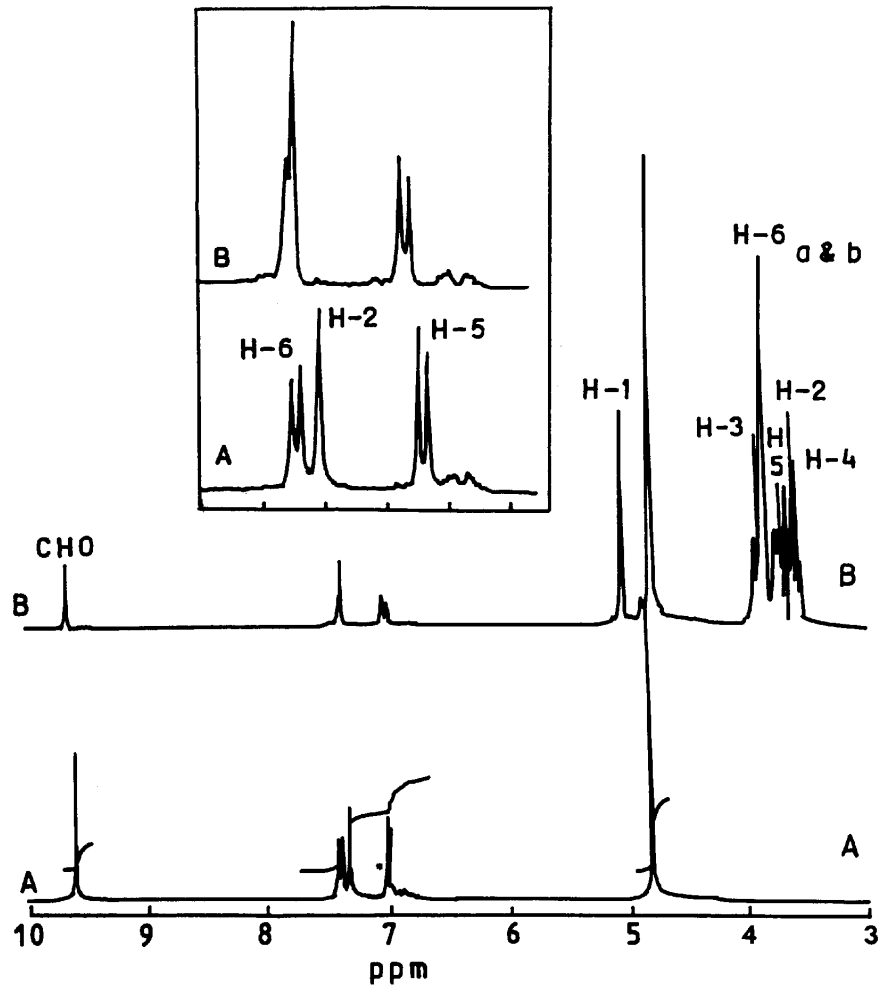


Figure 5. ^{1}H-NMR spectra of protocatechuic aldehyde (A) and its 1 : 1 complex with βCD (B) in D_2O at 270 MHz. [Protocatechuic aldehyde] = 0.016 M. A molar equivalent of βCD was added. Inset: Expanded region of aromatic portion 6.85–7.45 ppm. A-free and B-complex.

maximum (5.0 ppm) downfield shift and C–1 and C–6 adjacent to the aldehyde carbon showed a 4.0 ppm upfield shift. The aldehyde carbon signal was found to broaden out completely. The carbons next to OH groups, namely C–5 and C–2, are most affected (downfield shifts) with C–1 and C–6 affected in an opposite manner (upfield shifts) to the former indicating that the phenolic OH and aldehyde are in different environments. All the βCD carbons showed downfield shifts with maximum effects for C–1 and C–4, the carbons which constitute the apolar region of the βCD cavity. In addition all the signals from βCD especially C–1, C–2, C–3 C–5 and C–6, show changes in the splitting pattern when protocatechuic aldehyde

Table III. ^{1}H-NMR chemical shift values[a] of the substituted phenols in the free state and in the presence of βCD in D_2O.[b]

