

## $^{13}\text{C}$ -N.m.r. spectra of cyclomalto-oligosaccharides (cyclodextrins), their derivatives, and complexes with azo dyes\*

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### ABSTRACT

The nature of the inclusion complexes of several cyclomalto-oligosaccharides (cyclodextrins, CDs) with azo dyes has been studied on the basis of  $^{13}\text{C}$ -n.m.r. chemical shifts, relaxation times, correlation times, and broadening and doubling of the n.m.r. signals. All CDs show the azo dye-induced shifts at the narrow-rim side of the CD, indicating that the azo dyes protrude from the cavity. CD-induced shifts of azo dyes depend on the hydrophobic nature of the cavity, van der Waals forces, as well as ring-current and deformation effects, and suggest inclusion essentially from the hydrophobic site. The broadening and the doubling of the  $^{13}\text{C}$ -n.m.r. signals, the altered relaxation and correlation times, as well as the temperature dependence for these phenomena, also provide particular information about the characteristic host-guest interactions.

### INTRODUCTION

Cyclomalto-oligosaccharides (cyclodextrins, CDs), as is well known, can complex with guest molecules to form inclusion complexes. It is possible to synthesize water-soluble CD derivatives and polymers which have different diameters and heights of the hydrophobic torus, namely, heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose (DM- $\beta$ CD) and heptakis(2,3,6-tri-*O*-methyl)cyclomaltoheptaose (TM- $\beta$ CD), water-soluble epichlorohydrin (Ep), and ethyleneglycol-bis(epoxypropyl)-ether (DiEp) derivatives of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CDs<sup>1,2</sup>. Inclusion states with methyl orange (1) have been studied using chemical shifts of both  $^1\text{H}$ - and  $^{13}\text{C}$ -n.m.r. spectroscopy<sup>2,3</sup>, as well as *via* circular dichroism spectroscopy<sup>1</sup>.

In the previous paper<sup>4</sup>, the molecular motions of the inclusion complexes of five types of CDs with 1 and its analogues were studied at 333K on the basis of  $^{13}\text{C}$ -n.m.r. relaxation times. The correlation times were estimated to be the order of  $\sim 10^{-10}$  to  $10^{-11}$  s by the isotropic model. Azo dyes rotate up to twice as fast as the CDs upon complex formation. The internal rotations of the primary alcohol, methyl, and phenyl groups were shown to be preserved in the complexes.

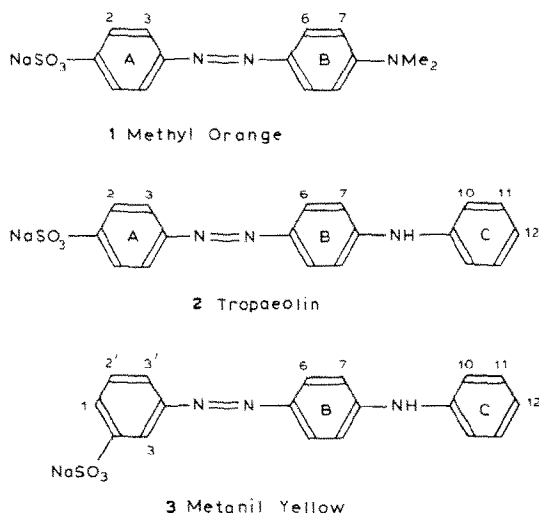
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In this paper,  $^{13}\text{C}$ -n.m.r. chemical shifts of the inclusion complexes of nine types of CDs and **1** and its analogues were measured to analyse the induced shifts and to investigate the mode of inclusion. Determination of the relaxation times were found helpful to this end.

#### MATERIALS AND METHODS

A JEOL JNM-GX 500 and a Hitachi R-900 F.t.-n.m.r. spectrometers, operating at 125 and 22.5 MHz, were used for measuring  $T_1$  and chemical shifts, respectively. Materials used were the same as those previously described<sup>1,3</sup> except that metanil yellow was purchased from Sigma Chemical Company (U.S.A.). Assignment of chemical shifts of **1**, tropaeolin (**2**) and metanil yellow (**3**) are as shown in Table I.



The calculated values were obtained by the additivity of substituent chemical shifts<sup>5</sup> where the following approximations were used:  $\text{Ph}-\text{N}=\text{N}-$  instead of  $p\text{-NaSO}_3\text{-Ph}-\text{N}=\text{N}-$ ,  $m\text{-NaSO}_3\text{-Ph}-\text{N}=\text{N}-$ , or  $-\text{N}=\text{N}-\text{Ph}-\text{NR}'\text{R}''$ . For example, **1** is expressed as  $p\text{-NaSO}_3\text{-Ph}- + -\text{N}=\text{N}-\text{Ph}$  on the benzenesulfonate side, and as  $\text{Ph}-\text{N}=\text{N}- + -\text{Ph}-\text{NMe}_2$  on the phenylalanine side of the molecule. The calculated values show a good agreement with the observed values (see Table I). Assignments were confirmed by  $T_1$  data for the tertiary carbons, and by  $^{13}\text{C}-^1\text{H}$  coupling constants obtained from proton-coupled  $^{13}\text{C}$ -n.m.r. spectra. Comparison with data of other azo dyes<sup>3</sup> was used for making assignments for the quaternary carbons. Assignments for each CD was reported in the previous paper<sup>2</sup>.

Since **2** is barely soluble in  $\text{D}_2\text{O}$ , the extrapolated induced shifts of the  $2\text{-}\gamma\text{CD}$  complex were used instead of actual chemical shift data for **2**. The complexes were 1:1 guest molecule to CD except the  $\gamma\text{CD}$  complexes which are 2:1. The complexes have

TABLE I

Assignment for the chemical shifts of azo dyes 1, 2, and 3<sup>a</sup>.

