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^{13}C Nuclear Magnetic Resonance Spectra of Cyclodextrin Monomers, Derivatives and their Complexes with Methyl Orange

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Abstract. Low molecular mass fractions of water soluble α-, β-, and γ-cyclodextrin epichlorohydrin polymer products (cdx-Ep) were characterized by ^{13}C nuclear magnetic resonance. The derivatives proved not to be polymers, but substituted cdx having one or two glyceryl groups per one glucose at the C-2, C-3 and C-6 positions. Spectra of analogous hydroxy-propyl β-cdx indicate that the degree of substitution is rather higher at the C-6 position. Methyl orange (MO) was included into nine kinds of cdx having different inner diameters and hydrophobic torus heights; α-, β-, and γ-cdx monomers, 2, 6-dimethyl and 2, 3, 6-trimethyl β-cdx, water soluble α-, β-, and γ-cdx-Ep and ethyleneglycol-bis(epoxy-propyl) ether products. The inclusion shifts were compared with each other and with the dioxane-induced solvent shift of MO. The *N, N*-dimethyl-aniline side of MO shifted to a higher field site with the increase of the inner diameter in cdx. By substituting cdx with ether groups of different length, the mechanism of inclusion formation remains substantially the same, but by lengthening the hydrophobic cavity, the hydrophobic interaction becomes stronger, as a better resemblance of inclusion shifts and solvent shifts can be observed.

Key words: Alpha, beta, gamma, cyclodextrin, epichlorohydrin, carbon NMR, methyl-β-cyclodextrin, methyl orange, inclusion shift, solvent shift.

1. Introduction

Water soluble cyclodextrin polymers are useful in the pharmaceutical area because of their high solubility and nontoxity. The characterization of the above products is worth studying because they can be studied in solutions and can be a clue to characterize the insoluble polymers. In the previous paper [1], the circular dichroism (CD) spectra of methyl orange (MO) were recorded in the presence of separated fractions of water soluble polymers prepared by reacting cdx with epichlorohydrin (cdx-Ep) or with ethyleneglycol-bis(epoxypropyl) ether (cdx-DiEp) as well as in the presence of the parent monomers, 2, 6-dimethyl β-cdx (DMβ) and 2, 3, 6-trimethyl β-cdx (TMβ). The complexes with α-cdx-Ep and α-cdx-DiEp showed an exciton splitting in the induced π–π^* band of the N=N group. The above phenomenon disappeared when larger host molecules and azo dyes were used. In the present work, ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on all the above compounds. Specific emphasis was placed on the following points;

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1. positions and degrees of substitution and polymerization of the products obtained.
2. whether the increase of the inner diameter in cdx and the lengthening of the hydrophobic torus by substituting with the linking agents and methyl groups affect the inclusion properties.
3. whether the included geometry may be deduced by comparing the inclusion shift with the chemical shift of the guest molecule in dioxane.

2. Materials and Methods

Cdx-Ep and cdx-DiEp products were prepared according to [1]. DMβ and TMβ were prepared according to [2]. Hydroxypropyl β-cdx was a generous gift from Dr J. Pitha. Other compounds used are the same as described previously [1, 3]. ^{13}C NMR spectra were recorded on a Hitachi R-22 CFT spectrometer (22.5 MHz) and a JEOL JNM–GX 500 FT NMR (125 MHz) at a temperature of 307 °K. Host molecules of the order of ~0.05M were added to ~0.05M guest molecules. Chemical shifts were measured in ppm downfield from external tetramethylsilane (TMS). A positive sign indicates a low field shift.

