[Chem. Pharm. Bull. 27(6)1343—1351(1979)]

UDC 547.556.33.08 : 543.422.45

Inclusion Compounds of Cyclodextrin and Azo Dyes. II.[1] ^{1}H Nuclear Magnetic Resonance and Circular Dicroism Spectra of Cyclodextrin and Azo Dyes with a Naphthalene Nucleus[2]

Miyoko Suzuki and Yoshio Sasaki

Faculty of Pharmaceutical Sciences, Osaka University[3]

(Received October 20, 1978)

Azo dyes with a hydroxylated α-naphthyl group form 1:1 complexes with cyclodextrin. The nature of the substituent, the bulkiness of the molecule, the change of the ^{1}H nuclear magnetic resonance (NMR) spectral pattern of cyclodextrin and the signs of the maxima in circular dicroism (CD) spectra indicate the specific orientation and freedom of motion in the cavity of the guest molecules after inclusion. For example, in orange II-β-cyclodextrin complex, the ^{1}H NMR spectrum of β-cyclodextrin rules out inclusion from the benzenesulfonate side of orange II. The CD spectrum suggests inclusion from the short molecular axis side and tight fitting with β-cyclodextrin.

The azo dyes investigated are longer than the depth of the cavity of cyclodextrin. Nevertheless, the inclusion shifts of the guest molecules are not localized at one side of the molecule. The whole H_2O structure around the azo dye is probably broken down upon inclusion, or the included azo group produces inclusion shifts at all the ring protons.

Keywords——azo dye; naphthalene nucleus; α-cyclodextrin; β-cyclodextrin; inclusion compound; NMR; complex formation ratio; orientation; position

Introduction

The cavity of cyclodextrin (cdx) can contain many organic molecules to form inclusion compounds. The binding state of the host and guest molecules has been studied by circular dicroism (CD), ultraviolet (UV) and X-ray[4–7] techniques and binding is thought to be mainly due to H-bonding, van der Waals forces and hydrophobic interaction. Recently, studies by nuclear magnetic resonance (NMR) have become practical;[8–13] the chemical shifts and relaxation times indicate the orientation of the guest molecule and its freedom of movement. Bergeron *et al.*[11] reported that sodium *p*-nitrophenolate enters from the uncharged, hydrophobic NO_2 group site and is partially included by α-cdx. Moreover, the guest molecule is fixed firmly in the cavity and cannot move. Saenger *et al.*[8] concluded from the results of both

1) Part I: M. Suzuki and Y. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **27**, 609 (1979).
2) A part of this work was presented at the 97th Annual Meeting in Tokyo, April, 1977 and the 98th Annual Meeting in Okayama, April, 1978.
3) Location: *133-1, Yamada-Kami, Suita, Osaka, 565, Japan.*
4) F. Cramer and H. Hetller, *Naturwissenschaften*, **49A**, 625 (1967).
5) F. Cramer, W. Saenger, and H-ch. Spats, *J. Am. Chem. Soc.*, **89**, 14 (1967).
6) *a*) M. Otagiri, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **23**, 188 (1975); *b*) *Idem, ibid.*, **23**, 201 (1975); *c*) *Idem, ibid.*, **23**, 1421 (1975).
7) *a*) K. Harata, *Bull. Chem. Soc. Jpn.*, **48**, 375 (1975); *b*) *Idem, ibid.*, **48**, 2409 (1975); *c*) *Idem, ibid.*, **48**, 3409 (1975); *d*) *Idem, ibid.*, **49**, 1493 (1976); *e*) *Idem, ibid.*, **49**, 2066 (1976); *f*) *Idem, ibid.*, **50**, 1259 (1977); *g*) *Idem, ibid.*, **50**, 1416 (1977).
8) D.J. Wood, F.E. Hruska, and W. Saenger, *J. Am. Chem. Soc.*, **99**, 1735 (1977).
9) K. Takeo, K. Hirose, and T. Kuge, *Chem. Lett.*, **1973**, 1233.
10) P. Demarco and A.L. Thkkar, *Chem. Commun.*, **1970**, 2.
11) *a*) R. Bergeron and R. Rowan, *Bioorg. Chem.*, **5**, 425 (1976); *b*) *Idem, ibid.*, **5**, 437 (1976); *c*) R. Bergeron, M.A. Channing, G.J. Giveily, and D.M. Pillor, *J. Am. Chem. Soc.*, **99**, 5146 (1977).
12) J.P. Behr and J.M. Lehn, *J. Am. Chem. Soc.*, **98**, 1743 (1976).
13) K. Uekama, F. Hirayama, and H. Koinuma, *Chem. Lett.*, **1977**, 1393.

