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Inclusion Compound of Cyclodextrin and Azo Dye. I. Methyl Orange[1]

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The complex formation molar ratio of methyl orange and cyclodextrin, position and freedom of motion after inclusion were examined by nuclear magnetic resonance (NMR).

1) Methyl orange forms 1:1 complexes with α- and β-cyclodextrin.

2) Methyl orange is included predominantly from the N,N-dimethylamino group side. α-Cyclodextrin includes firmly methyl orange at the N,N-dimethylaniline side and β-cyclodextrin includes methyl orange at the benzenesulfonate side with little perturbation.

3) The data of ^{1}H NMR showed that α- and β-cyclodextrin 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.

Keywords——methyl orange; α-cyclodextrin; β-cyclodextrin; inclusion compound; NMR; complex formation ratio; orientation; position; freedom of motion

Introduction

The cavity of cyclodextrin (cdx) can include many organic molecules to form inclusion compounds. Recently, studies by nuclear magnetic resonance (NMR) have become practical[3–9]; the chemical shift and the relaxation time can indicate the orientation of inclusion and the freedom of movement. Tabushi *et al.*[3] examined in their comprehensive works ^{1}H NMR of 1-anilino-8-naphthalenesulfonate-β-cdx complex and concluded that the phenyl part is the periphery of β-cdx. From the orientational specificity of the inclusion shift value, signal broadening, nuclear Overhauser effect (NOE) and the position of the substituents, Bergeron et al.[7] reported that sodium *p*-nitrophenolate enters from the uncharged, hydrophobic NO_2 group site and is partially included. Moreover, the guest molecule fixes firmly into the cavity and can not move. Saenger *et al.*[4] concluded from the results of both X-ray and NMR that α-cdx includes *p*-iodoaniline from the hydrophobic iodine site and the amino group remains at the periphery of the cavity. Behr *et al.*[8] examined the relaxation time of α-cdx and cinnamic acid derivatives and concluded that upon inclusion the orientation times of the guest molecule increase by a factor of *ca.* 4 and the guest-host molecules have different molecular motions and are weakly coupled from the dynamic point of view. Information from several analogues was described. Uekama *et al.*[9] 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 interiour and exteriour of the cavity of β-cdx interacts.

1) The part of this work was presented at the 27th Annual Meeting of Kinki Branch of Kobe, Nov. 1977 and the 98th Annual Meeting of Okayama, April 1978.
2) Location: *133-1, Yamada-Kami, Suita, Osaka, 565, Japan.*
3) I. Tabushi and T. Nishitani, *Kagaku* (Kyoto), **32**, 768 (1977).
4) D.J. Wood, F.E. Hruska, and W. Saenger, *J. Am. Chem. Soc.*, **99**, 1735 (1977).
5) K. Takeo, K. Hirose, and T. Kuge, *Chem. Letters*, **1973**, 1233.
6) P. Demarco and A.L. Thakkar, *Chem. Commun.*, **1970**, 2.
7) *a*) R. Bergeron and R. Rowan, *Bioorg. Chem.*, **5**, 425 (1976); *b*) R. Bergeron and M.A. Channing, *Bioorg. Chem.*, **5**, 437 (1976); *c*) R. Bergeron, M.A. Channing, G.J. Giverily, and D.M. Pillor, *J. Am. Chem. Soc.*, **99**, 5146 (1977).
8) J.P. Behr and J.M. Lehn, *J. Am. Chem. Soc.*, **98**, 1743 (1976).
9) K. Uekama, F. Hirayama, and H. Koinuma, *Chem. Letters*, **1977**, 1393.

In the present study, the inclusion compounds of methyl orange were examined by ^{1}H and ^{13}C NMR. Methyl orange is one of azo dyes which have simple structures and the complex with α-cdx has been studied in various fields; Tabushi *et al.*[10] published the important study as to the aspects of driving force of inclusion by α-cdx and concluded that van der Waals interaction, conformation energy and water clusters are the most important terms which govern the inclusion process as far as specifity in the substrate binding by cdx is concerned. Moreover they concluded that methyl orange in a 1:1 complex is assumed to preserve a portion of H_2O cluster and eighteen is the estimated difference in number of H_2O molecules of the cluster between uncomplexed and complexed states of methyl orange. Cramer *et al.*[11] showed from visible spectrum that methyl orange and α-cdx can form complexes with higher stoichiometric ratios besides a 1:1 complex when α-cdx concentration is raised. Harata[12] examined that methyl orange forms a 1:2 complex with α-cdx. The present study by NMR may be able to give information of the inclusion state concerning individual atom of methyl orange in solution and become the index of the following study of azo dyes which have the more complex structures. Special emphasis was placed on the differences of behaviour of the guest and host molecules caused by the difference between the diameter of the cavity in α- and β-cdx.

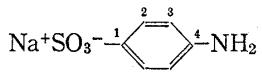
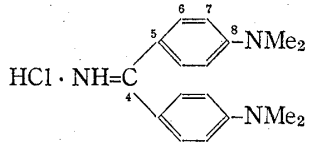
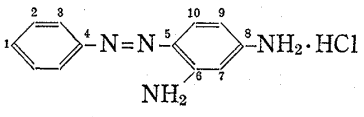
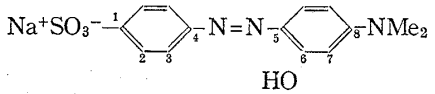
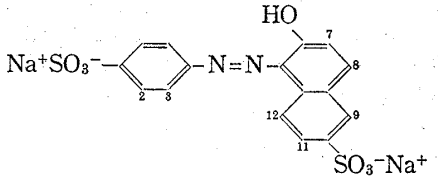
Experimental

α-cdx and guest molecules of guaranteed grade were purchased from Nakarai Chemicals Ltd., and β-cdx was supplied by Teijin Ltd.