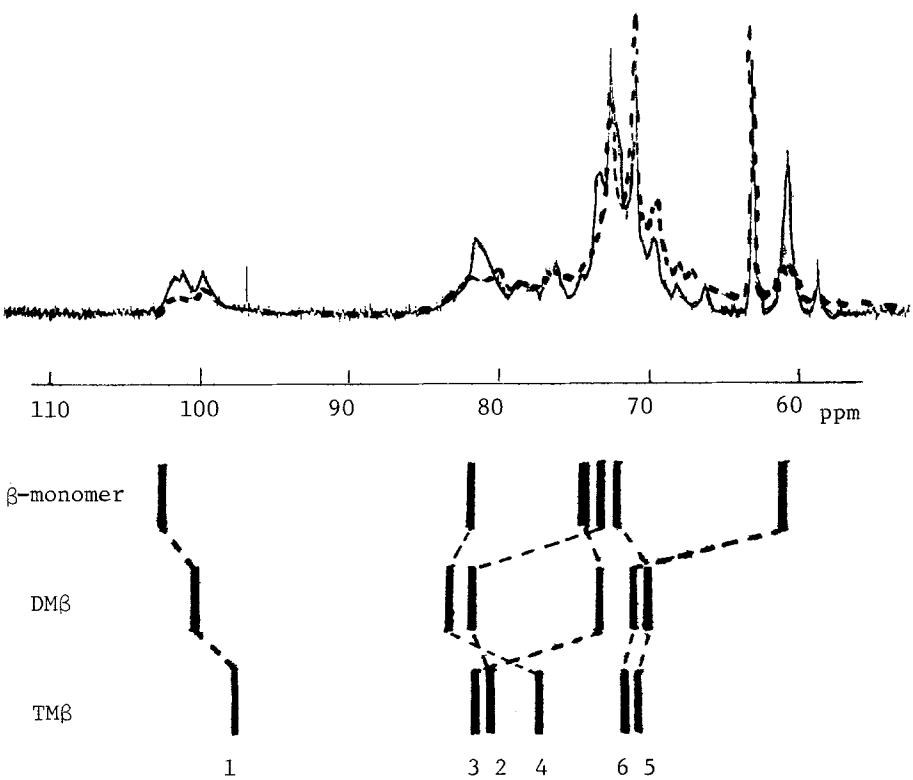

Fig. 1. ^{13}C NMR spectra of β-cyclodextrin epichlorohydrin products (125 MHz). ——— Low molecular mass; ----- high molecular mass.