1		2		3	
Carbon No. <sup>b</sup>	in D <sub>2</sub> O	Calc.	in D <sub>2</sub> O	Calc.	in D <sub>2</sub> O
1	143.1	145.8	143.8	128.8	126.9
2	127.1	126.8	127.0	143.8	144.1
2'				130.4	130.1
3	122.4	123.3	122.7	120.0	119.9
3'				126.2	122.8
4	154.3	155.8	153.8	153.2	152.4
5	142.6	143.8	141.0	143.8	140.8
6	126.0	123.7	125.6	123.7	125.5
7	112.4	118.1	115.0	118.1	115.3
8	153.9	146.8	148.6	146.8	147.8
9		143.9	145.0	143.9	145.1
10		117.2	121.0	117.2	119.9
11		129.6	130.1	129.6	130.0
12		120.1	123.6	120.1	122.8
Me	40.0				

<sup>a</sup> Measured in  $\delta$  units (p.p.m.) downfield from external Me<sub>4</sub>Si. <sup>b</sup> Refer to appropriate compound in figures.

large association constants ( $10^4$ – $10^5$  M<sup>-1</sup>)<sup>6a-6d</sup>. Host molecules (0.05M) were added to 0.05M guest molecules at 307K. Chemical shifts were measured in p.p.m. downfield from external tetramethylsilane (TMS). A positive sign indicates a low-field shift. T<sub>1</sub> data were obtained by the method as previously described<sup>4</sup>.

## RESULTS AND DISCUSSION

*Relaxation and correlation times.* — Previously, the molecular motions of the inclusion complexes of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CDs, DM- $\beta$ CD and TM- $\beta$ CD, and azo dyes at 333K have been studied on the basis of <sup>13</sup>C-n.m.r. relaxation times<sup>4</sup>. The correlation times ( $\tau_{\text{eff}}$  and  $\tau_G$ ) for the isotropic overall molecular reorientation and the additional internal rotation were calculated using the equations shown as follows<sup>7,8</sup>.

$$1/NT_1 = \hbar^2 \cdot \gamma_C^2 \cdot \gamma_H^2 \cdot r_{\text{CH}}^{-6} \cdot \tau_{\text{eff}} = \text{const} \cdot \tau_{\text{eff}},$$

and

$$1/NT_1 = \text{const} \cdot \tau_{\text{eff}} [A + B \cdot 6\tau_G / (6\tau_G + \tau_{\text{eff}}) + C \cdot 3\tau_G / (3\tau_G + 2\tau_{\text{eff}})],$$

$N$  is the number of directly bonded protons,  $\gamma_H$  and  $\gamma_C$  are the magnetogyric ratios of <sup>1</sup>H and <sup>13</sup>C nuclei, respectively,  $r_{\text{CH}}$  is the length of the C–H bond, and  $A = 1/4(3\cos^2\theta - 1)^2$ ,  $B = 3\sin^2\theta \cos^2\theta$ , and  $C = 3/4\sin^4\theta$ , where  $\theta$  is the angle between the C–H vector and the axis of rotation. For free CDs, the NT<sub>1</sub> values indicate the isotropic overall molecular reorientation for the pyranose ring and rapid internal rotation for the

CH<sub>2</sub>OH group of the CD, as well as for all the MeO groups of DM- $\beta$ CD and TM- $\beta$ CD. For azo dyes, the component aromatic rings are not necessarily coupled. Most  $\tau_{\text{eff}}$  values for CDs and azo dyes indicate a slowing of rotation upon the formation of complexes. The  $\tau_{\text{eff}}$  for azo dyes are faster than those for CDs, as azo dyes rotate faster than the host molecules. The methyl group in **1** and the phenyl ring in both **2** and **3** maintain their rapid internal rotation even in the complexed state.

In the present study, the  $T_1$  data were measured at 307K to correspond with the chemical shift data.  $T_1$  data previously measured at 333K were also used to determine the temperature dependence (Tables II and III).

In the **1**-series, the average  $NT_1$  values of the component aromatic rings are in the order  $A \geq B$ . These values in ring B at 307K decrease in the order DM- $\beta$ CD  $\sim$   $\gamma$ CD  $>$  TM- $\beta$ CD ( $0.32 \sim 0.30 > 0.26$  s). The ratios of the  $NT_1$  for ring A to ring B are nearly unity, except for the TM- $\beta$ CD complex, in which all the signals of the MeO groups show doublets. The  $NT_1$  values of the NMe<sub>2</sub> group are always ca. tenfold longer than those of ring B, indicating that the NMe<sub>2</sub> group maintains free rotation<sup>9</sup>. The  $\tau_G$  value of the CH<sub>2</sub>OH (CH<sub>2</sub>OMe) group in the CDs indicates a slower rotation upon the formation of complexes, and the ratios of  $\tau_{\text{eff}}$  for **1** to CDs indicate that **1** can rotate most rapidly in DM- $\beta$ CD (DM- $\beta$ CD =  $0.8 <$  TM- $\beta$ CD =  $\gamma$ CD =  $0.9$ ).