X-ray and NMR studies that α-cdx includes *p*-iodoaniline from the hydrophobic iodine site and the amino group remains at the periphery of the cavity. Uekama *et al.*[13] also examined the relaxation time of β-cdx and sulfathiazole. They concluded that when sulfathiazole is included, molecular movement is decreased, especially at the phenyl residue, and both the interior and exterior of the cavity of β-cdx are involved in the interaction. The above results indicate that NMR can give information on the inclusion state in solution down to the level of individual atoms.

In the previous paper, the complex formation molar ratio of methyl orange and cyclodextrin, as well as the position and freedom of motion of the former after inclusion were examined by NMR and the following results were obtained. 1) Methyl orange forms 1:1 complexes with α- and β-cdx. 2) Methyl orange is included predominantly from the NMe_2 side. α-cdx includes methyl orange firmly at the N,N-dimethylaniline side. β-cdx passes the N,N-dimethylaniline side and includes methyl orange at the benzenesulfonate side. 3) ^1H NMR showed that α- and β-cdx include methyl orange in the whole of the cavity, and the results of ^{13}C NMR showed that deformation at the linkage positions of glucose is detectable.

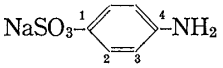
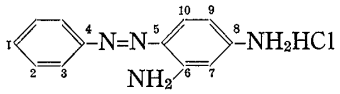
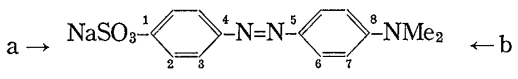
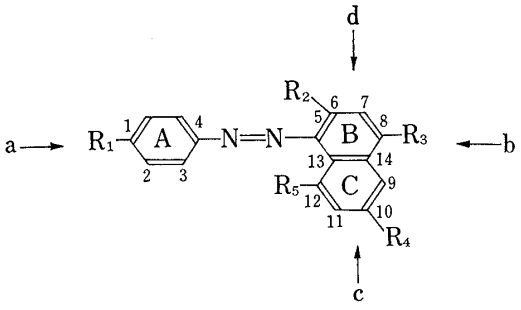
In the present study, inclusion compounds of azo dyes with the more bulky naphthalene nucleus were examined by NMR, CD and UV. Special emphasis was placed on the specific orientation of the guest molecules in the cavity.

Experimental

α-cdx of guaranteed grade was purchased from Nakarai Chemicals Ltd. β-cdx was supplied by Teijin Ltd. and was recrystallized from H_2O then dried over P_2O_5 *in vacuo*. Azo dyes used were standard samples distributed by the National Institute of Hygienic Sciences.

^1H NMR spectra were obtained using a Hitachi type R-22 (90 MHz) spectrometer at 35°. Host molecules were added to ~0.05 M D_2O solution of guest molecules. Tetramethylsilane (TMS) was used as an external reference.

Table I. Guest Molecules tested

	Compound					
I	*p*-Aminobenzenesulfonic acid sodium salt					
II	Crysoizine					
III	Methyl orange					
						
		R_1	R_2	R_3	R_4	R_5
IV	Orange II	SO_3Na	OH	H	H	H
V	Yellow V	SO_3Na	OH	H	SO_3Na	H
VI	Orange I	SO_3Na	H	OH	H	H
VII	Croceine orange	H	OH	H	SO_3Na	H
VIII	Orange G	H	OH	H	SO_3Na	SO_3Na

UV and visible spectra were measured with a Shimadzu Mps-50L spectrophotometer using a 0.1 cm cell.

CD spectra were measured with a Nihon Bunkō ORD/UV-5 spectropolarimeter with a CD attachment using a 0.5 cm cell.

In measurements of UV, visible and CD spectra, $\sim 10^{-4}$ M guest molecule and $\sim 10^{-3}$ M host molecule were dissolved in 0.1 M phosphate buffer at pH 6.0.

Guest molecules and their chemical shifts are shown in Tables I and II.

TABLE II. ^{1}H Chemical Shifts of Azo Dyes and Related Compounds (Hz from TMS)

Compound	1	2	3	6	7	8	9	10	11	12	Me
I		694	644								
II	(	669s[a]	)		504		569	660			
III		728	703	703	616						280
IV		682	619		549	651				682	
V		708	666		582	672	704		710	729	
VI		694	635	659	563						
VII	633s[a]	656br[a]	633s[a]		567	656	688		688	688	
VIII	686m[a]	731	686m[a]		627	719	750		804		

a) s, br and m indicate singlet, broad peak and multiplet, respectively.
Data were measured using a 90 MHz instrument.