^{1}H and ^{13}C NMR spectra were obtained using Hitachi R-22 Type (90 MHz) and R-22 CFT Type (22.6 MHz) spectrometers at 34°. Host molecules were added to 0.05 M D_2O (H_2O in the case of ^{13}C NMR) solution of guest molecules (containing suspension). Tetramethylsilane (TMS) was used as external reference.

^{13}C NMR spectra were measured with maximum frequency 200 ppm, 8 K sampling points, pulse angle 30°, band pass filter 4 KHz, pulse interval 5 sec, wide band decoupling ±500 Hz wide band, 9 watt. Resolution was ±0.06 ppm. A positive sign indicates a low field shift.

Table I. Guest Molecules used

	Compound	
I	Sodium sulfanilate	
II	Auramine	
III	Crysoizine	
IV	Methyl orange	
V	Yellow V	

10) I. Tabushi, Y. Kiyosuke, T. Sugimoto, and K. Yamamura, *J. Am. Chem. Soc.*, **100**, 916 (1978).
11) F. Cramer, W. Saenger, and H-ch. Spatz, *J. Am. Chem. Soc.*, **89**, 14 (1967).
12) K. Harata, *Bull. Chem. Soc. Jpn.*, **49**, 1493 (1976).

Assignment

1) ^{1}H NMR

The guest molecules and their chemical shifts are shown in Table I and II.

Table II. ^{1}H Chemical Shifts of Methyl Orange and Related Compounds (Hz from TMS)

Compound	1	2	3	6	7	8	9	10	11	12	Me
I		694	644								
II				674	614						270
III	(	669 s[a]	)		504		569	660			
IV		728	704	702	616						280
V		708	666		582	672	704		710	729	

a) s indicates singlet.

Coupling constant, peak height, additivity of substituent chemical shift, decoupling technique, comparison of the spectral data of related compounds and the change of a spectral pattern by inclusion have been used for the assignments. In IV, the signal pattern at both positions *ortho* to N=N (H-3 and H-6) broadens on the addition of α-cdx (*cf.* Fig. 2). Comparring the H-2 and H-7 signals in IV with those of I and II, protons *ortho* to the SO_3^- group are obviously situated at lower fields (*cf.* Table II). In V, H-2 and H-3 of the benzene ring with SO_3^- at the 1 position are assigned by coupling constants, integration values and by decoupling experiments. Moreover by comparison with IV, the chemical shift at lower field is assigned to H-2. The H-7 which is at the *ortho* position with respect to OH appears at the highest field. H-7 and H-8 were assigned by decoupling technique of each other. The ring protons of the SO_3^- substituted naphthalene nucleus were assigned by examination of the coupling pattern.

The assignment of α- and β-cdx is given by Demarco *et al.*[6]

2) ^{13}C NMR

C-1 and C-5, C-4 and C-8, C-2 and C-6 of IV in H_2O (34°) show almost the same chemical shifts, so assignment only by the additivity of the substituent chemical shift remains ambiguous. As shown in Table III, using the data from the analogues and from solvent effects, an assignment has been attempted. The closeness of C-4 (154.3 ppm) and C-8 (153.9 ppm) makes differentiation of C-4 and C-8 difficult. C-4 of IV and IV-a show deviations of 0.6 ppm in H_2O and 0.8 ppm in dimethylsulfoxide (DMSO), but the deviation of IV and IV-c reachs 2.5 ppm. Incorporation of a carboxylic acid group at the 3 position causes C-4 to shift to a high field. C-8 of IV, IV-b and IV-c show deviation of 0.7 ppm in DMSO, but that of IV-a shifts 4.3 ppm to high field. Moreover, judging from the acidity of the substituent, it seems that the N=N group experiences stronger solvent effect from the basic DMSO than is the case for the NMe_2 group. It seems reasonable that where $(DMSO-H_2O)_{34°}$ in IV=0.0 ppm the resonance can be assigned to C-8 and where it is 0.4 ppm to C-4.

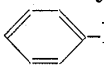
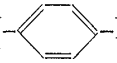
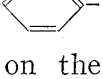
C-1 (143.1 ppm) and C-5 (142.6 ppm). C-1 of IV and IV-a show the same values in H_2O, but are quite different from IV-b and IV-c in DMSO. C-5 of IV, IV-b and IV-c show nearly the same values in DMSO. It is sufficient for interpretation that C-5 of IV and IV-a show different values in H_2O. Moreover SO_3^- with the higher acidity introduces the larger solvent effect $(DMSO-H_2O)_{34°}$=3.2 ppm at C-1 compared with 0.6 ppm at C-5.

C-2 (127.1 ppm) and C-6 (126.0 ppm). C-2 of IV and IV-a show the same chemical shifts, but those of IV-b and IV-c are different. On the other hand, C-6 of all compounds show almost the same values. I and II confirm the above results.

