NUCLEAR MAGNETIC RESONANCE STUDY OF COMPLEXES OF CYCLODEXTRINS WITH SOME AROMATIC COMPOUNDS

by

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A Thesis submitted in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE
(Pharmacy)

at the

UNIVERSITY OF WISCONSIN
(1977)

Acknowledgements

I wish to express my gratitude to my wife, Susie, for her support and understanding during the course of this study.

I also wish to express my gratitude to Professor Kenneth A. Connors for his guidance and suggestions in this investigation.

This study was supported by National Science Foundation Grant GP-36567.

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I. Introduction

A. Nature of Cyclodextrins

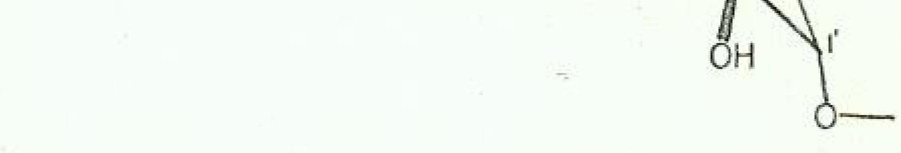
The cyclodextrins (also called cycloamyloses and Schardinger dextrins) are a series of homologous oligosaccharides that are formed from the breakdown of starch by the action of Bacillus macerans amylase. α-, β- and γ-cyclodextrins are respectively composed of six, seven and eight D-glucosepyranose residues linked at α-(1,4) in a macrocyclic form. The molecules are doughnut shaped and their cavities of fixed size and shape have led to considerable interest in their chemical properties (1,2,3). Table I lists some of the properties of the cyclodextrins and structures 1 and 2 give the structures of α- and β-cyclodextrins. The production, purification and chemistry of the cyclodextrins have been reviewed (4,5). The conformations of the cyclodextrins have been established with nuclear magnetic resonance (6,7,8), X-ray (9) and rotary dispersion (10) to be of C1 chair conformation for the glucose units. Structure 3 shows the conformation of two glucose units in a cyclodextrin.

Cyclodextrins thus have primary and secondary hydroxy groups crowning opposite ends of its torus with

Table I

Some properties of Cyclodextrins (20)

Cyclodextrin	Number of glucose residues	Water solubility (g/100cc)	Cavity dimension (Å)	
			Diameter	Depth
Cyclohexaamylose (α-cyclodextrin)	6	14.5	4.5	6.7
Cycloheptaamylose (β-cyclodextrin)	7	1.85	7.0	7.0
Cyclooctaamylose (γ-cyclodextrin)	8	23.2	8.5	7.0



Structure 3. Two glucose units of cyclodextrin

H-3, H-5 and H-6 directed towards its interior and H-1, H-2 and H-4 located on its exterior. The interior of the cavity is mainly composed of the symmetrically distributed bridging acetal oxygen atoms, flanked by four hydrogen atoms in a square array, with their lone orbitals orthogonal to the cylindrical axis of the molecule. Thus the interior of the cavity contains a region of high electron density (12) and might behave like a Lewis base (13). The secondary groups interact with one another by means of hydrogen bonds involving the C-3 hydroxyl of one glucose residue and the C-2 oxygen of an adjacent residue as shown in Structure 3. The notion that the cavity is hydrophobic in the classic sense of association, characterized by a favorable entropy of association, has been refuted on the grounds of spectroscopic (14) and thermodynamic evidences (15). Nevertheless, the cyclodextrins are hydrophobic in the sense that their solubilities are enhanced by the addition of small amounts of relatively nonpolar solvents such as ethanol (15). The solvation of the interior of the cavity by water is not ideal and the enthalpy initially required to create the solvent cavity for the cyclodextrin molecules cannot be fully regained through solvent-solvent interaction. In this sense, the cyclodextrin cavity can be considered enthalpy rich. The inclusion process can be viewed as a mutually favorable association of a relatively nonpolar substrate with an imperfectly solvated "hydrophobic" cavity. The driving force of the inclusion process is thought to be due to a favorable enthalpy change, with the transfer of the relatively nonpolar substrate from an aqueous environment to a "hydrophobic" cyclodextrin cavity. There are other speculations on different attractive forces like hydrogen bonding, van der Waal forces, dipole-dipole interaction and inductive effects in order to explain the association. However, these evidences are not conclusive. Cramer (16) considers covalent bonding to be important for the association when the cyclodextrin functionsas an assymetric enzymatic catalyst.

Cyclodextrinshave been found to form complexes with a wide variety of "guest" chemicals, effectively precipitating some and enhancing the solubilities of others. The "guest" molecules range from the highly polar reagents such as potassium acetate, aliphatic and aromatic carboxylic acids or amines to the highly nonpolar aliphatic and aromatic hydrocarbons and rare gases. In practically all cases, a correlation between the sizes of the "guests" and the cyclodextrin cavities can be established except where the molecular chain has become long enough to curl. The combining ratiosof the host to guest are often 1:1 in solution for short chain molecules and higher than 1:1 in crystalline form. Any ratio higher than 1:1 is thought to be due to the stack-effect of the cyclodextrins to form a channel (6,7,8).

Evidence suggesting inclusion of substrates in cyclodextrin cavities in solution has been derived from nuclear magnetic resonance measurements (18) in which the chemical shifts of the interior hydrogens were seen to move by small amounts. To date the best evidence supporting the theory of inclusion complexation of cyclodextrins with aromatic compoundswas given by Harata and Uredaira (33), who performed an X-ray determination of the p-iodoaniline-α-cyclodextrin complex structure. They found that the aromatic compound was fitted tightly into the cavity, with the iodine and the aromatic ring inside and the amine projecting outside.

Apart from inclusion, it is also speculated (20) that for polar substrates, the binding force of the complex can be due to intermolecular hydrogen bonding from outside the cavity or channel in addition to the inclusion binding. A large number of compounds have been screened for complexation with cyclodextrins. For example, Cramer and co-workers (21) have measured the rate constants and equilibrium constants for the association of p-nitrophenol and a series of azo dyes with α-cyclodextrin, while Lach and his co-workers (22,23,24) looked into the solubilities of a variety of pharmaceuticals in the presence of cyclodextrins. Demarco and

Thakkar (18) measured the chemical shifts of α-cyclodextrin complexed with a series of substituted benzoic acids and phenols.

