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Inclusion Compounds of Cyclodextrin and Azo Dyes. IV (b).[1] Nuclear Magnetic Resonance and Circular Dichroism Spectra of γ-Cyclodextrin and Orange II in the Solution State

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The behavior of orange II–γ-cyclodextrin (cdx) complex was examined in the solution state. ^{1}H-Nuclear magnetic resonance (NMR) data indicate that orange II can pass through γ-cdx. The molar ratios of orange II: γ-cdx as determined from Job plots was 1 : 1 (^{13}C-NMR) and 2 : 1 (^{1}H-NMR). Simulation of the equilibrium constants (K)'s was attempted with three systems. K's of the complex determined by the C–SO_3^- at 66 °C could be well represented by an A+B=AB, AB+B=AB$_2$ system and gave $K_1=74$, $K_2=5$, indicating the coexistence of 1 : 1 and 2 : 1 complexes. On the other hand, β-cdx complex showed a 1 : 1 ratio in Job plots of both ^{1}H and ^{13}C data, giving $K_1=2370$ in an A+B=AB system, no improvement was obtained by applying an A+B=AB, AB+B=AB$_2$ system. Generally, the ^{13}C-NMR signals of γ-cdx-induced orange II show high-field shifts compared to those of the β-cdx complex. This may be due to the superiority of the hydrophobic interaction to other factors. Judging from the circular dichroism (CD) spectra, γ- and β-cdx include orange II in different directions and there is no electrostatic interaction between γ-cdx and orange II.

Keywords——orange II; γ-cyclodextrin; solution; ^{1}H-NMR; ^{13}C-NMR; CD; equilibrium constant

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 dichroism (CD), ultraviolet (UV) and X-ray techniques[2-7] 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; for instance, Laufer *et al.* determined the equilibrium constants and thermodynamic parameters by ^{13}C-NMR analysis of inclusion compounds in their series[8] and concluded that the main binding force was dipolar interaction. Moreover, they assumed preferential binding of the guest molecules based on interpretation of the chemical shifts. The above results indicate that NMR can give information on the inclusion state in solution down to the level of individual atoms.

In the present series of studies, inclusion states of azo dyes and α-, β-cdx complexes have been examined by NMR and CD.[1,9] The sizes of azo dyes examined have limited complex formation to a 1 : 1 molar ratio, if it is allowed at all. The data obtained previously should provide a basis for analyzing more complicated complexes.

In the previous communication,[1] we reported that the complex of orange II and γ-cdx forms a colloid at room temperature. At that time, the molar ratio was estimated to be 1 : 1 by ^{13}C-NMR spectroscopy, but later, the possibility was considered of the coexistence of a complex having a different molar ratio.

In the present paper, we estimate the molar ratios and equilibrium constants of orange II–γ-cdx complex by using ^{1}H- and ^{13}C-NMR data, and we interpret the inclusion shift macroscopically. CD spectra can offer further information to aid in determining from which direction inclusion occurs.

Results and Discussion

1) ^{1}H-NMR

All the ^{1}H-NMR signals of orange II in the γ-cdx complex at 66 °C show parallel low field shifts (Fig. 1a), but those of H-2 and H-3 are decreased compared to those of the β-cdx complex (Table I). Job plots indicate that orange II–γ-cdx forms a 2:1 complex (Fig. 1b). Generally, an increase of the diameter of the cavity in cdx decreases included low field shifts.[10] Moreover, when two guest molecules are included in one cavity, the ring current effect of the aromatic ring in the other guest molecule cannot be neglected. In the case of γ-cdx complex, the benzene side in one orange II molecule will be subject to a large ring current effect of the naphthalene nucleus in the other one. On the γ-cdx side, H-6's show completely a high field shift, in contrast to the β-cdx complex which show a low field shift.[9b] This suggests a difference in the direction of inclusion and mol ratio of the guest molecule between γ- and β-cdx complexes.