Signal	Chemical shift values		Signal	Chemical shift values		Signal	Chemical shift values	
	Free	Phenol + βCD		Free	Phenol + βCD		Free	Phenol + βCD
Protocatechuic aldehyde			Catechol			Guaiacol		
CHO	9.59	9.67	H–4	6.92	6.90	H–5	7.03	6.97
			H–5					
H–6	7.39	7.40	H–3	6.84	6.80	H–3	6.94	6.90
	(J = 8.2)		H–6			H–4		
						H–6		
H–2	7.32	7.38						
H–5	6.98	7.02				OCH_3	3.84	3.83
	(J = 8.1)	(J = 8.5)						
βCD								
H–1	5.05	5.04			5.05			5.04
	(J = 4.6)	(J = 3.2)			(J = 0)			
H–2	3.59	3.63			3.63			3.62
	(J = 10.02, 3.5)	(J = 10.02, 3.5)			(J = 10.03, 3.2)			
H–3	3.95	3.89			3.93			3.91
	(J = 9.5)	(J = 9.6)			(J = 9.0)			
H–4	3.57	3.56			3.56			3.61
	(J = 9.3)	(J = 9.4)			(J = 9.1)			
H–5	3.85	3.72			3.76			3.77
					(J = 9.0)			
H–6a,b	3.87	3.83			3.84			3.87

[a] ^{1}H NMR recorded at 270 MHz. J in Hz.
[b] Values are for the 1 : 1 complex; Error in chemical shift value $\pm$ 0.01 ppm.

is included within the βCD cavity, probably due to a ring current effect of the phenyl ring on the βCD carbons.

Based on the above mentioned studies it can be concluded that in the case of catechol, guaiacol and eugenol the orientation of the phenolic hydroxyl should be outwards with the phenyl portion present inside the cavity (Figure 7). The double bond portion of eugenol, is present in the apolar region. The structures in these cases are very similar to the p-nitrophenol–βCD complex [18]. In protocatechuic aldehyde while the phenolic hydroxyl groups are present in the apolar cavity the aldehyde group partly projects outwards. A similar orientation is also possible for vanillin also although the presence of the OCH_3 group in the latter causes a slight variation in the disposition of the phenolic group due to steric interaction of the OCH_3 group, the C1-C4 axis of vanillin may be present at an angle to the perpendicular axis of the cavity [19]. This may explain the observed decrease in pK of the phenolic OH for vanillin in contrast to the increase in pK observed for