Two methods of determining the association constant have been commonly used by most workers. One is the solubility method, in which plots of the solubility of the substrates against the concentration of added cyclodextrins were usually linear and these slopes are used to calculate the association constant, K_{11} (5). The other method is the spectrometric method using the Benesi and Hildebrand equation(25). With either method, the association constants were found in the order of over 10^2, indicating strong association between the "guests" and host. Another interesting feature of the cyclodextrin is their ability to induce stereospecific precipitation. Optical purities as high as 84% for chiral sulfoxides have been attained (26). Recently, much attention has been given to cyclodextrin as a model for enzyme catalysis (20). A wide variety of cyclodextrin-induced rate accelerations and decelerations imposed by the cyclodextrins have been revealed and many of these can be explained quite adequately. For example, the reaction of derivatives of carboxylic acids and organophosphate compounds with cyclodextrin proceed to form covalent intermediates. Other types of reaction appear to be influenced by dielectric properties of the microscopic cyclodextrin cavity. Still other reactions can be affected by geometrical requirements of the inclusion process. In all cases, catalysis by the cyclodextrins displays specificity with respect to both the structure of the substrate and the sizes of the cyclodextrin cavities.

B. NMR Study of Complexes

The observed chemical shift (δ) for a given nucleus in a molecule in solution is not the same as the shift in the gaseous phase (δ_{gas}). The difference, sometimes called the solvent screening constant ($\delta_{solvent}$), is due in part to contributions from the bulk diamagnetic susceptibility difference of the solution and reference samples (δ_b), the solvent magnetic anisotropy (δ_a) and weak dispersion (van der Waal) interaction between solute and solvent molecules (δ_w). A polar effect (δ_E) is also experienced; this is generally interpreted in terms of a reaction field; i.e. the result of a secondary electric field in the solvent owing to its polarization by the permanent dipole of the solute. Any specific complex formation in solution will also make its contribution (δ_s) to the observed chemical shift. In general, we may therefore write:

$$\delta_{solvent} = \delta_{gas} - \delta = \delta_b + \delta_w + \delta_a + \delta_E + \delta_s$$

The appearance of the magnetic resonance spectrum of a nucleus which can exist in more than one chemical environment is dependent on its lifetime in these different environments. Let us take the simple case of a nucleus which can exist in two environments, I and II, in either of which alone it appears as a singlet. If the lifetime in the two states are long compared with $\sqrt{2}(\pi|\delta_I - \delta_{II}|H)^{-1}$ (where $|\delta_I - \delta_{II}|H$ is the frequency difference in Hz between the two absorptions at δ_I and δ_{II} if each appeared singly) then two lines are observed. If the lifetimes are short compared with $\sqrt{2}(\pi|\delta_I - \delta_{II}|H)^{-1}$, then only a singlet, time-averaged line is observed. In the latter case, the position of the line (δ) is dependent on the relative populations, P_I and P_{II}, in the two environments:

$$\delta = P_I\delta_I + P_{II}\delta_{II} \qquad ....(1)$$

For a nucleus rapidly exchanging between n different environments, a corresponding relationship holds. In the case of weak complex formation, under conditions for which equation (1) is valid, the environment I could represent an unassociated molecule and environment II the same molecule in a complexed state. In general, any change in the system which will cause an alteration in the relative populations of P_I and P_{II} will affect the observed time-averaged chemical shift (δ). Such changes will include concentration changes, temperature variations and alterations in the nature of the second component of the complex.

C.NMR Study of Cyclodextrins and their Complexes

Owing to the complexity of their spectra , very few nuclear magnetic resonance studies of cyclodextrin complexes have been carried out, and in most cases, it was the chemical shifts of the cyclodextrins rather than their "guest" molecules that were scrutinized. The earlier work in nuclear magnetic resonance (27,28) suggested that cyclodextrins are of C1 chair conformation, but it was Takeo and Kuge (22) who confirmed such conformation with the use of peracetylated cyclodextrins. Demarco and Thakkar (18,19) used magnetic resonance method to investigate the cavity of cyclodextrins. They argued that, with aromatic substrates, if inclusion occurred within or near the cavity, H-3, H-5 or H-6 will be strongly shielded, whereas if the association took place on the exterior of the torus then H-1, H-2 and H-4 will be more strongly shielded. It turned out that association of β-cyclodextrin with a series of benzoic acid derivatives, phenols and barbiturates produced change in chemical shifts ($\Delta\delta$) of appreciable value (~0.2 ppm) for H-5. This evidence suggested that the substrates were included in the torus of cyclodextrins. However, none of the references quoted above pays any attention to the chemical shifts of the substrates. It is expected that the complexed substrates, being in a different environment than the solvent, would have a different chemical shift than those in their free form in the solution.

Recently, MacNicol (34) reported in a short note that cyclodextrin could be used as an NMR shift reagent for hydrocarbons. After p-cymene had been heated in a solution of α-cyclodextrin, its spectrum was taken at 50 °C and it was found that the singlet of the aromatic protons split into four peaks covering a range of 20 Hz. When adamantane was observed in a α-cyclodextrin at 80 °C, a singlet (methine protons) and a triplet (methylene protons) were obtained, whereas in other solvents adamantane gives only a poorly resolved spectrum.

D.Plan of Study

In this study, attention was focused on the chemical shifts of some aromatic compounds in the presence of cyclodextrins. As indicated earlier, chemical shift is a function of the environment of the proton being observed. It was presumed that an included compound, being in a different environment, would exhibit a change in its chemical shifts. It is known that a change in solvent often results in a change in chemical shift for the solute. Aromatic solvents such as pyridine can cause a "solvent shift" of the order of 0.5 ppm when compared with a carbon tetrachloride solvent (31). It has also been reported (32) that a hydroxy group on a steroid can cause a change in chemical shift of a neighboring proton by about 0.17 ppm.

Since this was a preliminary study, the majority of the chemicals were screened without detailed study. A couple of these substrates were examined more closely in order to relate the change of the chemical shift of the substrates to the change in the concentration of cyclodextrin. For the other compounds studied, only the differences of their chemical shifts in the presence and absence of cyclodextrins were observed.

II. Experimental

A. Materials

All compounds were used as received except as noted*. Phenol was obtained from Mallinckrodt. Benzyl alcohol, ethyl benzoate, phenyl ethyl alcohol, nitrophenols, _trans_-cinnamic acid (recrystallized) and _p_-benzaldehyde were obtained from Baker; methyl benzoate was obtained from Matheson Coleman and Bell; _p_-dioxane, α-cyclodextrin, β-cyclodextrin and deuterium oxide (99,98%) were obtained from Aldrich; benzaldehyde and acetophenone were obtained from Fred Portz and Son.

B. Instrument

Nuclear magnetic resonance measurements were performed on a Bruker Model HX 90-E spectrometer interfaced with a Nicolet 1080 data system for use with Fourier Transform. Between 50 and 250 scans were made for each sample depending on its concentration. The

* Because of the sensitivity limitations of NMR, exceptionally high purity of substrates is not required, hence the best commercially available grade is generally satisfactory for direct use.

spectrum was locked to the D_2O signal. The temperature of the probe was about 30 °C.