2) ^{13}C-NMR

Figures 2a and 2b show concentration dependency of γ- and β-cdx-induced ^{13}C-chemical shifts of orange II at 66 and 35 °C. Job plots of the former complex indicate the formation of a 1:1 complex.[1] ^{13}C field shift may be derived from hydrophobic interaction and van der Waals interaction with the host molecule, stripping of bound solvent and substrate H-bonding.[11] Among the forces proposed for the interaction between cdx and the guest, hydrophobic binding seems to have the most solid basis. Van der Waals stabilization energy is also large in α-cdx complexes, but the importance of H-bonding is questionable.[12]

To examine the degree of hydrophobic interaction in inclusion shift, the behavior of orange II in hydrophobic solvents was examined. Functional groups SO_3^- and OH in hydrophobic solvents cause low- and high-field shifts, respectively. The effect is greater in highly hydrophobic solvents (Fig. 2c and Table II). Overalls, when Figs. 2a and 2b were compared with Fig. 2c, Fig. 2a clearly resembles Fig. 2c more than Fig. 2b. Job plots of both complexes indicate the formation of a 1:1 complex. Thus, it may be that orange II in the β-cdx complex is partially located in the interior of β-cdx, or is subject to van der Waals effect in the narrow cavity. A similar tendency is seen in methyl orange–cdx complexes. In these cases, increase of the inner size in cdx molecules increases the high-field inclusion shifts.[10] On the other hand, almost all the methyl orange ^{13}C signals of the α-cdx complex of methyl orange, which is reported to be tightly included (X-ray study),[7d] show low field shifts.

In general, ^{13}C inclusion shifts may be mainly divided into hydrophobic and van der Waals interaction shifts. The former may predominate in γ-complexes, and the latter in β-complexes.

3) Equilibrium Constants

To determine the mole ratios of γ-cdx complex, equilibrium constants were calculated from the concentration dependency of chemical shifts on chemical equilibria, and the curve-fitting method.[13] In the nonlinear least-squares method, the root-mean-square deviation is minimized by seeking optimum values of chemical shifts ϕ_{calcd},

$$\sigma=\left\{\sum_{i=1}^{n}(\phi_{\mathrm{obsd},i}-\phi_{\mathrm{calcd},i})^2\Big/n\right\}^{1/2}$$

Thus, equilibrium constants and chemical shifts intrinsic to species ϕ_{AB} and/or $\phi_{\mathrm{AB_2}}$ were calculated.

System I) $\mathrm{A}+\mathrm{B}\rightleftharpoons\mathrm{AB}$

The equilibrium constants K and initial concentrations C_{A}° and C_{B}° of components A (cdx) and

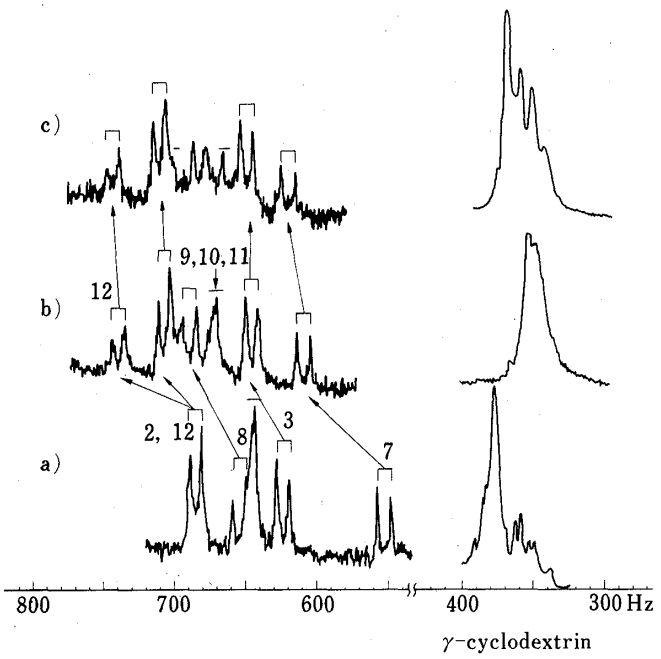

Fig. 1a. ^{1}H-NMR Spectra of Orange II and γ-Cyclodextrin

a) Orange II 0.04 M in D_2O.
b) +γ-Cyclodextrin 0.02 M.
c) +γ-Cyclodextrin 0.04 M.