TABLE III. Chemical Shifts of Methyl Orange and Related Compounds
(ppm from TMS)

			4	8	1	5	2	3	6	7	9	10	Me
$Na^+SO^-_3$-C$_6$H$_4$-N=N-C$_6$H$_4$-NMe_2	IV	Calcd.	155.8	153.8	145.8	140.4	126.8	123.3	123.6	113.7			
		in H_2O (95°)	155.2	154.3	144.9	143.7	127.7	122.9	126.3	112.9			
		in H_2O[a)]	154.3	153.9	143.1	142.6	127.1	122.4	126.0	112.4			40.0
		in DMSO	153.9	153.9	146.3	143.2	127.5	122.4	126.1	112.5			
$Na^+SO^-_3$-C$_6$H$_4$-N=N-C$_6$H$_4$-NH-C$_6$H$_5$	IV-a	in H_2O (95°)	154.6	148.9	144.9	141.6	127.4	122.8	125.8	120.9			
		in DMSO	153.1	149.6	145.6	142.1	127.5	122.3	125.7	120.2			
C$_6$H$_5$-N=N-C$_6$H$_4$-NMe_2	IV-b	in DMSO	153.2	153.2	130.2	143.4	129.9	122.6	125.4	112.3			
COOH-C$_6$H$_4$-N=N-C$_6$H$_4$-NMe_2	IV-c	in DMSO	151.4	153.7	130.1	143.2	{129.6 132.5	{118.1 129.9	126.1	112.4			
	I	Calcd.	152.4		132.1		126.7	115.2					
		in H_2O	142.5		135.3		127.8	123.3					
	II	Calcd.		149.9		128.3			127.5	113.3			
		in H_2O	176.2	155.3		116.5			134.3	111.8			
	III	Calcd.	152.4	130.8	131.6	140.9	129.6	122.8	143.4	108.6	110.5	123.7	
		in H_2O	161.2	127.0	126.3	141.9	130.0	116.6	157.7	93.4	119.3	121.6	

a) Temperature is 34°, unless specially described.

In Table III, the calculated values are obtained by the additivity of substituent chemical shifts[13] where the following approximations are used;  –N=N– instead of SO_3^-–  –N=N– and –N=N–  –NMe_2. For example, IV is expressed as SO_3^-–  – + –N=N–  on the benzenesulfonate side and  –N=N– + –  –NMe_2 on the dimethylaniline side. The calculated values show a good agreement with the observed values.

The assignment of α- and β-cdx is given by Takeo *et al.*[14]

Results and Discussion

1) ^{1}H NMR

Table IV shows α- and β-cdx-induced chemical shifts on IV and related compounds for a molar ratio of one cdx molecule per host molecules.

TABLE IV. α- and β-Cyclodextrin Induced ^{1}H Chemical Shifts of Methyl Orange and Related Compounds (Hz)

Compound	Add[a]	1	2	3	6	7	8	9	10	11	12	Me
II	α				5	7						6
	β				19	15						25
III	β	(	3	7)		15		13	17			
IV	g		0	−4	−4	−5						−1
	α		14	27	27	32						22
	β		18	18	32	19						−2
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	

a) Add indicates g; glucose α; α-cdx β; β-cdx. A positive sign shows a low field shift.

α- and β-cdx are composed of 6 and 7 D-glucopyranose residues respectively joined by α-, 1,4-linkages to produce a macrocyclic form. Owing to its simple symmetry, the conformational analysis of cdx is relatively simple, as it closely resembles its constituent monosaccarides. The chair conformation has been established for the constituent glucose units in cdx.[15] Thus it has primary and secondary OH crowding the opposite ends of its torus, H-3 and H-5 directed toward its interiour and H-1, H-2 and H-4 located on its exteriour. It might be expected that if inclusion occurs in the neighbourhood of protons located within or near the cavity, H-3, H-5 or H-6 should be strongly shielded by the ring current effect of the aromatic ring of guest molecule. Alternatively, if association takes place at the exteriour of the torus, H-1, H-2 and H-4 should be strongly affected.

When β-cdx is added to a suspension of IV, the effect on the host molecule is illustrated in Fig. 1a. It is evident that the H-3, H-6 and presumably H-5 signals shift to higher field from their initial positions when β-cdx is added. The behaviour of the chemical shifts establishes that the ring current affects the whole of the cdx cavity.

13) M. Mishima, Ph. D. Thesis, Osaka University (1976), p. 63.
14) K. Takeo, K. Hirose, and T. Kuge, *Chem. Letters*, **1973**, 1233.
15) *a*) A. Hybl, R.E. Rundle, and D.E, Williams, *J. Am. Chem. Soc.*, **87**, 2779 (1965); *b*) C.A. Glass, *Can. J. Chem.*, **43**, 3652 (1965).