C. Procedure

The samples were prepared by shaking 2 µl of a liquid substrate in 1 ml D_2O in a 1 ml volumetric flask. For each compound tested, two samples were prepared. To one of the samples was added about 0.04 g of α-cyclodextrin. Solid substrates were prepared by dissolving excess of the chemical in D_2O to make a saturated solution. Then α-cyclodextrin was added as described. All the substrates were slightly soluble in water. For samples where precipitation occurred, Millipore® filtration was used to obtain a solid-free sample for filling into the NMR tubes. In many cases, precipitation occurred on standing in the NMR tubes even after filtration. The samples were run and recorded nevertheless. No apparent effect due to the precipitation was observed.

For fear of interference to the complexation under study, an NMR reference compound was not used. Hence, in all measurements the HDO peak was used as the internal reference peak. A comparison between the chemical shift of p-dioxane in D_2O in the presence and absence of α-cyclodextrin showed only a difference of ~1.5 Hz, which is within instrumental error. This result was considered an indication that HDO peak was stable enough as a reference for qualitative work where $\Delta\delta$ is in the range of 10-35 Hz.

Since the ppm unit was awkward to use for such a small shift, the unit Hz was used in all cases. For this instrument, a shift of 90 Hz corresponds to 1 ppm. Chemical shifts that are downfield from the HDO peak are given positive signs. Those that are upfield from the HDO peak are considered negative.

III. RESULTS

Figure 1 shows a typical spectrum of benzoic acid in α-cyclodextrin solution. The doublet at about 330 Hz represents the *ortho* protons and the quintet at about 270 Hz is from the *para* and *meta* protons, which overlap one another and are impossible to distinguish from one another. The single peak at 0.0 Hz is the HDO peak with its spinning side bands at about ±29 Hz. The complex structure further up field to the HDO peak is from the protons of the α-cyclodextrin. For this particular spectrum, 250 scans were made to accumulate the data.

All the compounds observed exhibit some change in chemical shift ($\Delta\delta$) in the presence of α-cyclodextrin. $\Delta\delta$ ranged between 3-4 Hz for benzene and toluene up to 35 Hz for benzoic acid.

To check the reproducibility of the instrument, four samples of benzoic acid of identical concentrations were observed. The chemical shifts of the various signals are given in Table II. Peak assignments are indicated in Figure 1. The standard deviation of the chemical shifts ranges between 0.200 and 0.315 Hz. With the reasonable adoption of a confidence interval of mean ±3 standard deviations for δ, then a change in shift $\Delta\delta$ of greater than ±1.8 Hz (6x0.3 Hz) is considered significant.

Table II. Observed chemical shifts of the aromatic protons of benzoic acid for four spectra

	δ (Hz)			
	ortho		para	
Peaks	A	B	C	D
	298.339	290.039	256.836	249.267
	298.584	290.528	257.081	249.756
	298.339	290.039	256.836	249.511
	298.095	290.039	256.591	249.023
Mean	298.339	290.161	256.591	249.389
Standard deviation	0.200	0.245	0.200	0.315

Observed $\Delta\delta$ could be due to a "real" shift of the signal of the compound being observed, and/or to a shift of the reference peak. Since it is not possible to fix the chemical shift of the HDO peak, it is not known what the real $\Delta\delta$ value was for the compounds observed. However, it was observed that different proton signals of a given substrate shifted by different amounts, so it follows that there was some "real" shift for the signals of the substrate.

To measure the medium effect of the systems, glucose solution was used to simulate the α-cyclodextrin solution. Dextrose was weighed out to the same weight as the cyclodextrin in the other samples. A comparison between the benzoic acid signals in glucose and in cyclodextrin showed only a difference of 0.3 Hz. Thus, the medium effect was considered insignificant in these experiments.

Figure 2 shows the change in chemical shift of the aromatic protons of benzoic acid in different concentrations of α-cyclodextrin. A graphical representation of these shifts is given in Figure 3. It is seen that $\Delta\delta$'s of the <u>ortho</u> protons are larger than those of the <u>para</u> and <u>meta</u> protons and the curves start to level off at 0.02 <u>M</u> of α-cyclodextrin.

Figure 4 shows the spectra of benzaldehyde in the absence (a) and presence (b) of α-cyclodextrin and (c) in the presence of β-cyclodextrin. In (b) it is noted that two doublets are formed from the doublet of the <u>ortho</u> protons in (a). In the presence of β-cyclodextrin, only one doublet is obtained for the <u>ortho</u> protons. However, the peaks for the <u>meta</u> and <u>para</u> protons are better resolved.

Table III lists all the compounds studied in this investigation with their respective $\Delta\delta$'s in a solution of ~0.04 <u>M</u> α-cyclodextrin. Assignments of peaks were made according to the spectra in "Stardard NMR Spectra" by the Sadtler Research Laboratory (1975).