In the **2**-series, the  $NT_1$  values of the component aromatic rings are in the order  $C \geq A \geq B$ , which means that the average  $T_1$  of carbons on the central ring B are the shortest, and that the other rings have a contribution from internal rotation. In this series, the average  $NT_1$  values of ring B are of the order  $\alpha$ CD  $>$   $\beta$ CD  $>$  DM- $\beta$ CD  $>$  TM- $\beta$ CD complexes ( $0.39 > 0.33 > 0.28 > 0.19$  s), and the ratios of the  $NT_1$  for ring A to ring B are variable. In the  $\alpha$ CD complex, the average  $NT_1$  values of rings A and B are almost the same; hence, they are observed to couple with each other. On the other hand,  $\tau_G$  of ring C is the fastest in this series, indicating that ring C may protrude from the cavity. This situation with **2** does not change at 333K. In the  $\beta$ CD complex, the  $T_1$  values of the aromatic rings are in the order  $C = A > B$ , and it exhibits different values for  $T_1$  for each ring of **2**. The  $\tau_G$  value of ring C is the same order as  $\tau_{\text{eff}}$  ( $12$  and  $15 \times 10^{-11}$  s), but it becomes faster at 333K ( $4 \times 10 \times 10^{-11}$  s). The  $\tau_G$ s for CH<sub>2</sub>OH, on the other hand, are slow. In the DM- $\beta$ CD complex, the  $\tau_{\text{eff}}$  of **2** and DM- $\beta$ CD,  $\tau_G$  of the phenyl ring show the same value ( $18 \times 10^{-11}$  s), indicating that **2** rotates at about the same speed as does the host, DM- $\beta$ CD. At 333K, the  $\tau_G$  becomes fast ( $6 \times 10^{-11}$  s), and the component rings show the different  $T_1$  values, respectively. In the TM- $\beta$ CD complex, the  $T_1$  values are in the order  $A = C > B$ . The ratio of the  $NT_1$  for ring B to ring A is large. The  $\tau_{\text{eff}}$  of **2** is slower than that of TM- $\beta$ CD ( $26$  and  $18 \times 10^{-11}$  s).

In the **2**-series, the ratios of the average  $NT_1$  of component rings in azo dyes depend on the inner diameter of CDs, the temperature, and the substitution of Me on the inside of the cavity. Raising the temperature renders both  $\tau_{\text{eff}}$  and  $\tau_G$  faster. The latter gets slower in CDs as the overall length of the guest molecule increases. The ratios of  $\tau_{\text{eff}}$  of **2** to CDs were of the order TM- $\beta$ CD  $>$  DM- $\beta$ CD  $>$   $\beta$ CD  $>$   $\alpha$ CD ( $1.4 > 1.0 > 0.8 > 0.7$ ). It is apparent that the major factor which prevents the rotation of **2** in CDs is not the inner diameter of the cavity *per se*. It is the increase in the torus height

TABLE II

<sup>13</sup>C-n.m.r. (125 MHz) relaxation times ( $T_1$ ) for CDs, azo dyes, and their complexes<sup>a</sup>

Compound <sup>b</sup>	$NT_1$ (s)									
CDs	C-1	C-2	C-3	C-4	C-5	C-6	$\langle T_1 \rangle_{1-5}^c$	MeO-2	MeO-3	MeO-6
$\alpha$ CD	0.37	0.38	0.38	0.36	0.37	0.53	0.37			
2- $\alpha$ CD	0.27	0.27	0.27	0.26	0.27	0.30	0.27			
	0.29									
2- $\alpha$ CD	0.28	0.30	0.31	0.34	0.38	0.36	0.33			
	0.33									
3- $\alpha$ CD	0.28	0.32	0.25	0.31	0.31	0.30	0.29			
$\beta$ CD <sup>d</sup>	0.33	0.34	0.35	0.36	0.34	0.42	0.34			
1- $\beta$ CD	0.28	0.30	0.30	0.29	0.31	0.40	0.30			
	0.22									
2- $\beta$ CD	0.25	0.27	0.26	0.25	0.26	0.28	0.26			
2- $\beta$ CD	0.28	0.30	0.31	0.30	0.31	0.34	0.30			
DM- $\beta$ CD	0.33	0.34	0.33	0.30	0.32	0.38	0.32	4.38		4.44
1-DM- $\beta$ CD	0.26	0.26	0.26	0.26	0.26	0.30	0.26	3.72		3.60
	0.24		0.27					3.72		
1-DM- $\beta$ CD	0.28	0.29	0.26	0.27	0.29	0.34	0.28	3.99		4.47
2-DM- $\beta$ CD	0.24	0.30	0.28	0.28	0.27	0.32	0.27	3.12		3.57
2-DM- $\beta$ CD	0.26	0.27	0.29	0.28	0.24	0.32	0.27	3.48		3.93
TM- $\beta$ CD <sup>e</sup>	0.23	0.28	0.25	0.24	0.25	0.28	0.25	3.06	2.85	3.30
1-TM- $\beta$ CD	0.21	0.24	0.26	0.24	0.22	0.26	0.24	3.66	3.00	3.36
	0.22							3.63	2.85	3.45
1-TM- $\beta$ CD	0.25	0.31	0.27	0.25	0.27	0.32	0.27	3.51	3.09	3.87
	0.25									
2-TM- $\beta$ CD	0.26	0.25	0.29	0.29	0.24	0.26	0.27	3.27	3.00	3.72
	0.25							3.51	2.82	
2-TM- $\beta$ CD	0.26	0.27	0.28	0.29	0.24	0.36	0.27	3.63	3.03	3.84
$\gamma$ CD	0.33	0.35	0.35	0.34	0.34	0.40	0.34			
1- $\gamma$ CD	0.27	0.27	0.28	0.29	0.26	0.30	0.27			
1- $\gamma$ CD	0.28	0.28	0.30	0.27	0.25	0.30	0.28			
1	2	3	6	7	Me					
I <sup>f</sup>	0.76	0.74	0.58	0.59	6.81					
	[1.3]		[1.0]		[11.5]					
1- $\beta$ CD	0.53	0.56	0.49	0.51	6.30					
	[1.1]		[1.0]		[12.6]					
1-DM- $\beta$ CD	0.35	0.38	0.32	0.32	4.29					
	[1.1]		[1.0]		[13.4]					
1-DM- $\beta$ CD	0.54	0.54	0.36	0.51	6.69					
	[1.2]		[1.0]		[15.2]					
1-TM- $\beta$ CD	0.34	0.37	0.27	0.25	4.35					
	[1.4]		[1.0]		[16.7]					
1-TM- $\beta$ CD	0.45	0.38	0.33	0.33	5.31					
	[1.3]		[1.0]		[16.1]					
1- $\gamma$ CD	0.29	— <sup>g</sup>	— <sup>h</sup>	0.30	3.15					
	[1.0]		[1.0]		[10.5]					