To make structural assignments, we used the coupling constant, integral value, additivity rule for substituent chemical shifts, decoupling and nuclear Overhauser effect (NOE) techniques, comparison of the spectral data of related compounds and the change of spectral pattern on inclusion.

In IV—VI, H-2 and H-3 of the A ring were assigned on the basis of their coupling constants, integration values and the results of decoupling experiments. Moreover, by comparison with I and III, the signal at lower field can be assigned to H-2. As for the ring protons of the A ring in VII and VIII, H-2 can only be differentiated from H-1 and H-3. In the B ring, H-7 and H-8 in IV, V, VII, VIII and H-6 and H-7 in VI were assigned by the decoupling technique. The ring protons of the C ring in IV and VI could not be assigned. The ring protons of the C ring in V were assigned by examination of the coupling pattern. The H-9, H-11 and H-12 signals in VII were essentially singlet. It is difficult to differentiate H-9 from H-11 in VIII from their spectral patterns, but when the H-8 signal is irradiated, the singlet at higher field shows NOE (~10%), and is therefore assigned to H-9.[14]

^{1}H NMR spectra of α- and β-cdx were assigned by Demarco.[10]

Results and Discussion

1) ^{1}H NMR

In measurements on a 90 MHz instrument, complete assignment of cdx protons is impossible. In spite of the impossibility of individual assignment, the overall spectral pattern obviously suggests inclusion. As for the H-6 peaks, they show nearly the same shift values[8] and are the highest peaks in the cdx spectra, so their behavior can be followed readily when a guest molecule is included.

When β-cdx is added to IV, the H-3 signal of β-cdx may shift to high field, but the H-6 signal retains its original position or shows a marginal low field shift (Fig. 1). H-6 of cdx is a mixture of the *gauch-gauch* and *gauch-trans* forms with H-5.[7,8] Thus, if the aromatic ring is included in the neighborhood of H-6, H-6 would be affected by the ring current and ought to undergo a high field shift. In fact, the H-6 signal of the complex of III, which penetrates to the H-6 side of cdx according to X-ray[7a] and NMR[1] data, as well as those of V, VI and VII complexes undergo high field shifts. On the other hand, α-cdx complexes of benzenesulfonic acid sodium salt, *p*-iodoaniline and *p*-nitrophenol which are not extruded from the smaller diameter side of cdx according to X-ray data[7b,e,g] show the same behavior

14) R.H. Martin and J.C. Nouls, *Tetrahedron Lett.*, **1968**, 2727.

Table III. α- and β-Cyclodextrin-induced ^1H Chemical Shift of Azo Dye Compounds (Hz)

Compound	add[a]	1	2	3	6	7	8	9	10	11	12	Me
II	β	(3	7	)		15		13	17			
III	g		0	−4	−4	−5						−1
	α		14	27	27	32						22
	β		18	18	32	19						0
IV	g		6	5		5	5	5	5	5		
	α		7	12		10	7	9	9	9		
	β		37	58		65	33				58	
V	g		1	3		3	3	1		1	2	
	α		3	4		4	4	3		3	5	
	β		13	28		26	28	13		13	23	
VI	α		7	11	14	12					7	
	β		18	34	28	20					35	
VII	g	10	13	10		10	10	10		10	10	
	α	(	11	)		9	9	9		9	22	
	β	(	17	)		47	35	41		41	62	
VIII	β	6	10	6		10	10	8		4		

a) Add indicates addition of: α; α-cyclodextrin
β; β-cyclodextrin
g; glucose
A positive sign indicates a low field shift.