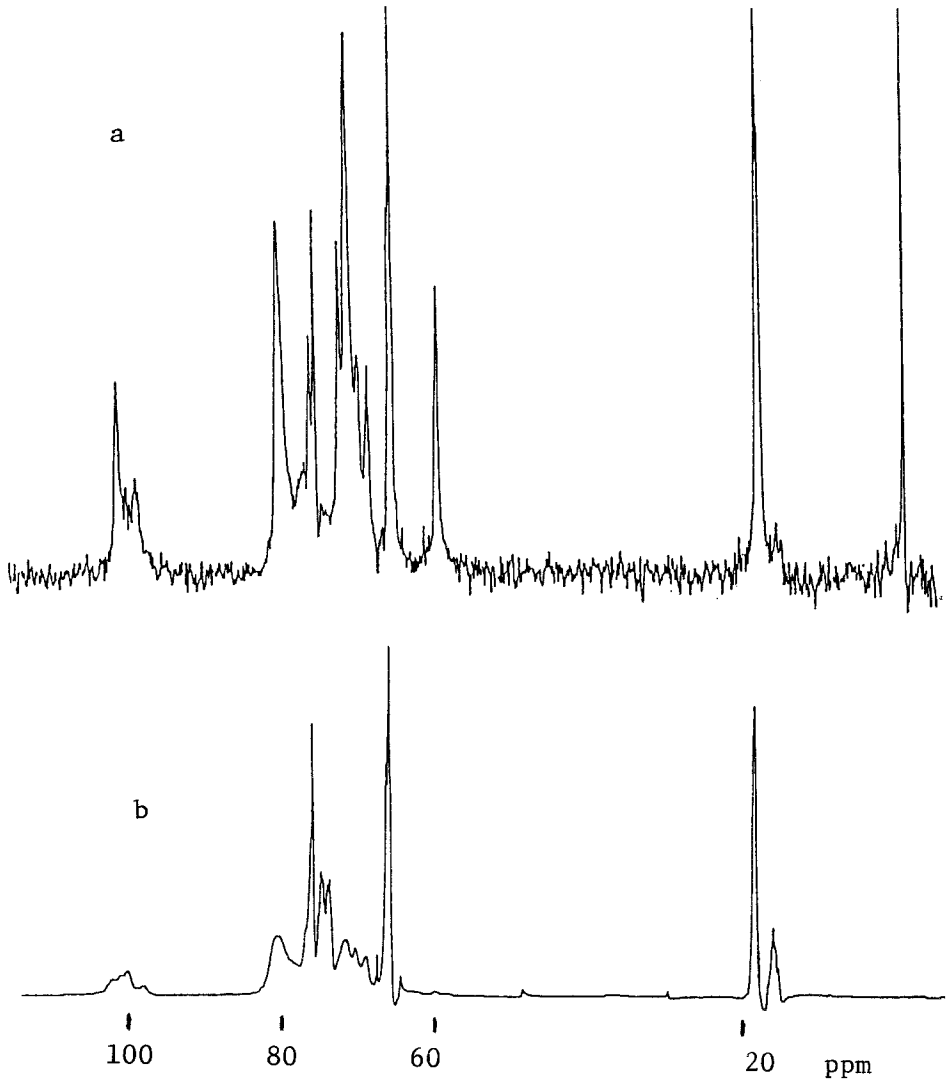

Fig. 2. ^{13}C NMR spectra of hydroxypropyl β-cyclodextrin. (a) Degree of substitution = 7.2; (b) Degree of substitution = 18.4.

3. Results and Discussion

3.1. ASSIGNMENT OF CDX-EP

When cdx is treated with Ep, substitution and crosslinking take place at the same time. So the product is a mixture of cdx glyceryl ethers of different degree of substitution (D.S.) and so called polymers containing 2 or more cdx rings.

$$\text{cdxOH} + \text{CH}_2\text{CHCH}_2\text{Cl} \longrightarrow \text{cdxOCH}_2\text{CHCH}_2 \longrightarrow \text{cdxOCH}_2\text{CHOHCH}_2\text{OH}$$

$$\text{cdxOCH}_2\text{CHCH}_2 \longrightarrow \text{cdxOCH}_2\text{CHOHCH}_2\text{Ocdx}$$

The above reactions resulted in rather low molecular mass products in the case of α-cdx and β-cdx (α-cdx-Ep and L-β-cdx-Ep, cdx contents 59 and 68%, mass average molecular mass 1400 and 2200, average D.S. = 9 and 7, respectively). In the case of γ-cdx, the product prepared by further crosslinking was a colloidal solution of higher molecular mass (γ-cdx-Ep, cdx content 59%, mass average molecular mass 7500, D.S. = 12).

The ^{13}C NMR spectrum of L-β-cdx-Ep was compared with those of β-cdx, DMβ and TMβ (Figure 1 and Table I). The C-1 signal gives a doublet-like pattern; C-1 and C-1′ (101.4 and 100.4 ppm). The C-1′ appears as a new band due to the substitution at C-2. The C-4 signal (81.8 ppm) becomes broad due to overlapping by the C-2′ and C-3′ signals. The new C-4′ signal (77.5 ppm) results from the 2, 3, 6-trisubstituted derivative. Three new sharp peaks appear at 72.8, 71.1 and 63.4 ppm. They were assigned to cdxO$\underline{C}H_2$, $\underline{C}$HOH and $\underline{C}H_2$OH of the substituent $CH_2CHOHCH_2OH$ and the linking agent $OCH_2CHOHCH_2O$. The substitution at C-6 induces the decrease in the peak height of the C-6 signal (61.0 ppm) and the appearance of the C-5′ signal (69.9 ppm).