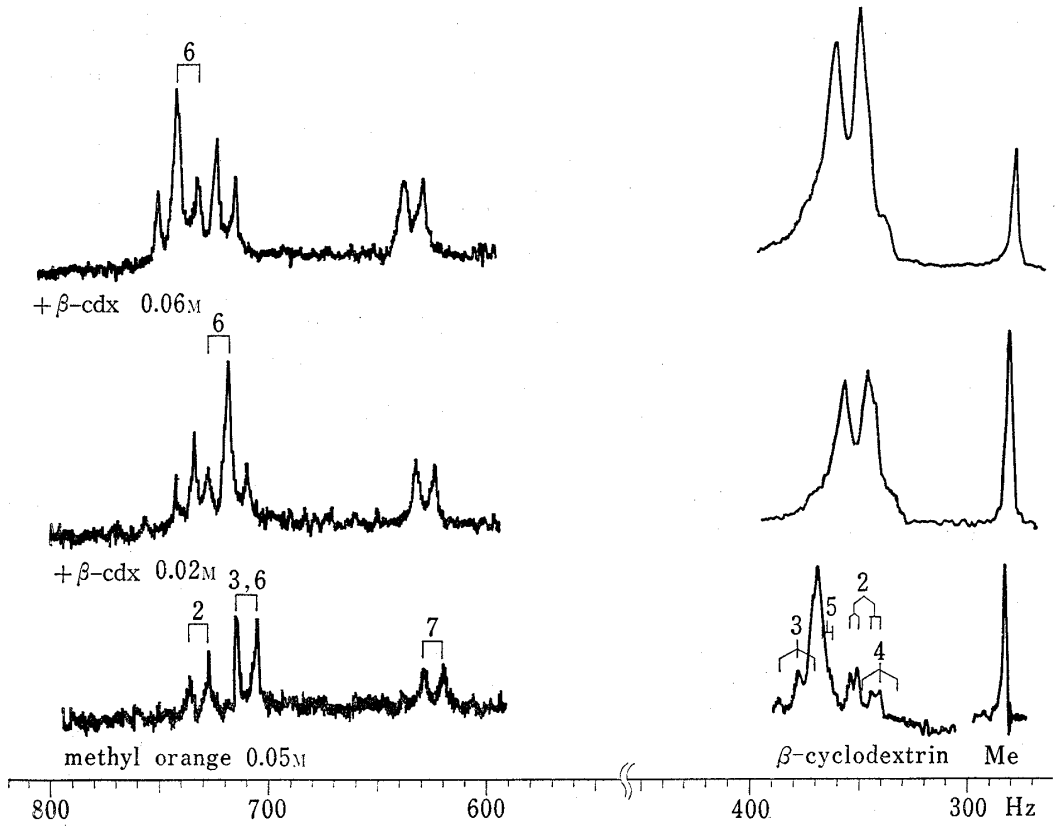


Fig. 1a. PMR Spectra of Methyl Orange and β-Cyclodextrin

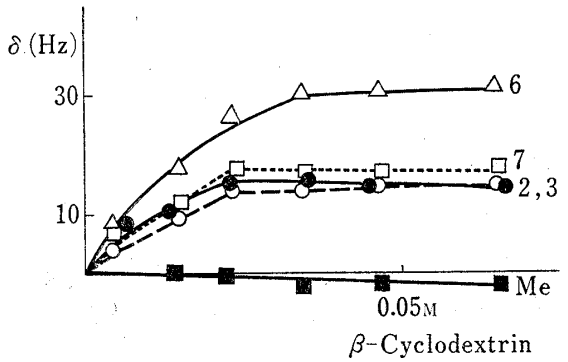


Fig. 1b. ^{1}H Chemical Shifts of Methyl Orange at Varying β-Cyclodextrin Concentrations
Methyl Orange 0.047 M Suspension
H-2, ○——○; H-3, ●——●; H-6, △——△;
H-7, □-----□; Me, ■——■.

On the other hand, ring protons of IV shift to a lower field site. When glucose is added instead of β-cdx, ring protons show a slight high field shift (*cf.* Table IV), so it seems certain that inclusion causes the low field shift. The low field shift might be induced by diamagnetic anisotropy of particular bonds or regions of the host,[16] van der Waals shift,[17] or steric perturbation.[18] When β-cdx is added to IV continuously, one portion *ortho* to the N=N positions shows a lower field shift than the other, and finally both portions separate clearly. When the H-7 signal is irradiated, the former is decoupled and is assigned to H-6. Originally, the solution of IV is red orange (concentrated colour), but when cdx is added, it turns yellow (diluted colour) on inclusion. This result also suggests that the N=N moiety is included by β-cdx.

Plots of chemical shifts of IV against variable β-cdx concentrations show the largest change at the H-6 signals (*cf.* Fig. 1b). Large change is not observed for the CH_3 signal. The inflexions suggest the possibility of a 1 : 1 complex though ambiguity remains. This treat-

16) J.W. Apsimon, W.G. Craig, P.V. Demarco, D.W. Mathieson, L. Saunders, and W.B. Whalley, *Tetrahedron* **23**, 2339 (1967).
17) B B. Howard, B. Linder, and M.T. Emerson, *J. Chem. Phys.*, **36**, 485 (1962).
18) B.V. Cheney and D.M. Grant, *J. Am. Chem. Soc.*, **89**, 5319 (1967).

ment for the host and guest molecules by ^{13}C NMR gives more distinct results. IV is nearly twice as long as the depth of cdx so that in the complex form, only partial inclusion of IV would be anticipated. However, both ring protons shift to low field. If inclusion of IV in the cavity were fixed more tightly, low field shift might occur in one benzene ring only and the other might retain the shift observed in aqueous media. As a next step, α-cdx (internal diameter~6 Å) which has a smaller diameter than β-cdx (~7.5 Å) was added[19] (*cf.* Fig. 2).