Figure 1. A typical spectrum of benzoic acid in α -cyclodextrin solution

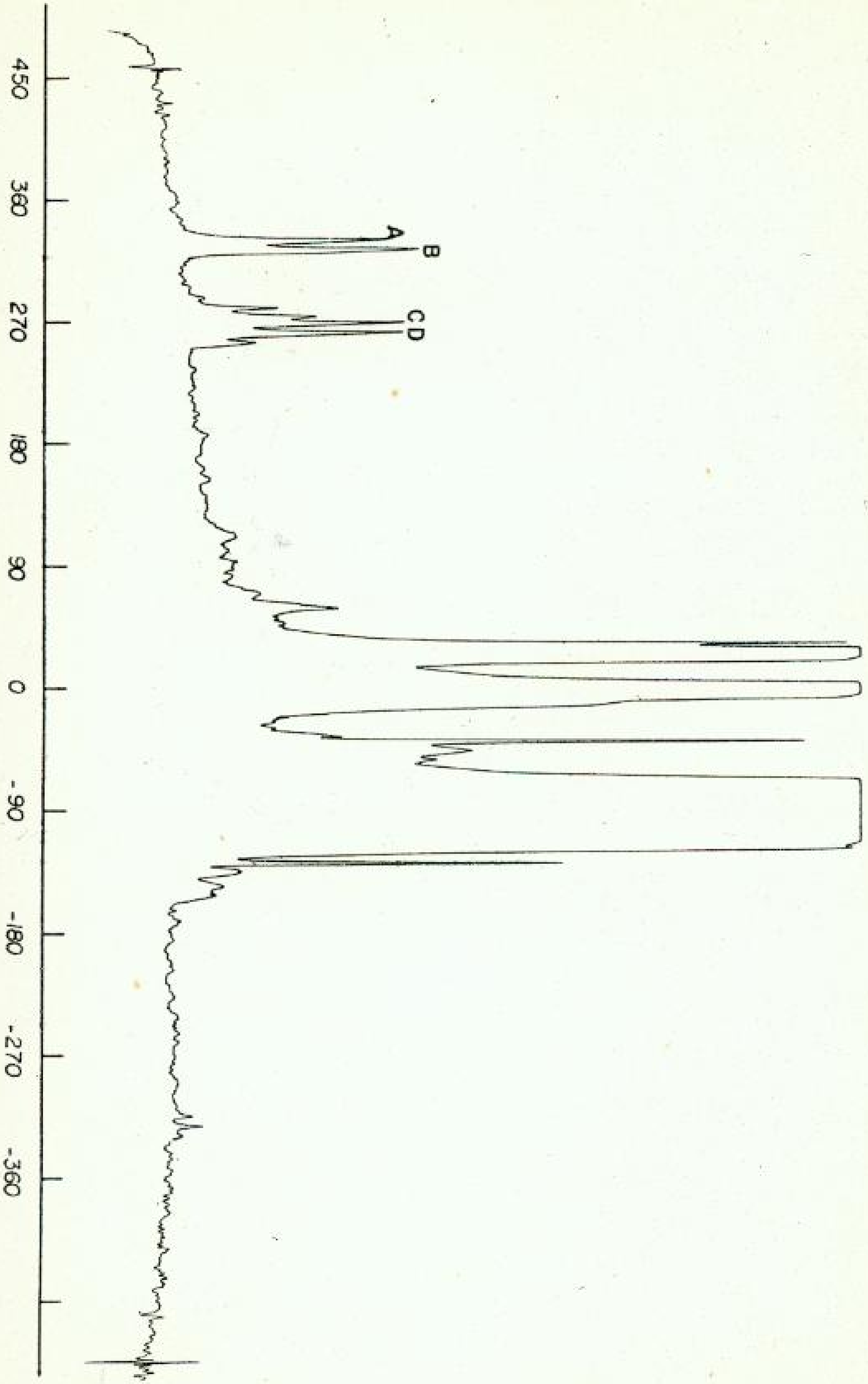
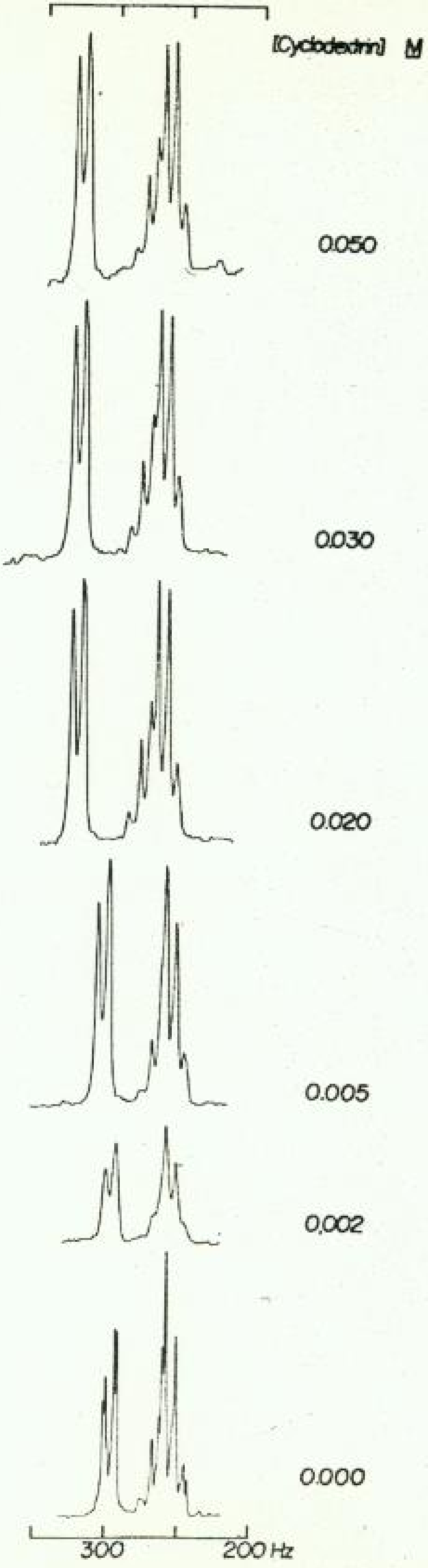