1- $\gamma$ CD	0.34 [1.0]	0.37	0.33 [1.0]	0.35 [11.4]	3.87				
2	2	3	6	7	10	11	12		
2- $\alpha$ CD	— <sup>a</sup> [1.0]	0.38	— <sup>b</sup> [1.0]	0.39	0.87	0.81	0.40		
2- $\alpha$ CD	0.65 [1.0]	0.61	0.57 [1.0]	0.64	1.42	1.32	0.62		
2- $\beta$ CD	0.42 [1.3]	0.45	0.34 [1.0]	0.31	0.45	0.40	0.30		
2- $\beta$ CD	0.71 [1.5]	0.68	0.47 [1.0]	0.48	0.89	0.78	0.45		
2-DM- $\beta$ CD	0.28 [1.0]	0.26	0.28 [1.0]	0.28	0.34	0.29	(0.24)		
2-DM- $\beta$ CD	0.41 [1.3]	0.41	0.29 [1.0]	0.33	0.45	0.45	0.22		
2-TM- $\beta$ CD	0.23 [1.5]	0.35	0.17 [1.0]	0.21	0.29	0.25	--		
2-TM- $\beta$ CD	0.34 [1.3]	0.31	0.26 [1.0]	0.25	0.37	0.36	0.33		
3	1	3	2'	3'	6	7	10	11	12
3	0.23	0.38 [1.1]	0.29	0.26 <sup>i</sup>	0.30 [1.0]	0.31	0.50 [1.7]	0.51	0.31
3- $\alpha$ CD	0.26	0.37 [0.9]	— <sup>j</sup>	0.33 <sup>j</sup>	0.37 [1.0]	0.40	0.77 [1.9]	0.71	— <sup>h</sup>

<sup>a</sup> The NT<sub>1</sub> values measured at 307K are indicated by numerals in Roman type, and those measured at 333K are indicated by numerals in italics. The values shown in brackets are average NT<sub>1</sub> ratios of ring A or C to ring B in 1-3. <sup>b</sup> See formulae for numbering of 1-3; 2 is solubilised only upon the formation of complexes. <sup>c</sup> Mean values for C-1 ~ C-5. <sup>d</sup> Measured at 0.05M. <sup>e</sup> Measured at 0.07M. <sup>f</sup> When glucose was added instead of the CD, the NT<sub>1</sub> value of the azo dyes did not change. <sup>g</sup> Overlapped with C-6. <sup>h</sup> Lines were too broad to get NT<sub>1</sub> values. <sup>i</sup> Overlapped with C-11. <sup>j</sup> The NT<sub>1</sub> values are smaller as the position is *para* to the SO<sub>3</sub>Na group.

TABLE III

Rotational correlation times ( $\tau_{\text{eff}}$  and  $\tau_G$ ) of CDs, azo dyes, their complexes and motional states of azo dyes in CDs<sup>a</sup>

Compound CD	Correlation Times ( $10^{-11}$ s)	
	CD overall ( $\tau_{\text{eff}}$ ) <sup>b</sup>	CH <sub>2</sub> OH internal ( $\tau_G$ )
$\alpha$ CD	13	12
2- $\alpha$ CD	18	78
2- $\alpha$ CD	15	65
3- $\alpha$ CD	17	199
$\beta$ CD	14	30
1- $\beta$ CD	17	21
2- $\beta$ CD	19	100
2- $\beta$ CD	16	64

DM- $\beta$ CD	15	39
1-DM- $\beta$ CD	19	50
1-DM- $\beta$ CD	18	30
2-DM- $\beta$ CD	18	44
2-DM- $\beta$ CD	18	44
TM- $\beta$ CD	20	61
1-TM- $\beta$ CD	21	105
1-TM- $\beta$ CD	18	44
2-TM- $\beta$ CD	18	-
2-TM- $\beta$ CD	18	22
$\gamma$ CD	14	42
1- $\gamma$ CD	18	78
1- $\gamma$ CD	18	78

1-3	Substrate overall ( $\tau_{\text{eff}}$ ) <sup>c</sup>	Phenyl internal ( $\tau_G$ )	$\tau_{\text{cdx-dye}}/\tau_{\text{cdx-dye}}$
1	8		
1- $\beta$ CD	10		0.6
1-DM- $\beta$ CD	15		0.8
1-DM- $\beta$ CD	11		0.6
1-TM- $\beta$ CD	19		0.9
1-TM- $\beta$ CD	15		0.8
1- $\gamma$ CD	16		0.9
1- $\gamma$ CD	14		0.8
2- $\alpha$ CD	13	3	0.7
2- $\alpha$ CD	8	2	0.5
2- $\beta$ CD	15	12	0.8
2- $\beta$ CD	10	4	0.6
2-DM- $\beta$ CD	18	18	1.0
2-DM- $\beta$ CD	16	6	0.9
2-TM- $\beta$ CD	26	- <sup>d</sup>	1.4
2-TM- $\beta$ CD	19	50	1.1
3	16	7	
3- $\alpha$ CD	13	- <sup>d</sup>	0.8