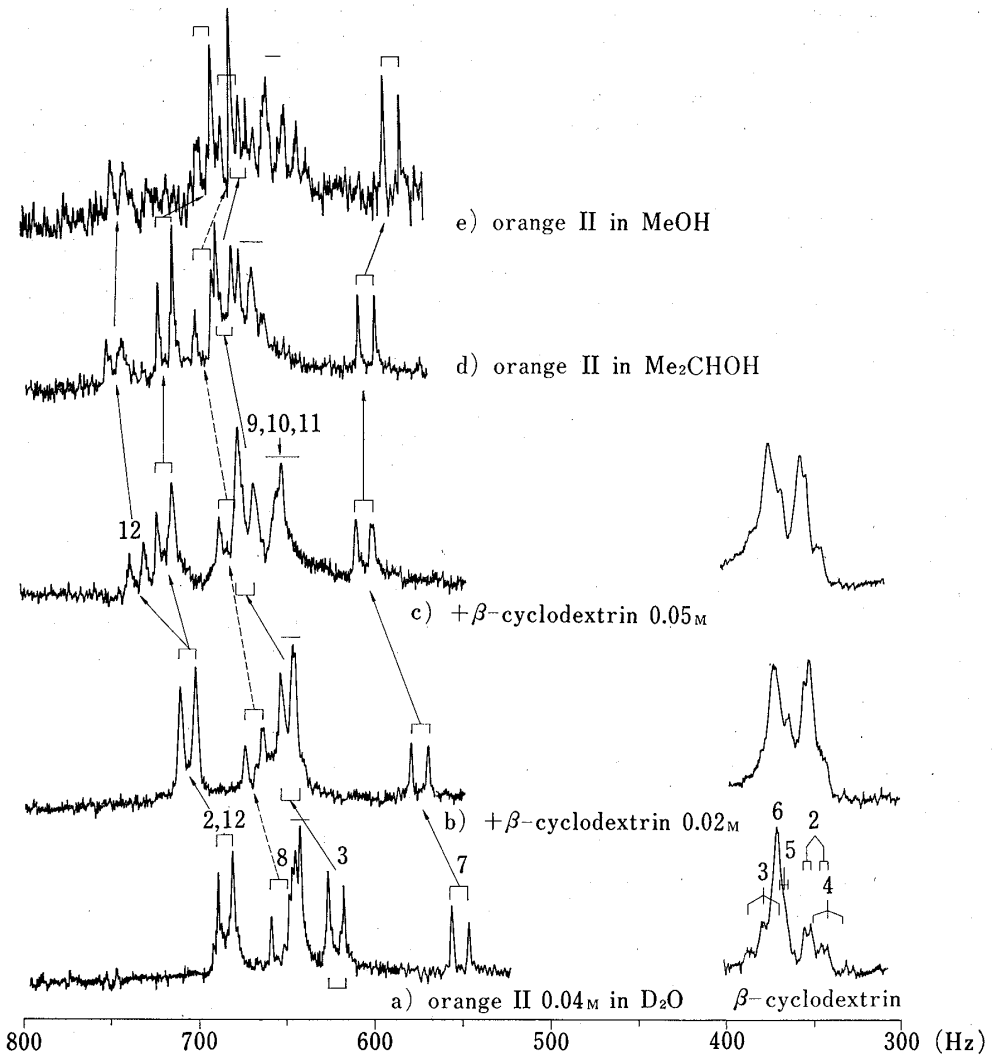


Fig. 1. PMR Spectra of Orange II and β-Cyclodextrin

as the IV-β-cdx complex. Thus, in the case of the IV-β-cdx complex, inclusion may not occur from the benzenesulfonate side of the molecule, which can extrude from the cavity.

On the other hand, the ring protons of IV show selective low field shifts (Fig. 1). Under the same conditions, when glucose and α-cdx are added instead of β-cdx, the ring protons show only a marginal low field shift, and selective low field shifts do not occur (Table III). It therefore seems clear that inclusion causes the low field shift. In Fig. 1, H-7 and H-3, which are at *ortho* positions with respect to OH and N=N, respectively, show large shifts. The H-12 signal, the *peri* position with respect to N=N, has the same shift value as the H-2 signal at first, but when β-cdx is added, it gradually shifts to low field and finally both protons separate completely (Fig. 1c). On the addition of β-cdx continuously, the total spectral pattern of IV becomes diminutive and the H-9, H-10 and H-11 signals which formerly were only two peaks separate and show the fine structure. IV and β-cdx form a 1:1 complex as determined from continuous variation plots for the chemical shifts of the signals of H-12 (Fig. 2) and other protons. The signals of H-2 and H-8, which are at a distance from N=N, show low field shifts of about half compared with those of H-3 and H-7. Thus, the naphthalene and benzene nuclei of IV show similar inclusion shifts (Table III). Based on the X-ray data for 1-*p*-nitrobenzeneazo-2-naphthol, which is an analog of the azo dyes used in the present work, the length along the long molecular axis of IV corresponds to about 1.5 times the depth of the cavity of cdx.[15] It is interesting to examine in what manner IV forms the complex with β-cdx. It is perceived that the inclusion effect of the guest molecule is mainly due to hydrophobic interaction with the host molecule.[16] The ^{1}H NMR spectrum of a solution composed of IV and β-cdx in D_2O (Fig. 1c) was compared with those of IV in D_2O, Me_2CHOH and MeOH (Fig. 1a, 1d and 1e). The former resembles the ^{1}H NMR spectra of IV in Me_2CHOH and MeOH rather than that in D_2O, especially as regards selective low field shift, the separation of the 12 position and the splitting of the C ring. When IV is included by cdx, the total spectral pattern shows the same effect as when IV is dissolved in a hydrophobic solvent. When β-cdx is added to VII, the former compound shows high field shifts at the H-3, H-5 and H-6 regions. This behavior resembles that of the III-α- and -β-cdx complexes[1] rather than that of the IV-β-cdx complex, though the behavior of the former compounds is more marked (*cf.* Fig. 3b). The hydrophobic benzene ring probably enters first and is partly extruded from the smaller diameter side of the cavity.