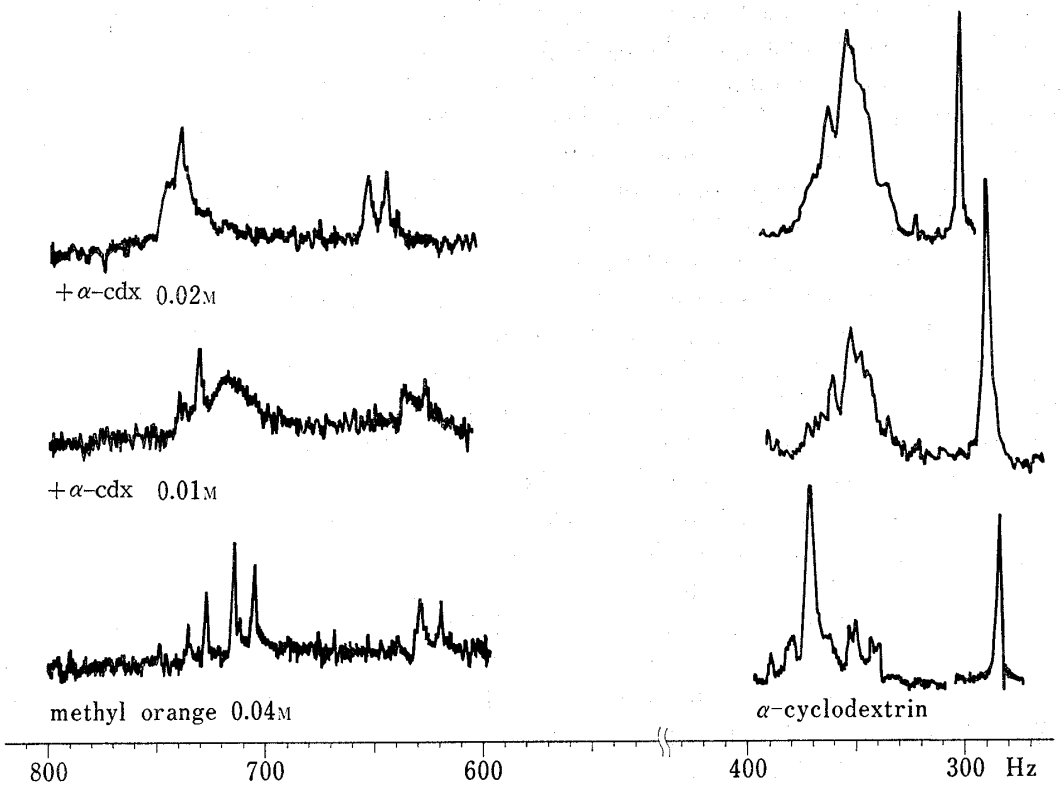


Fig. 2. PMR Spectra of Methyl Orange and α-Cyclodextrin

The spectrum of α-cdx shows a large high field shift when IV is included. It suggests that the whole framework of the inner cavity experiences the ring current effect of IV. On the other hand, the H-3 and H-6 signals of IV show signal broadening, accompanied by a low field shift. In the X-ray analysis of *p*-nitrophenol, *p*-hydroxybenzoic acid[20c] and *p*-iodoaniline[20d] complexes with α-cdx, several hydrogen–hydrogen contacts shorter than the ideal van der Waals contacts are found between α-cdx and the benzene ring. This indicates that the guest molecule is tightly packed in the α-cdx ring. Thus in the case of IV also, the smaller diameter of α-cdx causes exchange broadening in the process of chemical exchange[21] and greater transverse relaxation rates of the portions in the neighbourhood of N=N than those of others[7c] and signal broadening. Both the H-7 and Me-H signals show larger low field shifts than the H-2 signal. As to low field shift of the Me-H signal, II has three substituents with a bond angle of 110° around the center C-4. It ought, therefore, to be that Me-H here is included more certainly than in IV where there exists an almost linear framework. From Table IV, II–β-cdx causes shifts not only of ring protons, but also of Me-H. Hence the low field shift of the Me-H signal is also ascribed to inclusion.

19) F. Cramer and H. Hettler, *Naturwissenschaften*, **49A**, 625 (1967).
20) *a*) K, Harata, *Bull. Chem. Soc. Jpn.*, **48**, 3409 (1975); *b*) *Idem*, *ibid.*, **50**, 1259 (1977); *c*) *Idem*, *ibid.*, **50**, 1416 (1977); *d*) W. Saenger, K. Beyer, and P.C. Manor, *Acta Cryst.*, **B 32**, 120 (1976).
21) B.D. Sykes and M.D. Scott, *Annu. Rev. Biophys. Bioeng.*, **1**, 27 (1972).