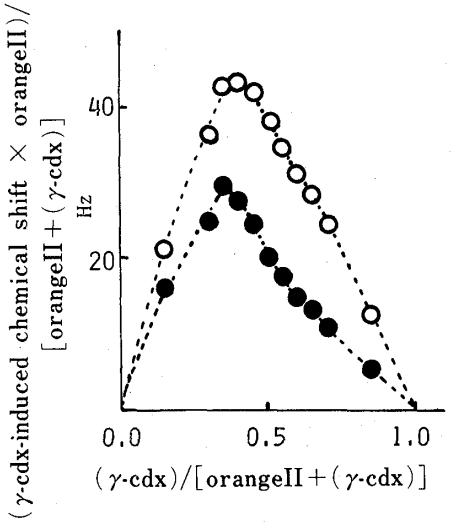

Fig. 1b. Continuous Variation Plots of the γ-Cyclodextrin-induced ^{1}H-NMR Chemical Shifts of Orange II at 66 °C

H-7, ○---○; H-8, ●---●.

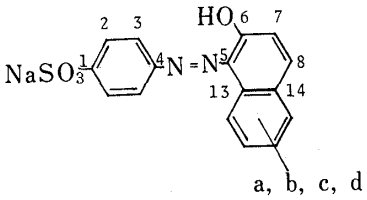

Chart 1

TABLE I. β- and γ-Cyclodextrin-Induced ^{1}H Chemical Shifts of Orange II (ppm)

Cyclodextrin	12	2	8	3	7
γ	0.69	0.24	0.33	0.27	0.78
β	0.87	0.47	0.44	0.77	0.83

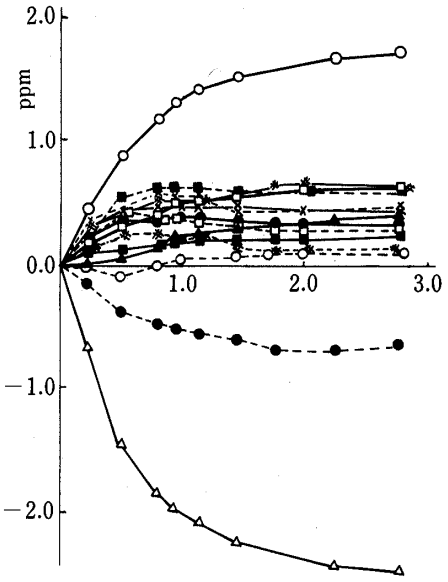

Fig. 2a. γ-Cyclodextrin-induced ^{13}C-NMR Chemical Shift Plotted as a Function of the Molar Ratio of γ-Cyclodextrin to Orange II (0.07 M) at 66 °C

C-1, ○—○; C-2, ●—●; C-3, ■—■; C-4, □—□; C-5, ▲—▲; C-6, △—△; C-7, ○---○; C-8, ●---●; C-a, ×—×; C-b, ×---×; C-c, ※—※; C-d, ※---※; C-13, □---□; C-14, ■---■.
A positive sign indicates a low-field shift.

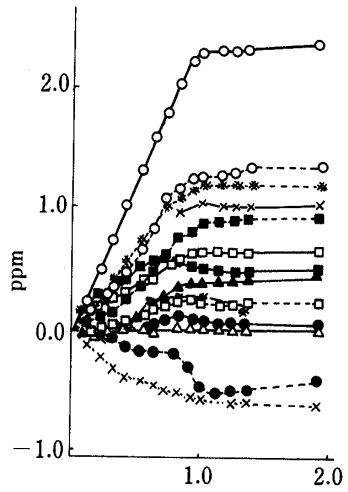

Fig. 2b. β-Cyclodextrin-induced ^{13}C-NMR Chemical Shift Plotted as a Function of the Molar Ratio of β-Cyclodextrin to Orange II (0.063 M) at 35 °C