<sup>a</sup> The results measured at 307K are indicated by the numerals in Roman type, and those measured at 333K are indicated by numerals in italics. <sup>b</sup> Calculated using the mean values of  $T_1$  for C-1 ~ C-5. <sup>c</sup> Calculated by using the mean values of  $T_1$  for the carbons of ring B of the azo dyes in Table II. <sup>d</sup> The signal of C-12 was too broad to obtain an exact  $T_1$  value.

brought about by substituting CDs with the Me group, as well as the increase of the viscosity<sup>4</sup>, that is responsible for limiting rotation. At 333K, the rotation of **2** becomes faster ( $1.1 > 0.9 > 0.6 > 0.5$ ), and decreasing the viscosity by raising temperature has more influence on  $\tau_{\text{eff}}$  values of **2** than on those of the CDs. The ratios, however, maintain about the same order of magnitude. The ratio of  $\tau_{\text{eff}}$  of azo dyes to CDs are sensitive to the following host-guest interactions: (i.) the overall lengthening of the guest molecule ( $1 = 0.8$ ,  $2 = 1.0$  in DM- $\beta$ CD), (ii.) substitution on the CD (1 in DM- $\beta$ CD =

TABLE IV

Azo dye-induced  $^{13}\text{C}$  chemical shifts of CDs<sup>a</sup> (p.p.m.)

<i>a</i>	<i>α-Ep</i> <sup>b</sup>	<i>β</i>	<i>β-Ep</i>	<i>DM-βCD</i>	<i>TM-βCD</i>	<i>γ</i>	<i>γ-Ep</i>
<b>Compound 1</b>							
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	-0.4 -0.2
6'				-0.4			0.3
2Me					-0.1	-0.1	
3Me						1.4	
6Me					-0.2	0.0	
CH <sub>2</sub> OR		0.0		-0.3			0.2
CHOH		0.1		-0.2			0.1
CH <sub>2</sub> OH		0.0		-0.3			0.0
<b>Compound 2</b>							
1	0.5	0.8	0.1	0.0	0.1	1.9	0.7
1'		0.6		0.3			
2	0.1		-0.4		-0.2	0.7	0.2
3	-0.2		-0.1		0.1	0.2	0.2
4	-0.1	1.4	-0.3	0.0	0.0	2.8	0.4 -0.1
5	0.0		0.0		0.0	0.3	0.1
6	-0.5	-0.2	-0.7	-0.5	-0.4	-0.2	-0.3 -0.5
6'				-0.3			-0.2
2Me					0.0	-0.1	
3Me						0.8	
6Me					0.2	0.0	
CH <sub>2</sub> OR		0.0		-0.1			0.0
CHOH		0.1		-0.1			-0.1
CH <sub>2</sub> OH		0.0		-0.2			-0.1
<b>Compound 3</b>							
1	0.6	0.5	0.3	0.2	0.1	1.8	0.6
1'		0.5		0.2			
2	0.0		-0.3		-0.2	0.7	0.1
3	-0.3		0.1		0.1	0.2	0.2
4	-0.2	1.5	-0.2	-0.1	-0.1	2.5	0.2
5	-0.1		0.1		0.0	0.1	0.0
6	-0.7	-0.6	-0.6	-0.5	-0.3	-0.2	-0.3 -0.3
6'				-0.2			-0.1
2Me					-0.1	0.0	
3Me						0.8	
6Me					0.2	0.0	
CH <sub>2</sub> OR		0.0		-0.1			0.1
CHOH		0.0		-0.1			0.1
CH <sub>2</sub> OH		-0.1		-0.2			0.0

<sup>a</sup> A positive sign indicates a low-field shift. <sup>b</sup> Ep means epichlorohydrin product. <sup>c</sup> The "\*\*\*\*" means new shifts induced by the substitution.



0.8, and in TM- $\beta$ CD = 0.9; **2** in  $\beta$ CD = 0.8, in DM- $\beta$ CD = 1.0; and in TM- $\beta$ CD = 1.4); (iii.) substitution at the *meta*-position of the azo dye (**2** = 0.5 and **3** = 0.8 in  $\alpha$ CD), (iv.) inclusion of more than one azo dye in the large cavity (**1** in  $\gamma$ CD) = 0.9), and (v.) lowering the temperature (**2** in  $\alpha$ CD) = 0.7 at 307K and 0.5 at 333K).

*Induced chemical shifts of CD-azo dye complexes. — A. Azo dye-induced chemical shifts of CDs.* Table IV shows the azo dye-induced chemical shifts of CD molecules with a molar ratio of 1.0 for host–guest molecules.

The  $\alpha$ CD complexes cause large low- and high-field shifts of almost the same magnitude at both the C-1 position of the ring juncture and at the narrow-rim side of the molecule. The  $\beta$ CD-,  $\beta$ CD-Ep-, and DM- $\beta$ CD complexes all cause the carbons of the narrow-rim side to move to higher field, while the carbons of the ring juncture and substituted Me and glyceryl ether groups shift only a small amount. DM- $\beta$ CD serves as a long and strainless hydrophobic environment (like dioxane solvent) for azo dyes. TM- $\beta$ CD induces the largest distortion in all CDs to the wide-rim side, to the MeO-3 signal, and to the ring juncture, but not to the narrow-rim side, as the signal of the MeO group still shows a doublet. The  $\gamma$ CD complexes show shifts at both the 1- and 6-positions. The behaviour resembles that of the  $\alpha$ CD complex, but in the former the shifts of the carbons of the narrow-rim sides are smaller than those of the ring juncture, indicating that the narrow-rim side is not as crowded as that of the  $\alpha$ CD complex.