In measurement on a 90 MHz instrument, complete assignment of cdx protons is impossible. Only the H-3 signal displays obviously assignable behaviour. A part of the H-3 resonance is situated to low field and separated from other protons, so a high field shift caused by inclusion can be perceived as disappearance of the signals. In spite of the impossibility of individual assignment, the total spectral pattern obviously suggests inclusion. The following tendencies are observed. 1) When sodium sulfanilate, *p*-phenylenediamine and N,N-dimethyl-*p*-phenylenediamine are included by β-cdx, the influences on the high field shift of β-cdx are in the order $NMe_2 > NH_2 > SO_3Na$. It is inferred that the effect of substituent on inclusion depends upon the hydrophobic nature of the substituent.[19] In the present study, NMe_2 induces stronger inclusion of the benzene ring than does SO_3Na. 2) When *p*-phenylenediamine and 4,4'-diaminobiphenyl, N,N-dimethyl-*p*-phenylenediamine and II are included by β-cdx, the guest molecules with longer frameworks induce larger shifts of β-cdx. It is clear that the length of IV promotes an ability for inclusion. 3) Oddly, the above molecules afford only marginal shifts to α-cdx in the complex, but IV–α-cdx gives a high field shift for all protons in the interiour of the cavity. Incorporation of an azo dye group in the center of the molecule may increase the ability to form inclusion compound. 4) IV has the possibility of inclusion from both sides of molecule, namely from the benzenesulfonate or N,N-dimethylaniline sides. IV–α-cdx gives a larger Me-H shift. Judging from the signal broadening of the spectral pattern in the neighbourhood of N=N, the freedom of movement of IV in the cavity must be much diminished. It is difficult for entry to occur from the benzenesulfonate side with passage through the cavity so that inclusion can occur at the dimethylaniline side. It seems that IV enters the cavity from the NMe_2 side and is included up to the neighbourhood of N=N. The middle part of the spectra of Fig. 2a supports the above fact. Compared with the broad H-3, H-7 and Me-H signals, H-2 situated *ortho* to SO_3^- holds its original pattern. Moreover, IV has the possibility of being included in α-cdx at different positions. H-3, H-5 and H-6 in α-cdx exhibit the ring current effect, so the aromatic rings of IV must fill the cavity. On the other hand, IV shows striking broadening at both *ortho* protons to N=N and Me-H signals. If they are always fixed in the same positions of the cavity, protons of cdx in the neighbourhood of NMe_2 and N=N ought not to experience the ring current effect. If contact within van der Waals radii in IV–α-cdx interaction does not permit motion, IV must be included in α-cdx at different positions; the one form may include the dimethylaniline group completely and the other form may include the neighbourhood of the N=N residue firmly. On the other hand, it is probable that IV–β-cdx is included with some free space, because III with a substituent at the *ortho* position to N=N forms a β-cdx complex. A large change is not observed at Me-H. Thus the N,N-dimethylaniline group passes the cavity and inclusion occurs at the benzenesulfonate side. The fact that inclusion of the hydrophobic NMe_2 is preferred to that of the hydrophilic SO_3^- is consistent with 1. 5) In a few cases a marginal inclusion occurs from the benzenesulfonate side. IV-a (orange IV, *cf.* Table III) with a bent form on the aniline side ought to be included from the aniline side only if the benzenesulfonate side is not be included at all. The latter shows a marginal low field shift accompanied by signal broadening. Moreover, V which has SO_3^- on both inclusion side shows a low field shift (Table IV) and an induced circular dichroism peak.[22]

2) ^{13}C NMR

^{13}C NMR is not so much effected by perturbations as in ^1H NMR, but it is sensitive to changes of conformation. In the case of the guest molecule, quarternary carbons, especially those with the SO_3^- group which is considered as the driving force for inclusion from the X-ray data add information to the results of ^1H NMR. As a result, it is possible to examine the behaviour of the whole molecule. In the host molecule, the sugar carbons can be detected

22) M. Suzuki and Y. Sasaki, *Chem. Pharm. Bull.* (Tokyo), accepted.

as separate individual spectral bands, so exact information can be obtained in cases which could not be differentiated from each other in ^1H NMR.

Table V shows α- and β-cdx-induced chemical shifts of the guest molecules with a molar ratio of one for cdx/host molecules. C-1 of the IV–β-cdx complex shows the larger value than

TABLE V. α- and β-Cyclodextrin Induced ^{13}C Chemical Shifts of Methyl Orange and Related Compounds (ppm)

Compound	Cyclodextrin	1	2	3	4	5	6	7	8	9	10	Me
I	β	0.3	0.0	−0.3	0.2							
II	α					0.3	0.2	0.2	0.2			0.2
	β				0.4	0.6	−0.1	0.1	0.3			0.3
III	β	−0.2	−0.2	0.1	−0.2	0.4	−0.1	0.2	0.7	0.2	0.4	
IV	α	0.9	−0.2	0.4	0.4	0.5	0.2	−0.1	0.2			0.4
	β	1.1	0.4	0.0	0.1	0.9	−0.5	−0.6	−1.0			0.1