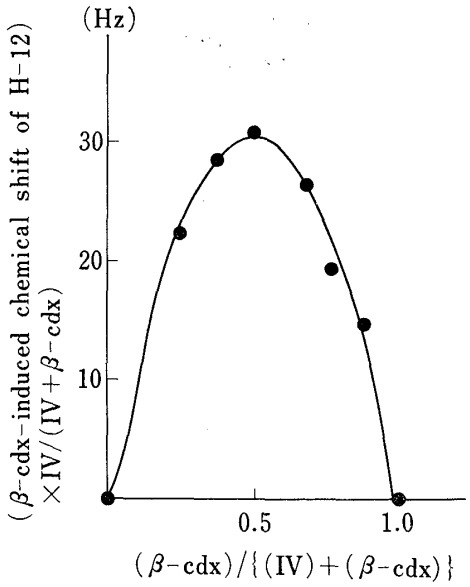


Fig. 2. Continuous Variation Plots of the β-Cyclodextrin-induced Chemical Shift of Orange II (H-12)

In VII, the H-9, H-11 and H-12 and the H-1, H-3 and H-8 signals show nearly the same chemical shifts, so the signal pattern is simple (*cf.* Fig. 3a). When β-cdx is added, the H-9, H-11 and H-12 signals separate and coupling appears. On the other hand, the H-1, H-2 and H-3 signals coalesce nearly into a singlet (*cf.* Fig. 3b). When the inclusion effect of VII is examined in terms of shift values only, that on the benzene side is as small as if inclusion has not occurred (Table III). However, a clear change of the spectral pattern occurs at

15) C.T. Grainger and J.F. McConnell, *Acta Cryst.*, **B25**, 1962 (1969).
16) *a*) T. Shibusawa and Y. Hirose, *Sen-i Gakkaishi*, **29**, 1 (1973); *b*) T. Shibusawa, T. Hamayori, and R. Sasaki, *Nihon Kagaku Kaishi*, 2121 (1975).