*B. CD-induced chemical shifts of azo dyes. —* Previously, the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD-induced chemical shifts of **1** were investigated<sup>2</sup>. Inclusion shifts in the  $\alpha$ CD complex cluster at low field. When the inner diameter of the cavity becomes large relative to the width of the guest molecule, inclusion shifts spread to higher field, especially on the *N,N*-dimethylaniline side of the guest. The fact that inclusion shifts in the  $\alpha$ CD complex appear together at lower field may be due to van der Waals contact effects.  $\gamma$ CD-Induced chemical shifts of **1**, on the other hand, cluster at higher field. Studies of this complex by circular dichroism<sup>6b</sup> suggest the parallel planar arrangement of the two **1**s induced to the annular axis of  $\gamma$ CD. In the <sup>13</sup>C-n.m.r. work, such a situation induces a ring-current effect of each molecule of **1** upon each other, resulting in the shift to higher field. Nine types of CDs having different inner diameters and heights of hydrophobic torus ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -CDs,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CDs-Ep,  $\alpha$ CD-DiEp, DM- $\beta$ CD and TM- $\beta$ CD) were used to include azo dyes. The torus heights of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CDs and DM- $\beta$ CD are  $\sim 8\text{\AA}^{10a}$  and  $\sim 11\text{\AA}^{10b}$ , respectively.  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CDs-Ep, which were proved to be substituted CDs having one or two glyceryl groups per one glucose at the C-2, C-3, and C-6 positions, have longer tori<sup>2</sup>. The length between the carbons of both ends on the component aromatic rings of **1** is  $\sim 11\text{\AA}$ , and those of **2** and **3** are  $\sim 15\text{\AA}$ .

Previously Shibusawa *et al.*<sup>11</sup> have examined the spectral shifts of 4-aminoazobenzene derivatives in various media and found that the formation of the complex with CD renders their absorption maxima almost the same as those observed in dioxane. Also, in their <sup>13</sup>C-n.m.r. spectra, the chemical shifts of the substrate in the strainless host–guest orientation appear much as the shifts measured in hydrophobic dioxane. In order to check the role of the hydrophobic nature of the cavity in the shifts of the

inclusion complexes,  $^{13}\text{C}$ -n.m.r. spectra of azo dyes were measured in dioxane. CD-induced chemical shifts of the guest molecules with a molar ratio of one for guest–host molecules and the dioxane-induced solvent shifts of azo dye are shown in Figs. 1–3. When the solvent shift of **1** was compared with inclusion shifts of **1** in  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CDs, the former resembles most closely the inclusion shift of the  $\beta$ CD complex. Thus the shifts observed upon inclusion of substrate in the  $\beta$ CD complex may be mainly due to hydrophobic nature of the inside of the cavity. Complexes with the CD-derivatives show almost the same inclusion shifts as those found in the complexes with the parent CDs.

For compounds **2** and **3**, the correlation between the inclusion shifts and the solvent shifts was not close. In the solvent shifts, C-1 and C-2 bearing  $-\text{SO}_3\text{Na}$  show the largest shifts, while C-9, C-5, and C-12 show the next largest shifts. In the shifts observed upon inclusion with  $\alpha$ - and  $\beta$ CDs, carbons in rings B and C show large low-field shifts, while in the **2**- $\gamma$ CD complex, all shifts move to the higher field location.

In the  $\beta$ -series, shift values between C-8, C-10, and C-1 in DM- $\beta$ CD and  $\beta$ CD complexes are smaller compared with those of the TM- $\beta$ CD and  $\beta$ CD-Ep complexes. In the **2**-DM- $\beta$ CD complex, DM- $\beta$ CD shows scarcely any shifts induced by complexation

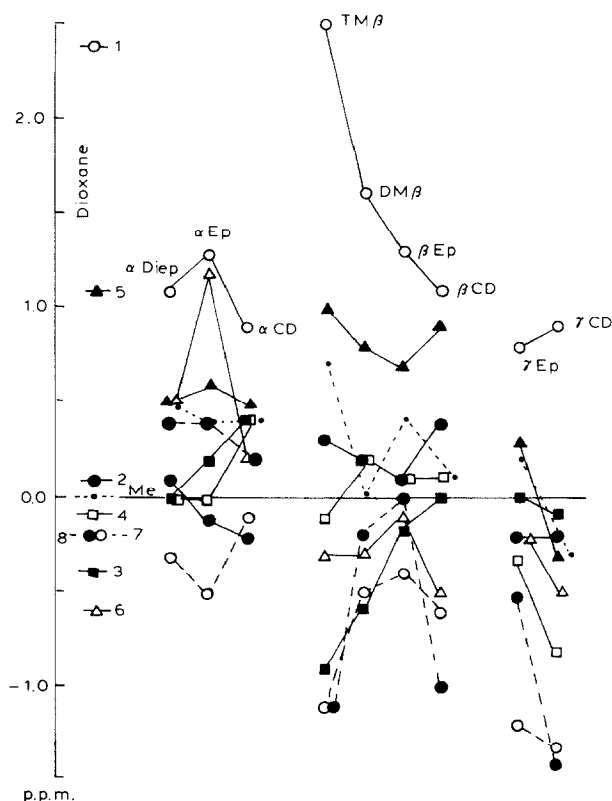


Fig. 1. Nine types of CD-induced  $^{13}\text{C}$ -n.m.r. chemical shifts and the dioxane-induced solvent shift of **1**.