450
360
270
180
90
0
-90
-180
-270
-360
(Hz)
A
B
C
D

Figure 2. Spectra of benzoic acid in different concentrations of α-cyclodextrin


[Cyclodextrin] M
0.050
0.030
0.020
0.005
0,002
0.000
300
200 Hz

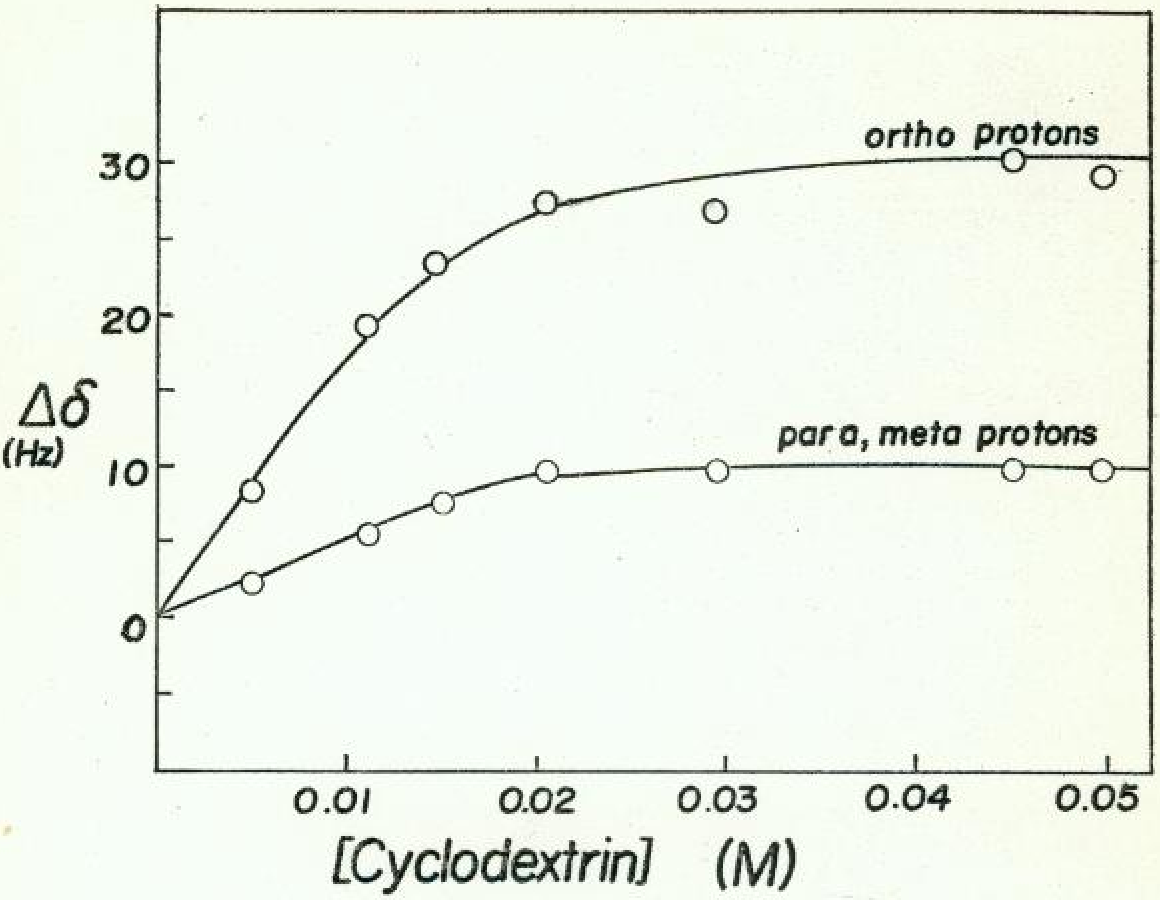


Figure 3. Dependence of Δδ of the aromatic protons of benzoic acid on the total concentration of α-cyclodextrin

Figure 4. Spectra of benzaldehyde in D_2O
(a) without cyclodextrin
(b) with α-cyclodextrin
(c) with β-cyclodextrin

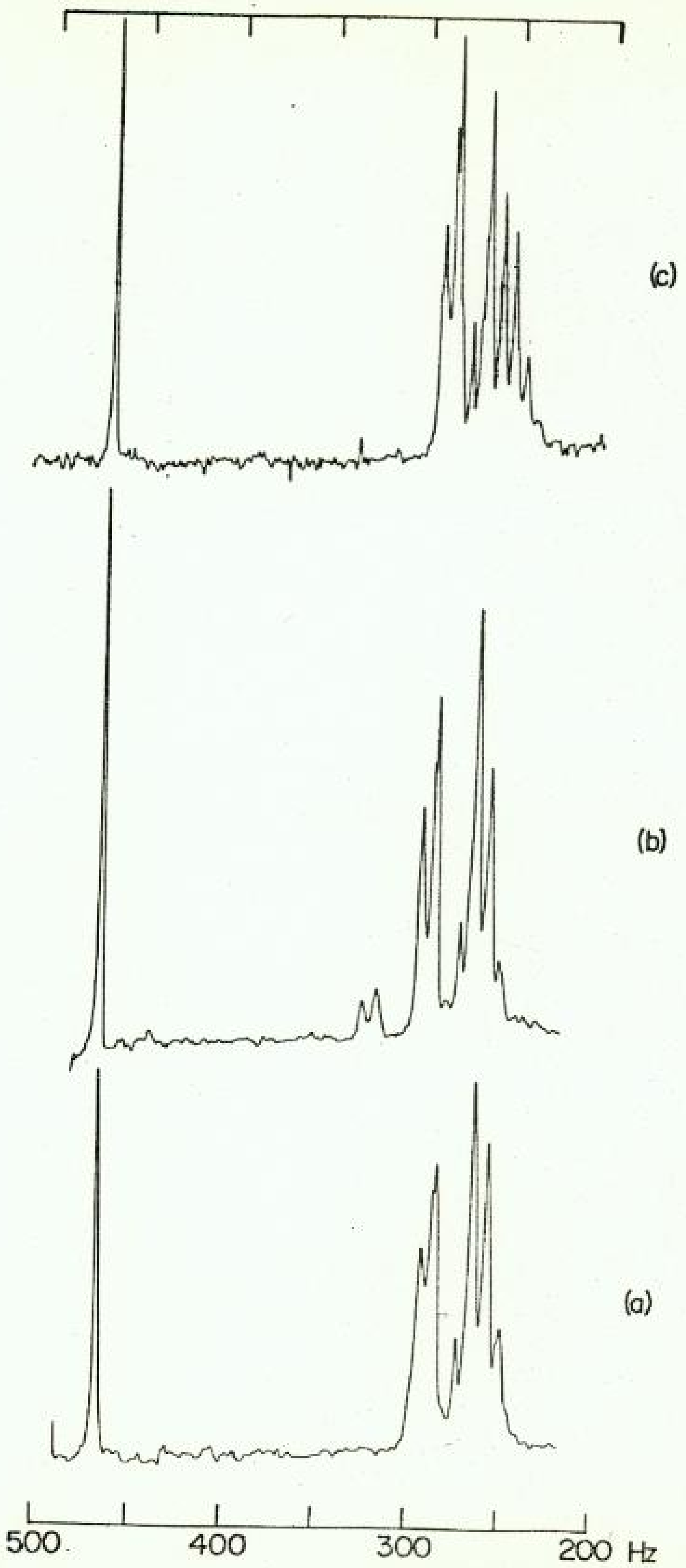
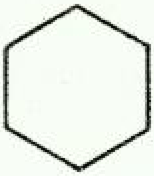
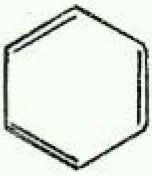
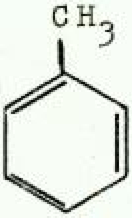
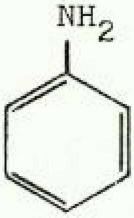
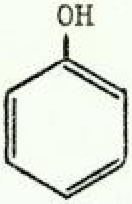
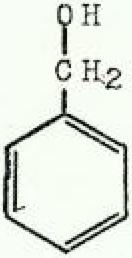
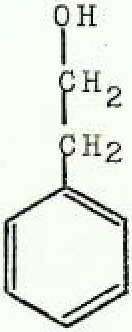
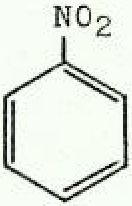
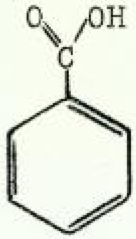
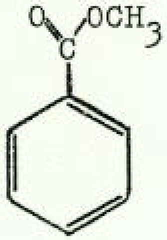
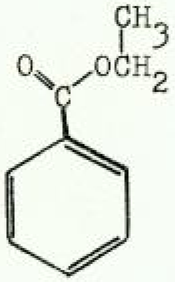
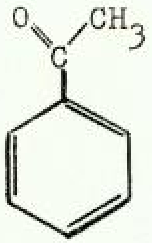
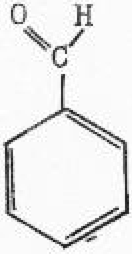
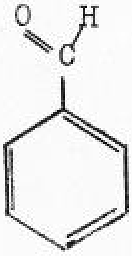
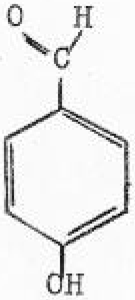