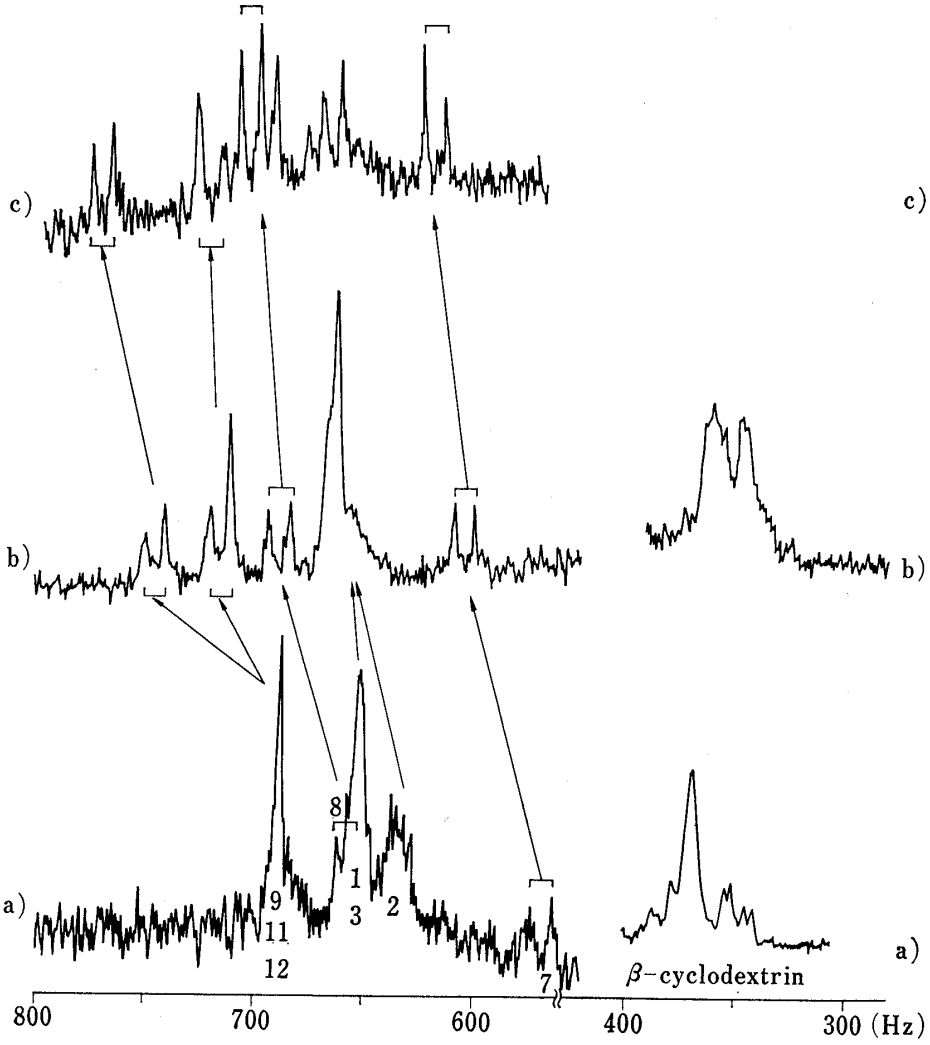


Fig. 3. PMR Spectra of Croceine Orange and β-Cyclodextrin

a) Croceine orange 0.04 M suspension in D_2O.
b) + β-Cyclodextrin 0.02 M.
c) Croceine orange in MeOH.

both the benzene and naphthalene sides of VII at the same time. The behavior of the H-6 signal in β-cdx suggests that the neighborhood is affected by the ring current, and this also confirms inclusion of the benzene side. As to the inclusion state, the CD spectrum supports the above considerations (see the next section). The signal pattern of a D_2O solution containing VII/β-cdx=1/2 resembles that of VII in MeOH except for the benzene nucleus region which is nearly singlet in the former, but multiplet in the latter.

Table III shows α- and β-cdx-induced ^1H chemical shifts of azo dye compounds for a ratio of one cdx molecule per guest molecule. The inclusion effect is in the order β-cdx> α-cdx>glucose, except in the case of III. Judging from the bulkiness of the benzene nucleus and the behavior of the III-β-cdx complex, which includes the benzenesulfonate side,[1)] partial inclusion of the benzene nucleus side in IV—VII should occur, even if the naphthalene nucleus side, which is too large to be included by α-cdx, cannot be included. In the α-cdx complex, the shift values of both the benzene and naphthalene sides of the molecule are not large compared with those of mixtures of the guest molecule and glucose. In the β-cdx complexes also, a tendency for inclusion of the whole molecule was noted. When one SO_3^- is present at either the 1 or the 10 position in the molecule (IV and VII), the ring protons show nearly the same shift values. When the amount of SO_3^- increases (V and VIII), the shift values are decreased,

especially in VIII, which has SO_3^- at the 12 position, and which shows scarcely any inclusion shift.

2) CD and Absorption Spectra

In the ^1H NMR of the cdx complex, it was impossible to obtain information from the guest molecule about the direction of inclusion, because the inclusion shift spreads over all the protons. However, in some host molecules, it was possible to determine the region that excludes inclusion (for example, the benzenesulfonate side in the IV-β-cdx complex). CD

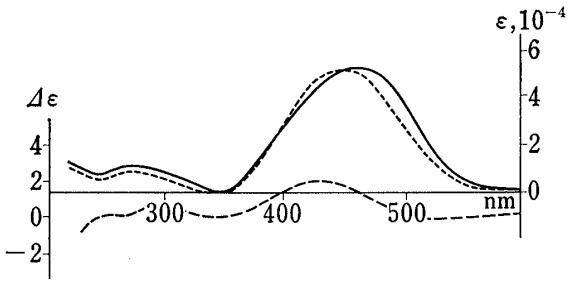


Fig. 4. CD and Absorption Spectra of Methyl Orange and β-Cyclodextrin-Methyl Orange

Absorption spectrum of methyl orange 1.5×10^{-4} M ——
+ β-cyclodextrin 1.5×10^{-3} M ---------.
CD spectrum of methyl orange+β-cyclodextrin -------.

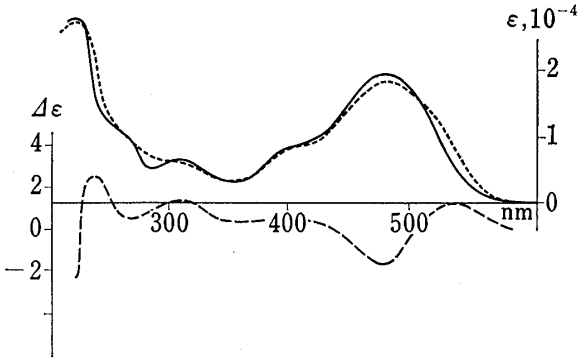


Fig. 5. CD and Absorption Spectra of Orange II and β-Cyclodextrin-Orange II

Absorption spectrum of orange II 2.0×10^{-4} M ——
+ β-cyclodextrin 2.0×10^{-3} M ---------.
CD spectrum of orange II+β-cyclodextrin. -------.