The spectral pattern becomes broader in the high molecular mass β-cdx-Ep (H-β-cdx-Ep, cdx content 59%) prepared by further crosslinking. The solution becomes colloidal, so it was impossible to separate fractions of different molecular

Table I. Chemical shifts of β-cyclodextrin derivatives[a] (ppm from TMS)

	β	DMβ	TMβ	*L*-β-Ep	D.S. of Hydroxypropyl β-cdx			
					2.5	5.1	7.2	18.4
1	102.3	100.4	97.5	101.4	102.4	102.4	102.2	102.4
1′				100.4			100.9	101.3
							100.0	100.4
1″								98.6
2	72.7	82.0	80.6		72.5	72.6	72.5	
2″				~82				
3	73.7	73.2	81.5	73.6	73.7	73.7	73.6	72.5
4	81.6	82.9	77.5	81.8	81.7	81.7	81.5	81.3
4′				77.5				
5	72.3	70.4	70.9		72.5	72.6	72.5	
5′				69.9	69.8	70.1	70.0	70.0
6	61.0	71.3	71.4	61.0	60.9	60.9	61.0	
6′				71.2	71.3	71.4	71.1	71.3
2-Me		60.1	58.6					
3-Me			60.2					
6-Me		58.6	58.9					
CH_2OR				72.8	77.6	77.7	77.5	76.7
					76.8	76.9	76.8	75.7
								74.5
CHOH				71.1	67.1	67.2	67.2	67.3
					66.8	66.8	66.8	66.7
								68.4
Me					19.0	19.1	19.0	19.0
					18.7	18.8		16.5
CH_2OH				63.4				

[a] Assigned by selective decoupling, off resonance and peak height.
′ Indicates new shifts induced by the substitution.

Table II. Methyl orange-induced ^{13}C chemical shifts of cyclodextrin (ppm)

	α	α-Ep	β	β-Ep	DMβ	TMβ	γ	γ-Ep
1	0.6	0.6	1.0	0.3	0.0	2.7	0.7	
1′		0.6		0.1				
2	0.2		0.1		−0.2	1.0	0.0	
3	0.1		0.3		0.0	0.6	0.1	
4	−0.2	1.5	−0.1	−0.4	0.0	4.1	0.1	0.0
5	−0.1		0.3		0.1	−0.3	−0.1	
6	−0.5	−0.2	−0.4	−0.6	−0.4	−0.1	−0.6	−0.2
6′				−0.4				0.3
2-Me					−0.2	−0.1		
3-Me						1.4		
6-Me					−0.1	0.0		
CH_2OR		0.0		−0.3				0.2
CHOH		0.1		−0.2				0.1
CH_2OH		0.0		−0.3				0.0

mass by gel chromatography to calculate mass average molecular mass. The C-3 signal (73.6 ppm) disappears. The intensities of the C-2′ and C-5′ signals (81 ~ 80 ppm and 69.9 ppm) increase and those of the C-4 and C-6 signals (81.6 and 61.0 ppm) decrease. The degree of the substitution increases. Comparison of the peak heights of cdxO$\underline{C}H_2$ and $\underline{C}H_2OH$ in the glycerylether group may indicate the degree of polymerization. Now the $\underline{C}H_2$Ocdx signal in H-β-cdx-Ep is better separated from those of the unreacted carbons of cdx (C-2, C-3, C-5) than that in the case of L-β-cdx-Ep in the measurement at 125 MHz. Qualitatively, in the case of H-β-cdx-Ep, the peak height $\underline{C}H_2OH$ is rather higher than that of cdxO$\underline{C}H_2$. This fact means that the soluble fraction measured in H-β-cdx-Ep does not contain the polymer part, but only an increase in the degree of the substitution. The spectra of α-cdx-Ep and the soluble fraction in γ-cdx-Ep were almost the same as that of L-β-cdx-Ep, though the latter obviously contains the fraction above 10 000 molecular mass weight.