(c)
(b)
(a)
500
400
300
200 Hz

Table III. Δδ's of the substrates in ~0.04 <u>M</u> of α-cyclodextrin

Structure	Δδ (Hz)	Sadtler Reference
	23	5678
	3	3429
	4 (methyl) 4 (aromatic)	16943
	4	191

	3	3152
	4	9374
	8 (methylene) 4 (aromatic)	113
	9 (ortho) 10 (meta, para)	4

	32 (ortho) 10 (meta,para)	57
	28 (ortho) 12 (meta,para)	78
	2 (methyl) 3 (methylene) 17 (ortho) 10 (meta,para)	1204
	4 (methyl) 7 (ortho) 6 (meta,para)	10480

	9(aldehydic) 35 (ortho,adjacent to carbonyl) 13 (ortho, adjacent to aldehydic hydrogen) 10 (meta,para)	17061
	*6 (aldehydic) 3 (ortho) 10 (meta,para)	17061
	2 (aldehydic) 6 (ortho) 6 (meta)	7349

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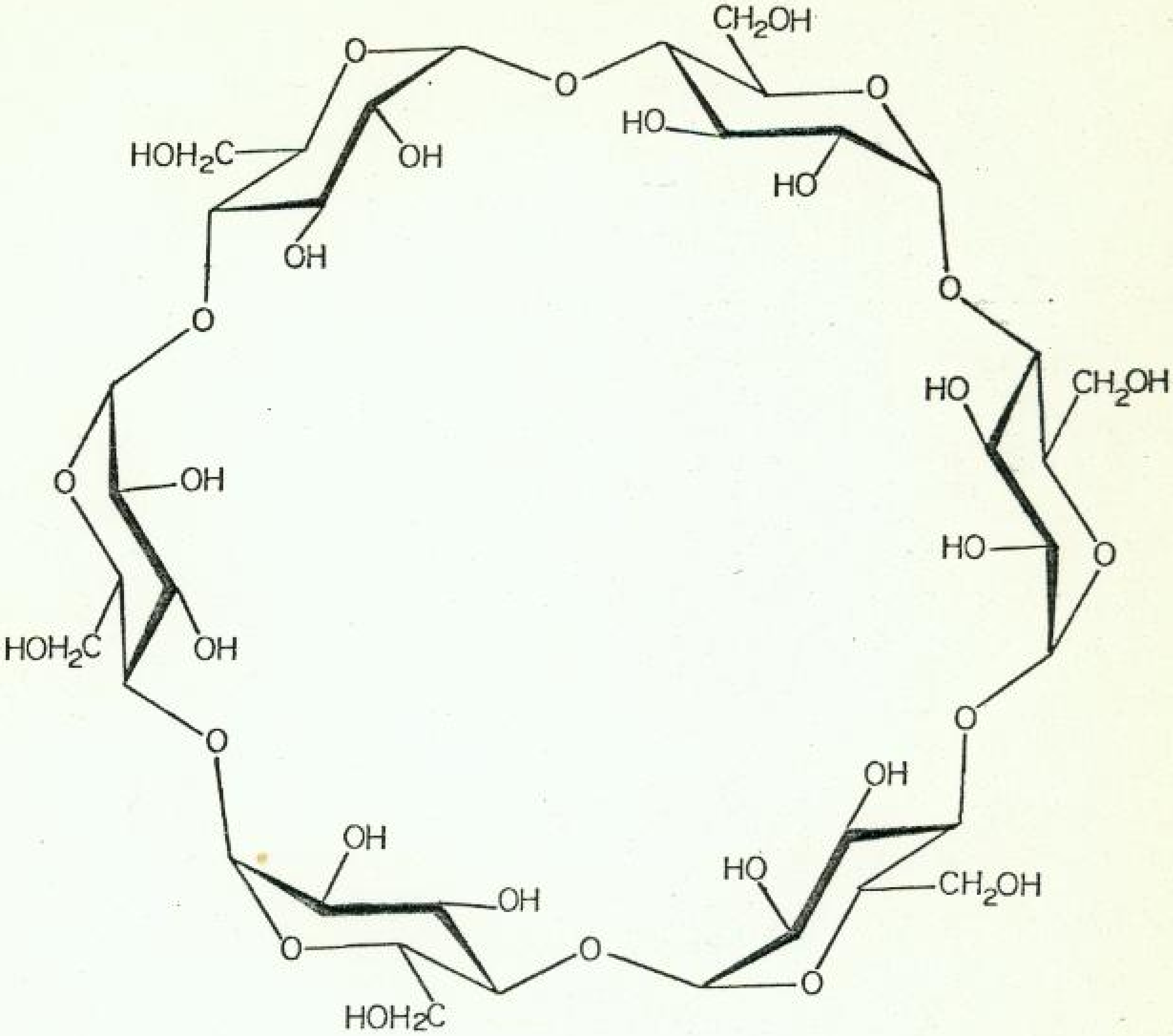
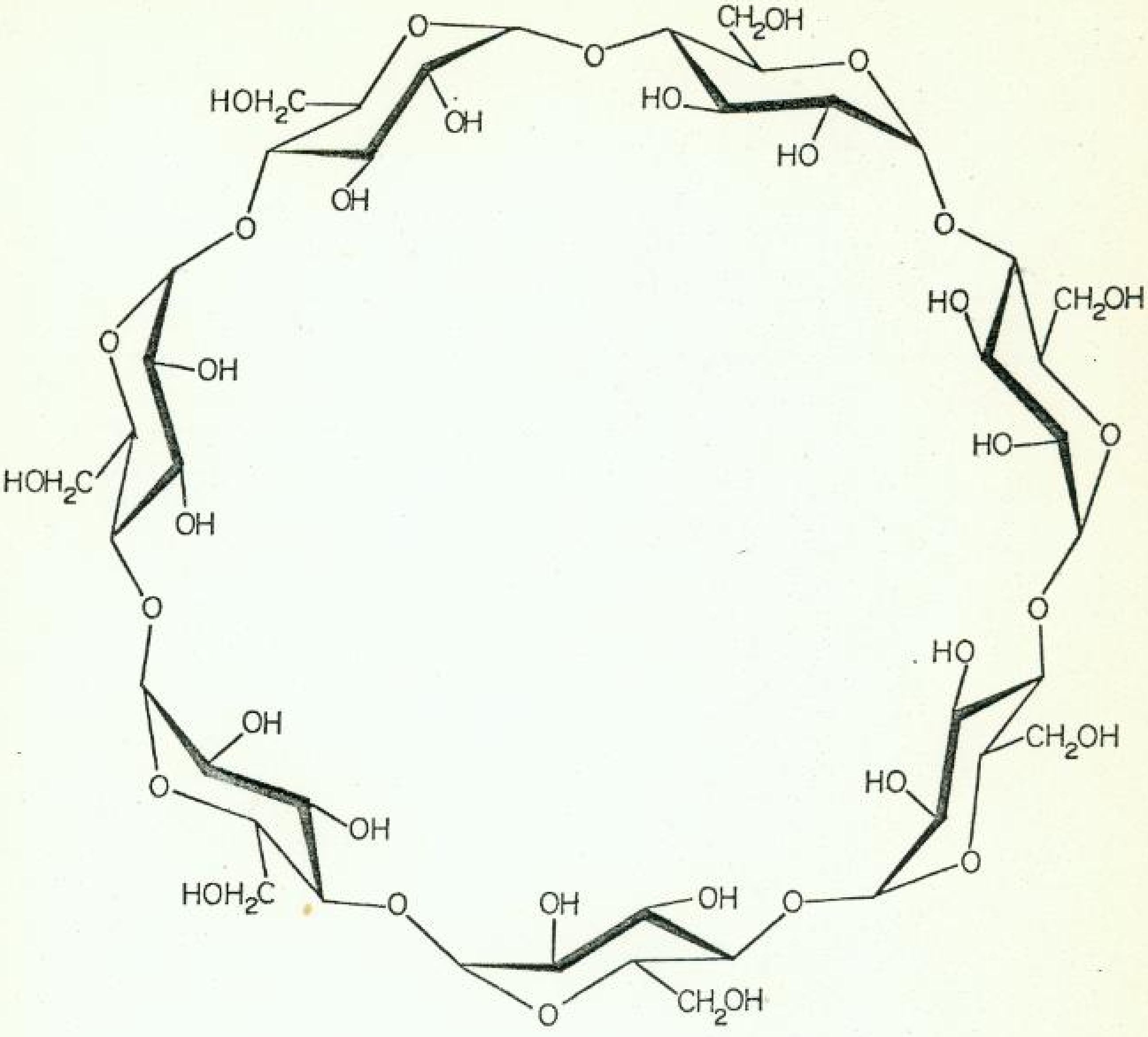
* Measured in 0.01 M β-cyclodextrin