TABLE IV. Induced CD Values of Cyclodextrin-Azo Dye Compounds

Compound	Cyclodextrin	CD_{max}	$\Delta\varepsilon$	$\varepsilon\times10^{-4}$	$g\times10^{4}$
III	α	258	+1.20	0.76	+1.58
		286	+1.81	0.82	+2.11
		416	+2.88	1.89	+1.52
	β	240	+0.53	0.67	+0.79
		290	+0.66	0.81	+0.81
		421	+2.27	2.07	+1.10
IV (pH 6.0)	β	242	+2.58	1.50	+1.73
		310	+1.20	0.67	+1.79
		479	−1.90	1.83	−1.04
		533	+1.20	0.69	+1.74
(pH 12.0)	β	243	+2.86	2.10	+1.36
		289	+0.80	0.93	+0.86
		314	+1.41	0.67	+2.10
		415	+0.64	0.65	+0.98
		482	−1.83	0.96	−1.91
		536	+1.12	0.57	+1.96
V	β	478	+1.28	1.92	+0.67
VI	α	465	+1.18	2.29	+0.52
	β	465	+1.28	2.26	+0.57
VII	α	499	+0.35	0.91	+0.39
	β	316	+0.42	0.51	+0.81
		470	+0.65	0.91	+0.71
		536	−0.29	0.19	−1.56

Dissymmetry factor $g=\Delta\varepsilon/\varepsilon$
where $\Delta\varepsilon$ and ε are the molar circular dichroism and absorption coefficient, respectively, of the complex formed with an azo dye molecule at the given wavelength.

spectra should add further information to aid in determining from which direction inclusion occurs.[7a)]

In IV—VII, inclusion from four directions may be anticipated (Table I). The charged, hydrophilic SO_3^- and the hydrophobic aromatic ring are unfavorable and favorable substituents for inclusion, respectively.[5)] Moreover, the bulkiness of substituents may sometimes fix and sometimes prevent inclusion. Using a Corey–Pauling Koltun molecular model, IV and VI appear to permit inclusion in the cavity from a, b and c directions, but that from d direction seems to be ruled out. In V and VII, SO_3^- at the 10 position prevents inclusion from the b direction. However, from both directions, guest molecules can be situated at the rim of cdx.

As shown in Fig. 4, the III-β-cdx complex has a large positive maximum at 421 nm in the CD spectrum (the N-π^* transition). The inclusion effect of α-cdx is larger than that of β-cdx in all regions (Table IV). It is not surprising that α-cdx should include III more firmly than β-cdx. When β-cdx is added to IV, IV shows CD peaks at all wavelengths. Both negative and positive maxima appear (Fig. 5) especially in the N-π^* transition and UV region. Such phenomena do not appear when α-cdx is added. N-π^* transitions in the V and VI-β-cdx complexes show the same behavior as in the III-β-cdx complex rather than in the IV-β-cdx complex as regards the signs of the maxima. The N-π^* transition of the VII-β-cdx complex resembles that of the IV-β-cdx complex in wavelength and intensity of the maxima, but the signs are reversed so the spectrum is nearly a mirror image. It is known that III in the III-α-cdx complex is fixed firmly in the cavity.[1)] Thus, azo dyes having a positive maximum in the N-π^* transition, such as the III-α-cdx complex, may be included from the a or b direction. The V, VI and VII-cdx complexes are such cases.

On the other hand, reversed signs of the CD spectrum in the IV-β-cdx complex indicate that N=N is included from other directions. Congo red (sodium diphenyldiazo-bis-α-naphthylamine sulfonate) does not shift protons in the interior of α-cdx in ^{1}H NMR, but in the CD spectrum α-cdx gives an induced CD peak in the N=N region which has a negative maximum at 495 nm and a positive maximum at 550 nm.[17)] In this case α-cdx cannot include congo red completely, especially at the N=N region, because α-naphthylaminesulfonate at the end of the molecule is too large. Congo red may therefore be on α-cdx (*c* or *d* direction). In this case the induced CD peak in the N-π^* transition shows the same pattern as that of the IV-β-cdx complex. In the latter case also, inclusion from the c or d direction may occur. The ^{1}H NMR of β-cdx in the IV-β-cdx complex suggests that inclusion might not occur from the benzene nucleus side, while inclusion from the c or d direction is suggested by the CD spectrum. The CD spectrum and the nature of the substituent suggest that III will be