Hydroxypropyl β-cdx of different D.S. will give clearer spectra, because the $\underline{C}H_3$ and $\underline{C}$HOH signals in the substituent are separated from those of cdx. It is therefore possible to examine the behavior of the substituent (Figure 2 and Table I). In the spectrum of D.S. = 2.5, new signals appear; C-6′ and C-5′ (71.3 and 69.8 ppm), $\underline{C}H_2$Ocdx (77.6 and 76.8 ppm), $\underline{C}$HOH (67.1 and 66.8 ppm), $\underline{C}H_3$ (19.0 and 18.7 ppm). With increasing D.S. values, the above peaks increase in intensity, but remains split. In the spectrum of D.S. = 7.2, the C-1 signal begins to separate (102.2, 100.9 and 100.0 ppm). The neighborhood of the C-4 signal becomes more intense and broader due to the overlapping by the C-2′, and C-3′? signals. In the spectrum of D.S. = 18.4, the C-1 signal becomes broader (102.4, 101.5, 100.4 and 98.6 ppm) and the C-6 signal disappears. The former may explain the substitution to C-3. All signals in the substituent group split. This fact must be due to the substitution to the different positions of β-cdx. Judged from the pattern of the C-1 signal, the substitution at C-3 cannot be perceived in the D.S. = 2.5 spectrum. So the splitting of the signal in the substituent may be ascribed to the substitution at C-2 and C-6; 77.6 and 67.1 ppm are ascribed to cdx-C-2-O$\underline{C}H_2$CHOHMe and cdx-C-2-OCH$_2\underline{C}$HOHMe, 76.8 and 66.8 ppm are ascribed to cdx-C-6-O$\underline{C}H_2$CHOHMe and cdx-C-6-OCH$_2\underline{C}$HOHMe. From comparison of the above peak heights, it is

possible to estimate the ratio of the substitution (C-6/C-2): it is roughly 3/2. The ratio is unchanged in the spectrum of D.S. = 7.2 (Figure 2b).

The glyceryl ether parts of α-, β-, and γ-cdx substituted at the 2, 3 and 6 positions at which D.S. are above seven and the ratio of substitution (C-6/C-2) may be rather higher at the C-6 position have been used in the further studies.

3.2. INCLUSION SHIFTS OF MO IN COMPLEXES BETWEEN α-, β-, AND γ-CDX AND MO

Figure 3 gives the α-, β-, and γ-cdx-induced chemical shifts of MO. Inclusion shifts in the α-cdx-complex gather together to low field. When the inner diameter of the cavity becomes large for the guest molecule, inclusion shifts spread to high field, especially on the N, N-dimethyl aniline side. By CPK molecular model, MO does not show any compression with β-cdx, but it shows the compression in the neighborhood of N=N with α-cdx. The fact that inclusion shifts gather together to low field may be due to some strain of the substituents. Both complexations consist of a 1 : 1 molar ratio [3]. γ-cdx-induced chemical shifts of MO gather together to high field. Plots of a molar ratio of γ-cdx/MO *vs.* change in chemical shifts of MO in Figure 3c are almost zero below a molar ratio of 0.5 for γ-cdx/MO and increase gradually after that (Figure 3c). Job plots give a 1 : 1 molar ratio (Figure 4), but the shape is distorted. These facts suggest the co-existence of the 2 : 1 (MO : γ-cdx) complex. In the investigation of this complex by CD spectra [1], the red shift and the absence of the exciton splitting of the maximum peak in the π-π^* region of the N=N group suggest the parallel planar arrangement of the two MO molecules with respect induced to the