Structure 1. Structure of α-cyclodextrin



Structure 2. Structure of β-cyclodextrin

IV. Discussion

It is apparent that the cyclodextrin can cause a change in chemical shift for hydrocarbon protons in solution, whether they are aliphatic, alicyclic or aromatic protons. It is surprising to observe that cyclohexane should have a much larger change in shift than benzene, even though cyclohexane, from a CPK model, seems to be too large for the cavity, whereas benzene can fit comfortably into the cavity. If inclusion is the critical process of complexation and all other effects are equal, one would expect the change in chemical shift for both chemicals to be of the same magnitude. Even more surprising is the aliphatic heptane which has a significant $\Delta\delta$ (15 Hz), compared with benzene (3 Hz).

It is not known exactly what causes these changes in chemical shifts. Two factors may be important. Either or both factors can contribute to the changes. One factor is the magnetic field effect, which could be due to the bulk anisotropy of the medium or to the effect of the cyclodextrin cavity when the substrate is included. The other factor is the redistribution of the electron density in the substrate molecule caused by some charge effect of the cyclodextrin. For aliphatic and alicyclic compounds, the magnetic field effect would be expected to be the only factor causing the shift, while for the aromatic compounds, where the π electrons are easily delocalized, redistribution of electron density for the aromatic ring is possible. However, evidence is still inadequate to allow a definite conclusion.

Potassium acetate and p-iodoaniline have been shown by X-ray to form inclusion complexes with cyclodextrins. However the systems studied here have not been examined by X-ray. Thus it is not known if inclusion complexation is the only mode of complexation. Other modes of complexation should not be neglected. For example, the substrate could be on either end of the cavity or on the outside of the rim. It is conceivable that these modes of complexation can also cause a change in chemical shifts.

From the results of Behr and Lehn (36), it is apparent that the methyl groups of the meta-and para-methyl cinnamates were included in the α-cyclodextrin cavity or at least in contact with the macrocycle because their rotations were hindered. However, the bulky tert-butyl of p-tert-butylphenolate were not much affected, indicating a non-included complex.

The aromatic compounds studied can be divided into two categories according to their substituents: those with electron withdrawing groups and those with electron releasing groups.

From the data, it is the $\Delta\delta$ of the aromatic protons for the systems with electron releasing groups that are small, in the order of 4 Hz, comparable to that of benzene itself. On the other hand, aromatic protons of systems with electron withdrawing groups have large $\Delta\delta$, ranging from 17-35 Hz for ortho protons and about 10 Hz for para and meta protons. At this point it is not known why the systems should behave so differently. Perhaps electronic density distribution is different for the two systems. For the system with electron releasing group,' a deshielded aromatic proton can upset the effect of deshielding by drawing electrons from the π electrons of the aromatic ring. This process is assisted by the electron releasing group. However, this same process is opposed by the electron withdrawing groups. Thus more deshielding was seen in the latter system than in the former. As to the cause of the deshielding, it is possible that, if inclusion did occur, the acetal oxygen atoms lining the cavity could behave like Lewis bases and deshield the aromatic protons.

Let us focus on the systems with electron withdrawing groups. All of them have larger $\Delta\delta$ for ortho protons than for meta and para protons. If the structure proposed by Harato and Uredaira (33) is correct, then it seems that the benzene ring with its substituent is inserted into the cavity of the cyclodextrin along its axis of rotation (26). It should be noted that their complex was in solid form and the systems studied here were in solutions. The mode of complexation could be different for the two states. Assuming the two complexations are similar and inclusion complexes were formed, then, the ortho protons, being located deeper into the cavity, are affected more than the meta and para protons. Thus, a larger $\Delta\delta$ was observed. This conjecture requires confirmation from other physical methods, for example, X-ray studies.

Furthermore, it seems that the bulkiness of the substituent can also affect the $\Delta\delta$. In the case of benzoic acid and its esters, it is seen that the larger the ester, the smaller was $\Delta\delta$ for the ortho protons. However, it is puzzling to see that the $\Delta\delta$ for the meta and para protons were not affected correspondingly.

The results obtained for the benzaldehyde system are interesting. When benzaldehyde was placed in an aqueous solution of α-cyclodextrin, not only large $\Delta\delta$ was observed for the ortho protons, but also a new doublet appeared. One doublet had a $\Delta\delta$ of 35 Hz while the other set had a $\Delta\delta$ of 13 Hz. The para and meta protons changed their chemical shifts by 10 Hz. The twosets of doublets are thought to result from the nonequivalence of the ortho protons when the system was complexed with α-cyclodextrin. This nonequivalence could be caused by the "freezing" of the C—C single bond of the aldehyde functional group. It is possible that when the aldehyde gooup was inserted into the α-cyclodextrin cavity, it was fixed and the rotation stopped. Now the two ortho protons became nonequivalent —— one being close to the aldehyde proton and the other close to the carbonyl group. It is known that C=O group could shift its neighboring group downfield more than a proton could; so the proton with larger $\Delta\delta$ was assigned to be the one that was closer to the carbonyl group.