Table V. Correlation between g and δ (Hz)

Cyclodextrin		III	VI	VII		
α	CD	1.5	0.5	0.4		
	^{1}H NMR H-3	27	11	11		
	H-6, H-7	27, 32	14, 12	9		
	H-12		7	22		
		IV	VII	III	V	VI
β	CD	−1.1	0.7	1.1	0.7	0.6
		1.7	−1.6			
	^{1}H NMR H-3	58		18	28	34
	H-6, H-7	65	47	32, 19	26	20
	H-8	33	35		28	
	H-9		37		13	
	H-12	58	62		23	35

17) K. Sense and F. Cramer, *Chem. Ber.*, **102**, 509 (1969).

included from the b direction; though the CD spectrum cannot indicate from which direction (a or b) inclusion occurs, inclusion of the hydrophobic NMe_2 at the b side will be preferred to that of the hydrophilic SO_3^- at the a side. CD spectra and the presence of SO_3^- at the 10 position suggest that V and VII will be included from the a direction.

Thus when complexes between azo dyes and cdx are formed, it is evident that CD spectra cannot only act as an index of complex formation, but can also indicate the orientation of inclusion. It seems clear that IV is included in a quite different manner from the other complexes.

It is interesting to compare CD values (g) in the N-π^* transition with the inclusion shift in ^1H NMR. The g values are in the order III>VI—VII in α-cdx complexes and III—IV—VII>V—VI in β-cdx complexes (Table V). Inclusion shifts of ring protons are in almost the same order as the g values. This order is obviously related to the bulkiness of guest molecules suitable for inclusion.

3) Conclusion

Azo dyes tested form a 1:1 complex with cdx. The nature of the substituent, the bulkiness of the molecule, the changes of the ^1H NMR spectral pattern of cdx and the signs of the maxima in CD spectra indicate the specific orientation and freedom of motion in the cavity of the guest molecules after inclusion. The azo dye tested are longer than the depth of the cavity of cdx. Nevertheless, the inclusion shifts of the guest molecules are not localized at one side of the molecule. The following points were noted.

1) If azo dyes are included loosely, they can move in the cavity and as a result, may show inclusion shifts in the whole molecule. However, in our experiments the inclusion shifts appeared in the whole molecule even in the firmly fixed IV-β-cdx complex.

2) It is thought that the whole H_2O structure around azo dyes is broken down upon inclusion. In this case, the whole molecule may exhibit the inclusion shifts. In the results of NOE[11] and T_1[12] also, the guest molecule shows the inclusion effect over a region larger than the depth of cdx.

3) In general, the reconstruction of the H_2O structure around the exposed parts of an azo dye may occur after the inclusion process.[18] However, in such a case, localization of the inclusion shift must occur, and this was not noted in our experiments.

4) Guest molecules may be included in channel- or the cage-type structures of cdx as in the crystal structures (a 1:2 complex), but the arrangement of host molecules in definite directions in solution in difficult. Continuous variation plots for the inclusion shifts of ring protons in the IV-β-cdx complex suggest the formation of a 1:1 complex (Fig. 2) and do not support the existence of channel- or cage-type structures.

5) When the substituents of an azo dye are introduced into the hydrophobic environment in the cavity, it is expected that different inclusion shifts may occur at the *ortho*, *meta* and *para* protons. For example, in the sodium *p*-nitrophenolate-α-cdx complex, irradiation of the H-3 signal of α-cdx produces intensity enhancements of 9% for the *meta*-H and of 3% for the *ortho*-H. At the same time, low field shifts of 30 Hz for the *meta*-H and of 10 Hz for the *ortho*-H were observed. Based on these results, Bergeron *et al.*[11b] suggested that NO_2 and the *meta*-H are included in the cavity and O^- and the *ortho*-H remain at the periphery of the cavity. Namely, when NO_2 is introduced into the hydrophobic environment, it produces different substituent shifts at the *ortho*-H and the *meta*-H, so even if the *ortho*-H is not included it experiences a low field shift. In our case also, the included azo group may produce inclusion shifts of all the ring protons.

When factors 2 and 5 play a major role, there may be a tendency for the whole molecule to be included.

18) I. Tabushi, Y. Kiyosuke, T. Sugimoto, and K. Yamamura, *J. Am. Chem. Soc.*, **100**, 916 (1978).