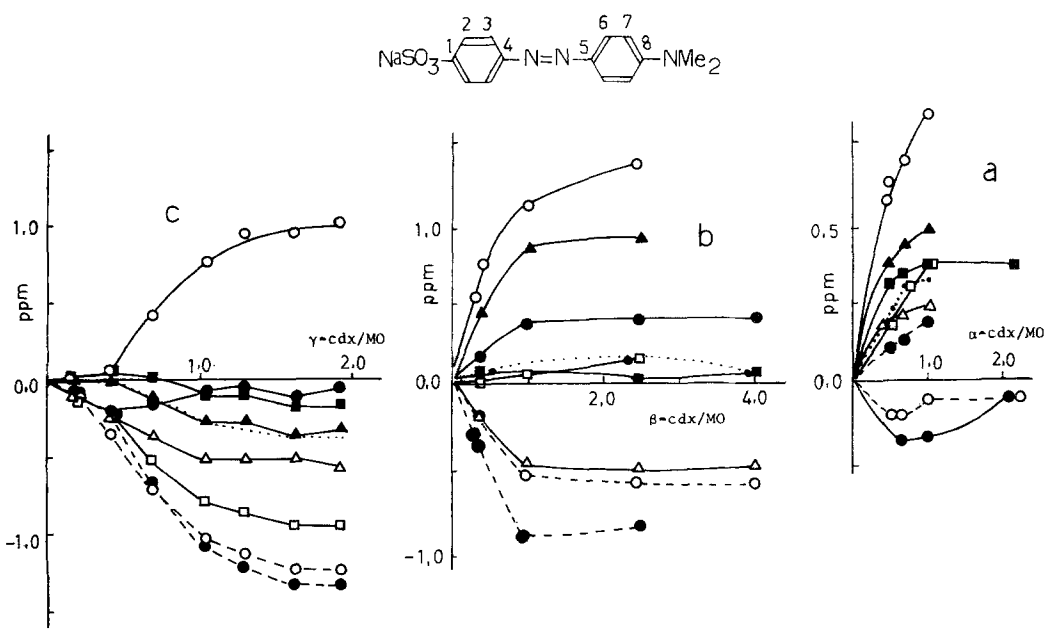

Fig. 3. γ-, β-, α-Cyclodextrin-induced shifts of methyl orange plotted as a function of the molar ratio of γ-, β-, α-cyclodextrin to methyl orange: C-1, ○——○; C-2, ●——●; C-3, ■——■; C-4, □——□; C-5, ▲——▲; C-6, △——△; C-7, ○- - -○; C-8, ●- - -●; Me, ... α-Cyclodextrin complex plots decrease above a molar ratio = 1 for α-cyclodextrin/methyl orange, because a precipitate appears.

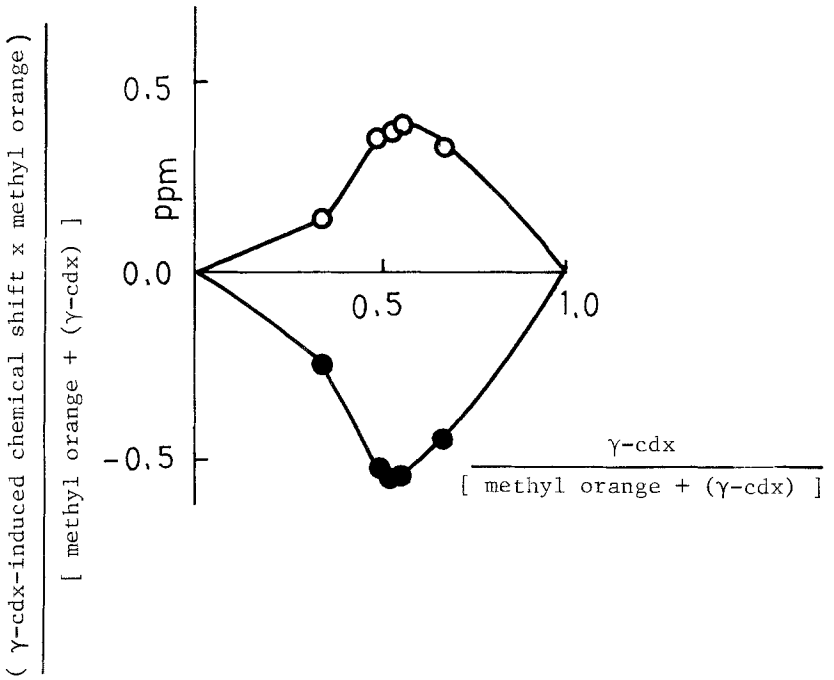

Fig. 4. Continuous variation plots of γ-cyclodextrin-induced chemical shifts of methyl orange at 53°C. C-1, ○; C-8, ●.

annular axis of γ-cdx. In the present ^{13}C NMR work, such a situation induces a ring current effect of MO on each other and may increase the high field shift.