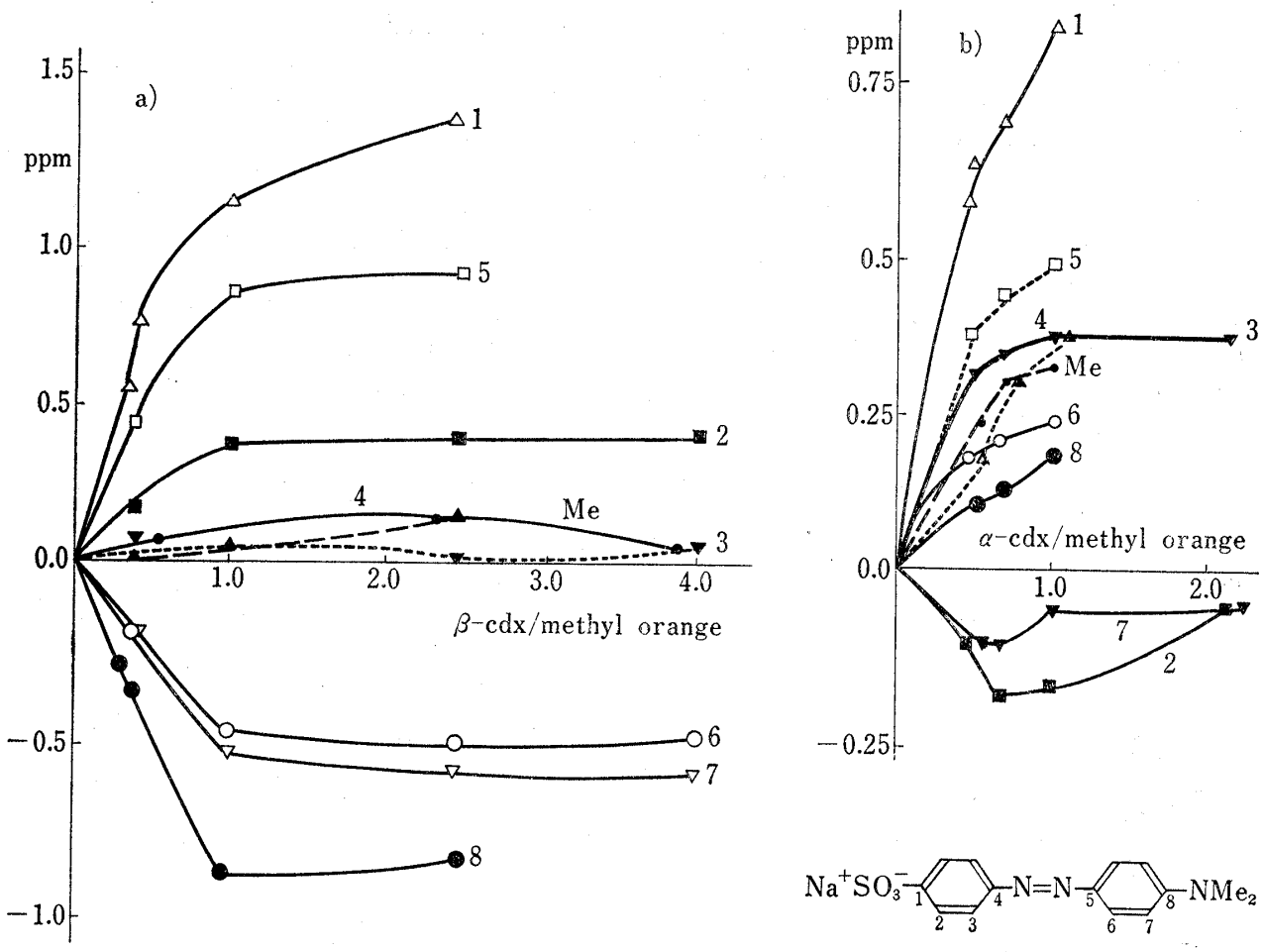


Fig. 3. α- and β-Cyclodextrin-induced Shift

a) β-Cyclodextrin-induced Shift plotted as a Function of the Molar Ratio of β-Cyclodextrin to Methyl Orange

C-1, △—△; C-2, ■—■; C-3, ▼-----▼; C-4, ▲-----▲; C-5, □—□; C-6, ○—○; C-7, ▽—▽; C-8, ●—●; Me, •—•.

b) α-Cyclodextrin-induced Shift plotted as a Function of the Molar Ratio of 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. A positive sign indicates a low field shift.

that of the I–β-cdx complex. Incorporation of an azo dye group in the center of the molecule may increase the inclusion ability of the SO_3^- group. C-8 of the II–β-cdx complex shows the same behaviour from that of IV–α-cdx complex, but quite different from that of the IV–β-cdx complex. The following treatment was tried.

Plots of molar ratio of β-cdx/IV *vs.* change in chemical shifts of IV suggest that a 1: 1 complex is formed (*cf.* Fig. 3a). Above a molar ratio of one for β-cdx/IV, no further substantial changes of the chemical shifts occur. It shows both high and low field shifts. Generally speaking, carbons on the dimethylaniline side which is inclined to extrude from the cavity from the result of ^{1}H NMR data, show high field shifts and those on the benzenesulfonate side with a high probability to be included in the cavity show low field shifts. Above low field shift may be derived from hydrophobic interaction and van der Waals interaction with the host molecule, stripping of substrate solvent and substrate H-bonding.[7b] In ^{1}H NMR, 2,3 and 7 positions of IV show almost the same behaviour, but in ^{13}C NMR they give different signs and magnitudes. ^{13}C and ^{1}H NMR spectral behaviour may be ascribed to different shift mechanisms in the aromatic ring.

Plots in Fig. 3b decrease above a molar ratio of one for α-cdx/IV, because a precipitate appears, where the stoichiometry of the complex has been proved to be 2: 1 by X-ray.[12] The chemical shift of Fig. 3b ought to show the properties of the 1: 1 complex just before the precipitation occurs (This fact is illustrated in Fig. 4a also). The α-cdx complex shift is displaced to low field on the N,N-dimethylaniline side of the molecule compared with the β-cdx complex, where the H-3, H-6 and Me-H signals of the α-cdx complex show significant broadening in ^{1}H NMR. These results may be ascribed to closer van der Waals interaction between the host and guest molecules than in the case for the β-cdx complex. The C-1 signal shows low field shift, but rather higher shift compared with that in the IV–β-cdx complex. The C-2 signal shows high field shift. The benzenesulfonate side in the α–cdx complex may have a higher probability outside the cavity than in the β-cdx complex.