It could be argued that the complexation could occur in other modes such that the aldehyde group was similarly frozen. However if inclusion and the size of the cavity were the determining factors of the "freezing" of the C—C single bond, then a larger cavity would allow the free rotation of the aldehyde group and consequently onlyone doublet would be observed. On the other hand, if other modes were the case, then two doublets should be observed as in the case of α-cyclodextrin. The result of using β-cyclodextrin as a complexing agent showed that no new doublet appeared and the $\Delta\delta$ for the ortho protons was small. The absence of an extra induced doublet in β-cyclodextrin complexation was taken as an indication that inclusion of the aldehyde functional group occurred in the α-cyclodextrin complexes.

Complexation of α-cyclodextrin with acetophenone did not lead to the emergence of a new doublet for the ortho protons. Probably in this case, the substituent was too large for a "good" inclusion. The much lower value of $\Delta\delta$ for the ortho protons is surprising when compared with other systems with electron withdrawing groups. This may be because the substituent was not a very strong electron withdrawing group.

Another interesting feature in the spectrum of benzaldehyde-β-cyclodextrin complex was that five peaks instead of four were observed for the para and meta protons. The new peak that appeared was thought to be due to a change in the coupling constant, J. Thus overlapping peaks in the α-cyclodextrin were now resolved in β-cyclodextrin. It is not clear what caused the change of coupling constant. However, from this result, it seems that cyclodextrins have the potential of a shifting reagent, as also proposed by MacNicol (24).

The $\Delta\delta$ of the aromatic protons of benzoic acid with concentration of α- cyclodextrin appears to have the same features as the same system observed potentiometrically (28). This system was chosen for study because it was known 1:1 complex is formed under this circumstance. In both cases, the measured quantity

($\Delta\delta$ in nuclear magnetic resonance study and ΔpKa in potentiometry) can be related to the concentration of the α-cyclodextrin and both curves level off at about 0.02 M of α-cyclodextrin. Unfortunately, the NMR measurement at this point is not under enough rigorous control so as to produce quantitative measurements of the stability constant K_{11} of the complex. Furthermore, the narrow range of $\Delta\delta$ also put a limitation to the accuracy of the value obtained.

One disubstituted benzene was run in this study to see what effect the combined influence of the two groups had on the $\Delta\delta$. p-Hydroxy benzaldehyde was used as the substrate because it had one electron withdrawing group and one electron releasing group located para to each other. In this case, no new doublet appeared as with benzaldehyde and the aldehydic proton had a nonsignificant $\Delta\delta$ (2 Hz), compared with that of the benzaldehyde system (9 Hz). It is possible that inclusion was difficult if not impossible and the low $\Delta\delta$ for all the aromatic protons indicated the dominance of the phenolic group over the aldehydic group. More disubstituted benzenes have to be studied in the future to gain better insight in these system.

V. Conclusion

It is obvious from the preceding discussion that many hydrocarbons form complexes with cyclodextrins in solution and further study will be necessary to determine the exact conformation, structure and mode of complexation of the cyclodextrins with hydrocarbons. However, the present preliminary data seem to suggest that, for aromatic compounds with electron withdrawing groups, exemplified by benzaldehyde, inclusion complexation with the substituents inside the cavity is the mode of complexation. This agrees with Harato's findings of p-iodoaniline. It is not clear from the present data how aromatic compounds with electron releasing groups complex with cyclodextrins.

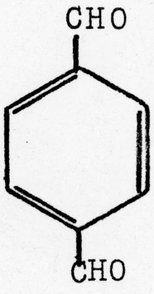
Furthermore, it seems that $\Delta\delta$ is related to the electron releasing and withdrawing power of the substtuents. $\Delta\delta$ is also related to the concentration of the α-cyclodextrin through the complexation constant K_{11}. However calculationof K_{11} for the system from $\Delta\delta$ is not very sensitive because of the narrow range of $\Delta\delta$ (~35 Hz or ~0.4 ppm for the benzoic acid protons, which has the largest $\Delta\delta$ in the series studied).

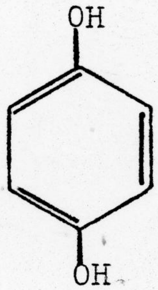
VI. Direction of Future Work

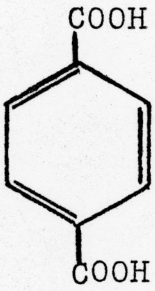
From the data of the three types of hydrocarbons, aliphatic (heptane), alicyclic (cyclohexane) and aromatic (benzene), it is seen that complexation occurs irrespective of the size of the substrates as long as it is not too bulky. The difference between the $\Delta\delta$'s of the three compounds are quite noticeable —— 15 Hz for heptane, 23 Hz for cyclohexane and only 3 Hz for benzene. Is the difference between the $\Delta\delta$'s of heptane and cyclohexane protons due to the proximity of each substrate to the cavity wall? This can be tested by using smaller cyclohydrocarbons and branched aliphatic hydrocarbons. Does the low $\Delta\delta$ of benzene indicate a "resistance" to the localdeshielding effect by the benzene? The protons on benzene are bonded by sp^2 orbitals. Any attempt to deshield its protons could be compensated by the mobile π electrons from the aromatic ring. To test the effect of the π electrons on the deshielding of the protons, cyclohexene and cyclohexadiene could be used as substrates.

A "resistance" of the aromatic protons to change their chemical shift is also demonstrated by the other systems with electron releasing groups. More substrates with electron releasing substituents should be studied to confirm this observation. If possible, a correlation between the strength of the electron withdrawing group (perhaps as measured by Hammett substituent constant) and the $\Delta\delta$ of the aromatic protons should be made. Similar experiments should be performed for the aromatic compounds with electron releasing groups.

Other disubstituted aromatic compounds should also be examined. Investigation of a series of 1,4 disubstituted benzenes with identical substituents (for example terphthalic acid) could prove to be productive. These compounds have only a single peak for their aromatic protons in their free form. However, if inclusion does occur and is assymetrical, then the two sets of neighboring protons will become nonequivalent and consequently two kinds of signals may appear. This could constitute a definite proof of inclusion.







To gain a better understanding of the structure of the complex form, a correlation between the $\Delta\delta$'s of the cyclodextrin protons and the aromatic protons of the substrates should be made. From this information, one might be able to infer the position and the direction of the insertion of the substrate in the cavity.

To furthur investigate the complexes, relaxation studies with nuclear magnetic resonance can be done. The ultimate goal will be to ascertain the conformation and structure of one system with nuclear magnetic resonance and other physical methods. Hence one can investigate the other systems with this one as a reference.

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