3.3. SOLVENT SHIFTS OF AZO DYES

In general, ^{13}C NMR inclusion shifts may be divided into hydrophobic, van der Waals, dipolar, ring current and steric compression interactions with the various substrate species [4]. In the spectroscopic experiments, the complex formation makes the absorption spectrum of the substrate almost the same as that in dioxane [5], and strengthens the fluorescence degree of 1-anilino-8-naphthalene sulfonate in the fluorescence measurement [6]. In ^{13}C NMR spectroscopy [7], inclusion shifts of the substrate in the strainless host-guest complexes give shifts similar to that in hydrophobic dioxane. Now, to check the role of the hydrophobic interaction in the inclusion shifts, ^{13}C NMR of MO in dioxane was measured (Figure 5). When Figure 5 was compared with Figure 3, the former resembles Figure 3b the most; the inclusion shifts of the β-cdx complex may be mainly due to hydrophobic interaction. On the other hand in the case of the p-(2-hydroxy-1-naphthylazo) benzenesulfonic acid sodium salt (orange II) which has a larger width, the solvent shifts agree with the γ-cdx-induced shifts rather than those of β-cdx [7]. Concerning longer guest molecules (for example, $NaSO_3$—⬡—N=N—⬡—NH—⬡), the above resemblance was not observed [8]. Thus, the inner size of cdx and the width and the length of the guest molecule sensitively affect the resemblance between both shifts.

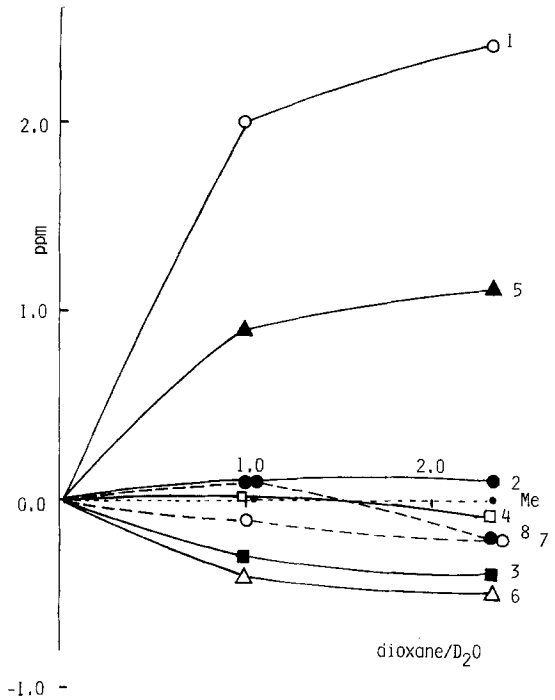

Fig. 5. Solvent shifts of methyl orange in dioxane + D_2O at 53 °C.

3.4. NINE KINDS OF CDX-INDUCED ^{13}C CHEMICAL SHIFTS AND THE DIOXANE-INDUCED SOLVENT SHIFTS OF MO

From the comparison between the inclusion shifts and the solvent shifts, it may be possible to deduce roughly the situation of the included molecule. The lengths of the skeleton carbons on MO and orange II are ~9Å. The torus heights of the monomers and DMβ are ~8Å [9] and ~11Å [2b], respectively. α- and β-cdx-Ep have a longer torus. Thus, nine kinds of cdx having different inner diameters and torus heights were used to include MO. The inclusion shifts were compared with each other and with the dioxane-induced solvent shifts (Figure 6).