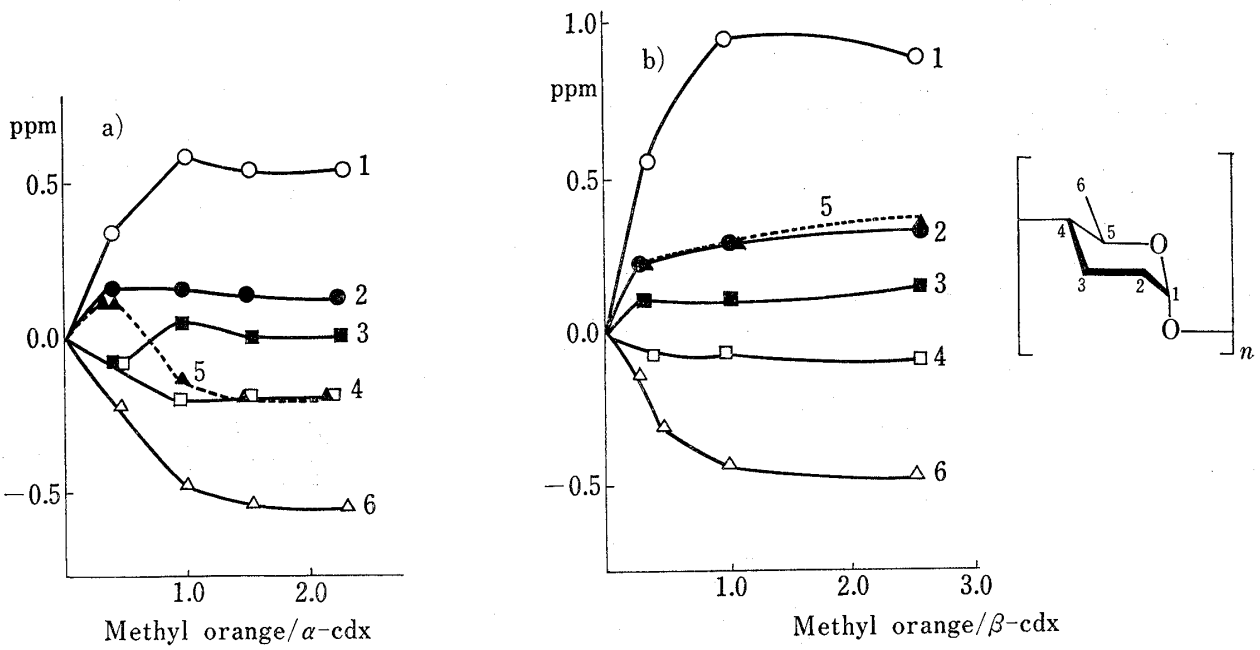


Fig. 4. Methyl Orange-induced Shifts of α- and β-Cyclodextrin
a) Methyl Orange-induced Shift plotted as a Function of the Molar Ratio of Methyl Orange/α-Cyclodextrin
b) Methyl Orange-induced Shift plotted as a Function of the Molar Ratio of Methyl Orange/β-Cyclodextrin
C-1, ○—○; C-2, ●—●; C-3, ■—■; C-4, □—□; C-5, ▲-----▲; C-6, △—△.

This high field shifts are consistent with the behaviour of the N,N-dimethylaniline side in Fig. 3a. Even in the case, C-1 with the SO_3^- substituent shows the lowest shift. In other azo dyes which can not form the α-cdx complex, carbons with the SO_3^- substituent do not show such a shifts, so it is apparent that inclusion causes the low field shift (for example, 0.1 ppm in the V–α–cdx complex, 0.7 ppm in the V–β-cdx complex low field shift, respectively). IV must be included in α-cdx at different positions; the one form may include the dimethylaniline group completely and the other form may include the neighbourhood of the N=N residue firmly.

In the host molecule, ^{13}C NMR plays an important role in assignment. On the whole, large change is not recognized, but the reproducibility is satisfactory.

Table VI. Methyl Orange and Related Compounds-Induced ^{13}C Chemical Shifts of α- and β-Cyclodextrin (ppm)

Compound	Cyclodextrin	1	2	3	4	5	6
II	α	0.1	0.1	0.2	0.1	0.1	0.1
	β	0.2	0.4	−0.1	0.2	0.2	−0.2
IV	α	0.6	0.2	0.1	−0.2	−0.1	−0.5
	β	1.0	0.3	0.1	−0.1	0.3	−0.4

When guest molecule-induced shift is plotted as a function of the molar ratio of guest molecule/cdx, inflexions appear at a ratio of 1 : 1 (*cf.* Fig. 4). Their values are shown in Table VI. In ^1H NMR, the H-3, H-5 and H-6 signals shift to the same point but in ^{13}C NMR, they show different behaviour. C-5 position shows high field shift in the α-cdx complex and low field shift in the β-cdx complex. C-6 gives a higher field shift than other carbons. This may be possibly due to the ring current effect. But for the sodium *p*-nitrophenolate-α-cdx complex which includes NO_2^- near the 5 and 6 positions of α-cdx, C-5 and C-6 show the same high field shift.[7a] Thus the ring current effect may not play a major role in ^{13}C NMR. By Corey-Pauling Koltun molecular model, IV does not show anywhere the compression with β-cdx, but show in the neighbourhood of N=N the large compression and the conformational change with α-cdx. So the difference of the behaviour of C-5 between α- and β-cdx complexes may be ascribed to above effect. C-1 and C-4 show low and small high field shifts, respectively. When a more bulky azo dye with a naphthalene nucleus is included,[22] C-1 and C-4 show almost the same low field shift values in the β-cdx complex. This low field shift may be due to changes of the bond angle of the glycosidic linkage loop arrangement. From X-ray[12] and optical rotatory dispersion[23] data, it has been stated that changes of cdx on inclusion are mainly due to changes about the C-O bonds joining each glucose unit. The ^{13}C NMR data herein add detailed information about the behaviour of the interiour of the cavity.

23) D.A. Rees, *J. Chem. Soc.* (*B*), **1970**, 788.