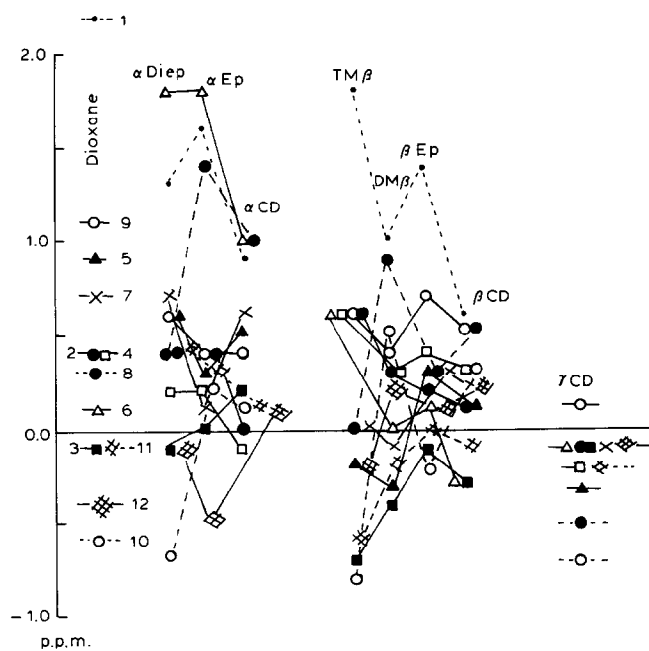


Fig. 2. Eight types of CD-induced  $^{13}\text{C}$ -n.m.r. chemical shifts and the dioxane-induced solvent shift of 2.

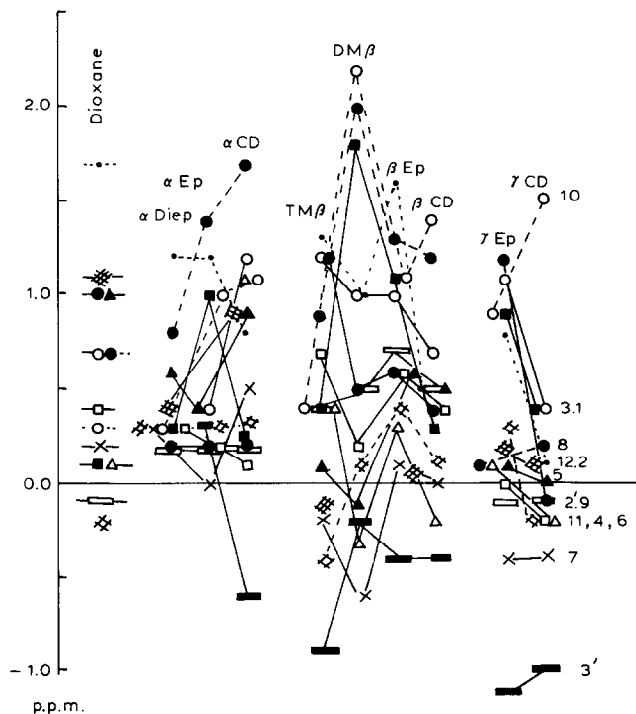


Fig. 3. Nine types of CD-induced  $^{13}\text{C}$ -n.m.r. chemical shifts and the dioxane-induced solvent shift of 3.

with **2**, and, as judged from  $T_1$  data, the whole of **2** bears the influence of inclusion. Thus the chemical shift of **2** in DM- $\beta$ CD can be regarded as the inclusion mode, in which the whole molecule is included. The inclusion pattern of the  $\beta$ CD complex resembles that of the DM- $\beta$ CD complex, but  $T_1$  data suggest a looser fit of **2** in the  $\beta$ CD, and, indeed, the A-ring may exist outside of the cavity. TM- $\beta$ CD and  $\beta$ CD-Ep give large plus and minus inclusion shifts, respectively, for **2**. In the **2**-TM- $\beta$ CD complex, **2** induces a large shift at the ring juncture, on the wide-rim side, and at the MeO-3 position. The signals of the MeO group at the wide-rim side show a doublet. The  $\tau_{\text{eff}}$  value of **2** in TM- $\beta$ CD is slower than that of TM- $\beta$ CD. The circular dichroism spectrum also shows anomalies<sup>6c</sup>, indicating that, for TM- $\beta$ CD complexes, more detailed studies are mandated.

The  $\alpha$ -series also shows the deformation shifts between rings B and C. In the **2**- $\alpha$ CD complex, the C-3 and C-6 signals (especially the C-6 signal) broaden and move to lower field. This is due to the fact that the small internal diameter of the  $\alpha$ CD causes exchange broadening in the process of chemical exchange and greater transverse relaxation rates of the portions in the neighbourhood of N=N than those of others, resulting in signal broadening<sup>12</sup>. Ring B is restricted on the narrow-rim side of the CD. The chemical shifts of ring C cluster closely together, and the  $T_1$  data indicate that rings A and B couple and that ring C is outside of the cavity. In the **2**- $\alpha$ CD-Ep and **2**- $\alpha$ CD-DiEp complexes, the shifts upon inclusion are more exaggerated, and, in the latter complex, the C-8 and C-10 signals move to the higher field location. Thus the substituent DiEp group may cause some inhibition for **2** to be included into the cavity, because the same phenomenon appeared with the **2**- and **3**-TM- $\beta$ CD complexes.

Concerning the  $\gamma$ CD series, all shifts move to the higher field side in the **2**- $\gamma$ CD complex. On the other hand, in the **3**- $\gamma$ CD and **3**- $\gamma$ CD-Ep complexes, the C-10 and C-8 signals show shifts to lower field. The above complexes exist in a 1:1 ratio of host to guest. A comparison of complexes of **2** and **3** shows that substitution at the position *meta* to the azo group in ring A induces a larger deformation of rings B and C upon inclusion.