Figure 6 shows the cdx-induced chemical shifts of MO with a molar ratio of one for cdx/host molecules. The behavior of the carbons at both ends of MO coincides with those of the parent cdx; in the α-cdx series the C-8 signals move to low field and in the β- and γ-cdx series move to high field. The lengthening of the torus gives the following change in the β-cdx series; inclusion in DMβ and β-cdx-Ep causes the general pattern of MO better to resemble that of the solvent shifts and inclusion in TMβ causes the change to the N, N-dimethylaniline side of MO.

Table II shows the MO-induced chemical shifts of cdx molecules with a molar ratio of one for cdx/host molecules. The values are smallest in DMβ and largest in TMβ. TMβ induces the largest distortion of all the cdx at the wide rim side, and the smallest one at the narrow rim side.

Figure 6 and Table II suggest that DMβ serves as a long and strainless hydrophobic environment (like dioxane solvent) to MO.

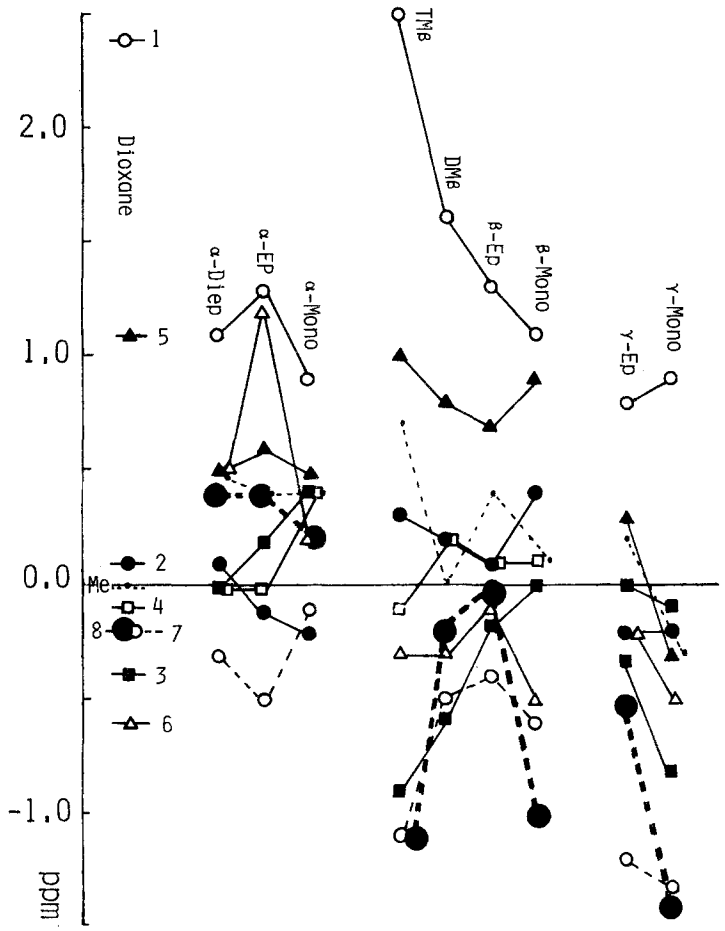

Fig. 6. Nine kinds of cyclodextrin-induced ^{13}C chemical shifts and the dioxane-induced solvent shifts of methyl orange.

4. Conclusion

1. Water soluble α-, β-, and γ-cdx-Ep polymer products proved to be not polymers, but substituted cdx having one or two glyceryl groups per one glucose at the C-2, C-3 and C-6 positions. The degree of the substitution may be rather higher at the C-6 position. 2. Comparison between nine kinds of cdx-induced inclusion shifts and dioxane-induced solvent shifts proved that the hydrophobic interaction plays an important role in inclusion. Moreover, the inclusion shifts in the α-cdx-MO series induce van der Waals and/or compression effects at the N, N- dimethylaniline side and those in γ-cdx-MO series induce the anisotropic ring current effect by the parallel planar arrangement of the two MO molecules included parallel to the annular axis of γ-cdx. 3. By substituting cdx with ether groups of different length, the mechanism of inclusion formation remains substantially the same, but the hydrophobic interaction becomes stronger.

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