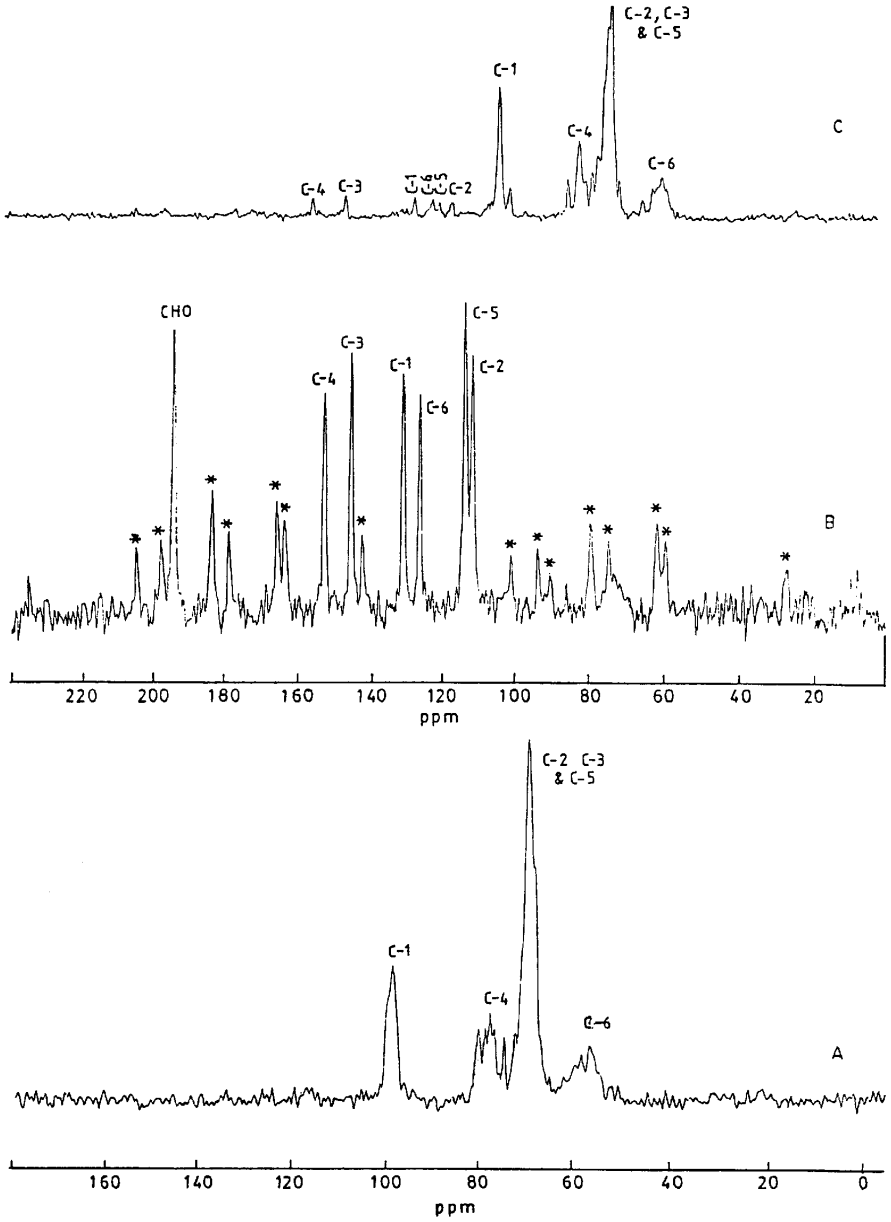


Figure 6. Solid-state ^{13}C-NMR spectra of A-βCD, B-protocatechuic aldehyde and C- 1 : 1 complex of protocatechuic aldehyde and βCD at 75 MHz. The source of the peaks marked with asterisk is not known.

the OH groups of protocatechuic aldehyde. In the case of ferulic and caffeic acids while the phenyl ring with the OH and OCH_3 groups is present inside the cavity, the trans double bond may be partly present inside the cavity with the carboxyl

Table IV. Solid-state ^{13}C NMR data of the protocatechnic aldehyde in the free state and in the presence of βCD.[a,b]

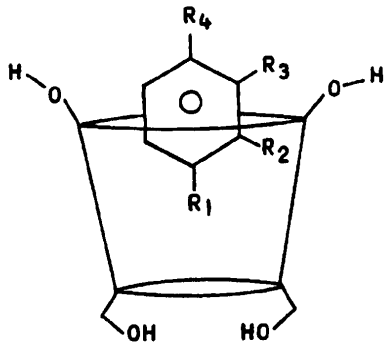
Signal	^{13}C-NMR	
	Free ppm	Phenol + βCD ppm
Protocatechuic aldehyde		
C-1	131.1	127.2
C-2	111.3	115.5
C-3	144.6	145.1
C-4	152.3	153.9
C-5	113.2	119.4
C-6	125.3	121.4
βCD		
C-1	98.5	102.9
C-2	70.0	72.1
C-2, C-5		
C-4	77.0	81.1
C-6	56.3	59.2

[a] ^{13}C-NMR recorded at 75 MHz.
[b] The values are for the 1 : 1 complex.

group projecting outwards hydrogen bonded to the secondary hydroxyl groups of βCD.

In all these cases, the relative polarity of the ends decide which end of the guest molecule goes in, the preference being for the non-polar end to be included inside the cavity. In protocatechuic aldehyde and vanillin, the aldehyde end is polar and the OH and OCH_3 end is non-polar. In eugenol guaiacol and catechol, the phenyl end is less polar than that containing OH and OCH_3. In caffeic and ferulic acids the phenyl end containing OH and OCH_3 groups is apolar and the carboxyl portion is polar.

These structures can explain the observed increase in para substituted products when guaiacol, catechol and 2,4-dihydroxybenzaldehyde were subjected to Reimer-Tiemann, hydroxymethylation and methylation reactions [9]. The attacking species-dichlorocarbene or formaldehyde or methyl iodide which also get included in the cavity from the narrower end finds the para position more susceptible for attack. While the Reimer-Tiemann reaction of guaiacol gave vanillin (*para* to OH) as the major product, hydroxymethylation gave isovanillyl alcohol (*para* to OCH_3) as the major product and methylation gave 2-hydroxy-4-methoxybenzaldehyde as the major product. Hence it is probable that in guaiacol two orientations are possible, one in which the OH is nearer to the βCD secondary hydroxyl and another in which the OCH_3 is nearer to the βCD secondary hydroxyl.



i) $R_1 = R_2 = H$; $R_3 = R_4 = OH$ Catechol
ii) $R_1 = R_2 = H$; $R_3 = OCH_3$, $R_4 = OH$ Guaiacol
iii) $R_4 = CH = CH-COOH$, $R_3 = H$, $R_1 = OH$ $R_2 = OH$ Caffeic acid
iv) $R_4 = CH = CH-COOH$, $R_3 = H$, $R_2 = OCH_3$, $R_1 = OH$ Ferulic acid
v) $R_1 = CH_2-CH = CH_2$, $R_2 = H$, $R_3 = OCH_3$, $R_4 = OH$ Eugenol
vi) $R_1 = R_2 = OH$, $R_3 = H$, $R_4 = CHO$ Protocatechuic aldehyde
vii) $R_1 = OH$, $R_2 = OCH_3$, $R_3 = H$, $R_4 = CHO$ Vanillin

Figure 7. Disposition of substituted phenols within the βCD cavity.

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