## CONCLUSIONS

All CDs show the azo dye-induced shifts on the narrow-rim side, and the  $\tau_G$  of the CH<sub>2</sub>OH group gets slower as the guest molecule becomes long and bulky, indicating that a part of azo dye protrudes from the cavity, and, in some cases, the sulfonate group may be hydrogen-bonded to the CH<sub>2</sub>OH group of the adjacent CD. Some of the C-1 signals show doublets, and the CD does not preserve the same conformation on the component glucose unit. DM- $\beta$ CD shows the smallest azo dye-induced shifts in chemical shift.  $\alpha$ - And  $\gamma$ -CDs induce the shift of the carbons at the ring juncture. Substituted methyl and glyceryl ether groups result in shifts to small degrees upon the formation of complexes, although the rotation of the methyl is inhibited to some degree. Only complexation of TM- $\beta$ CD induces large distortions at the wide-rim side, at the ring juncture, and at the MeO-3 position (whereby the signals of the MeO group show a doublet).

Comparisons between the inclusion shift data and that of the solvent shifts in dioxane-D<sub>2</sub>O solution of azo dyes, and comparison among the inclusion shift of each of the inclusion complexes make possible an analysis of the inclusion shifts and suggest both the direction and mode of inclusion. For the complex of **1**, the major factor inducing the chemical shifts is the hydrophobic nature of the cavity in the  $\beta$ -series, the van der Waals contact effect in the  $\alpha$ -series, and the ring-current effect in the  $\gamma$ -series. Moreover, for the complexes of both **2** and **3**, the deformation shift must be considered. CD-induced shifts of azo dyes indicate that the hydrophobic site is included in the cavity, and that substitution host and guest molecules with long and bulky groups exacerbate the deformation of the molecule, sometimes causing the inclusion ratio to change.

Inner diameters and heights of the torus of CDs change the inclusion mode of azo dyes. In the **2**- $\alpha$ CD complex, the values of  $T_1$  and  $\tau$  and the broadening of the signal suggest the partial inclusion of rings A and B, with ring C protruding from the cavity. In the **2**- $\beta$ CD complex, the fact that the ratio of  $T_1$  values for ring A to ring B is large, together with the fact that  $\tau_{\text{eff}}$  and  $\tau_G$  values are of the same order of magnitude, suggests that ring A is likely to be outside of the cavity and that ring C inside the cavity. In the **2**-DM- $\beta$ CD complex, the inclusion mode of the whole part can be observed.

The ratio of  $\tau_{\text{eff}}$  of azo dyes to CD is also sensitive to the host-guest interaction and indicates the fact that azo dyes are included by various modes which do not necessarily depend on the inner diameter of the cavity. Upon raising the temperature, most chemical shifts of azo dyes induce only shifts to lower field, but  $\tau$ -values give us more detailed information. For example, in the **2**-DM- $\beta$ CD complex, all  $\tau$ -values (rings A, B, C, and DM- $\beta$ CD) at 307K are  $18 \times 10^{-11}$  s, but at 333K the  $\tau_{\text{eff}}$  of ring A, B, and DM- $\beta$ CD are 12, 16,  $18 \times 10^{-11}$  s, and the  $\tau_G$  of ring C is  $6 \times 10^{-11}$  s, respectively, suggesting that the component rings of **2** can rotate in DM- $\beta$ CD. In this series, some molecules of the dye self-associate, and some complexes become colloidal at 307K.  $T_1$ ,  $T_2$ ,  $\tau_{\text{eff}}$ ,  $\tau_G$ , chemical shifts, broadening and doubling of the signal, as well as the temperature dependence in <sup>13</sup>C-n.m.r. spectra, can give information about all these phenomena.

## REFERENCES

- 1 M. Suzuki, E. Fenyvesi, M. Szilasi, J. Szejtli, M. Kajtar, B. Zsador, and Y. Sasaki, *J. Incl. Phenom.*, **2** (1984) 715-724.
- 2 M. Suzuki, Y. Sasaki, J. Szejtli, and E. Fenyvesi, *J. Incl. Phenom.*, **5** (1987) 459-468.
- 3 M. Suzuki and Y. Sasaki, *Chem. Pharm. Bull.*, **27** (1979) 609-619.
- 4 M. Suzuki, J. Szejtli, and L. Szenté, *Carbohydr. Res.*, **192** (1989) 61-68.
- 5 M. Mishima, *Memoirs. Fac. Sci. Ser. C*, **11**, (1978) 97-118; **12**, (1980) 227-236; **14**, (1983) 211-220; **15**, (1985) 99-110.
- 6 (a) H. Hirai, N. Toshima, and S. Uenoyama, *Polym. J. (Tokyo)*, **13** (1981) 607-610.  
(b) R. J. Clarke, J. H. Coates, and S. Lincoln, *Carbohydr. Res.*, **127** (1984) 181-191.  
(c) M. Komiya, E. Hirai, and K. Kobayashi, *Proc. Symp. Cyclodextrins*, 5th, Kyoto, 1986, p. 12.  
(d) J. Pitha, L. Szenté, and J. Szejtli, in S. D. Bruck (Ed.), *Controlled Drug Delivery*, Vol. 1., CRC Press, Boca Raton, FL, 1983, p. 124.  
(e) M. Suzuki, M. Kajtar, E. Fenyvesi, M. Vikmon, and J. Szejtli, unpublished results.
- 7 A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.*, **55** (1971) 189-198.

- 8 D. Doddrell, V. Glushko, and A. Allerhand, *J. Chem. Phys.*, 56 (1972) 3683–3689.
- 9 D. E. Woessner, B. S. Snowden, and G. H. Meyer, *J. Chem. Phys.*, 50 (1969) 719–721.
- 10 J. Szejtli, *Cyclodextrin and Their Inclusion Complexes*, Akademiai Kiado, Budapest, 1982, (a) p. 25, (b) p. 151.
- 11 Y. Shibusawa, T. Hamayori, and R. Sasaki, *Nippon Kagaku Kaishi*, (1975) 2171–2177.
- 12 R. Bergeron, M. A. Channing, G. J. Giverily, and D. M. Pillor, *J. Am. Chem. Soc.*, 99 (1977) 5146–5151.