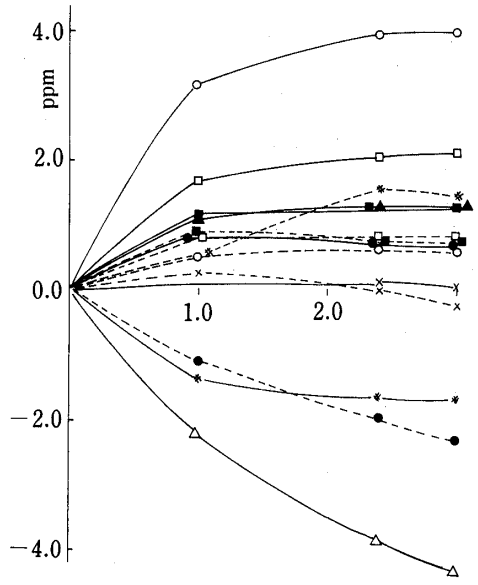

Fig. 2c. Solvent Effect of Orange II (0.07 M) in Dioxane + D_2O at 34 °C

TABLE II. ^{13}C Chemical Shifts of C–SO_3^- and C–OH of Orange II in Hydrophobic Solvents (ppm)

	D_2O : Dioxane	D_2O : $CH_3CH_2CH_2OH$	D_2O : $(CH_3)_2CHOH$	D_2O : CH_3OH
C–SO_3^-	3.2	2.6	3.0	1.9
C–OH	−2.1	−2.9	−2.9	−0.4

D_2O : hydrophobic solvent = 1 : 1 v/v.

B (orange II) are expressed as follows.

$$K_I = C_{AB}/(C_A C_B) \qquad C_A^\circ = C_A + C_{AB}, \quad C_B^\circ = C_B + C_{AB}$$

From these equations, ϕ_{calcd} for component B can be obtained as follows.

$$\phi_{calcd} = (C_B\phi_B + C_{AB}\phi_{AB})/C_B^\circ = \phi_B + \{1 + K(C_A^\circ + C_B^\circ) - R^{1/2}\}(\phi_{AB} - \phi_B)/2KC_B^\circ$$

$$R = \{1 + K(C_A^\circ + C_B^\circ)\}^2 - 4K^2 C_A^\circ C_B^\circ$$

System II) $A + 2B \rightleftharpoons AB_2$

$$K_{II} = C_{AB_2}/(C_A C_B^2) \qquad C_A^\circ = C_A + C_{AB_2}, \qquad C_B^\circ = C_B + 2C_{AB_2}$$

From these equations,

$$\phi_{calcd} = (C_B\phi_B + C_{AB_2}\phi_{AB_2})/C_B^\circ = \phi_B + C_{AB_2}(\phi_{AB_2} - \phi_B)/C_B^\circ$$

System III) $A + B \rightleftharpoons AB\ (K_1), \qquad AB + B \rightleftharpoons AB_2\ (K_2)$

$$K_1 = C_{AB}/(C_A C_B) \qquad K_2 = C_{AB_2}/(C_{AB} C_B) \qquad C_A^\circ = C_A + C_{AB} + C_{AB_2} \qquad C_B^\circ = C_B + C_{AB} + 2C_{AB_2}$$

From these equations,

$$\phi_{calcd} = (C_B\phi_B + C_{AB}\phi_{AB} + C_{AB_2}\phi_{AB_2})/C_B^\circ$$

$$= \phi_B + C_{AB}(\phi_{AB} - \phi_B)/C_B^\circ + C_{AB_2}(\phi_{AB_2} - \phi_B)/C_B^\circ$$

In the case of orange II–γ-cdx complex, there is a possibility of 1 : 1 and/or 2 : 1 complex formation judging from Job plots of ^{13}C- and ^{1}H-NMR. Thus, systems I and III for C-1, and systems II and III for H-7 were applied.

Simulation of K's in system III was satisfactory in both nuclei and gives a small σ value (less than the resolution of the computer) in the case of ^{13}C-NMR. In the case of ^{1}H-NMR the σ value in system III is rather larger, but it is obvious that system III is superior to system II. Thus, Job plots and Table III show that 1 : 1 and 1 : 2 complexes coexist in the γ-cdx complex. It is considered that 1 : 1 and 1 : 2 complexes coexist in solution, and that formation of the 1 : 1 complex affects ^{13}C signals more than ^{1}H and *vice versa* for the formation of the 1 : 2 complex. It is not obvious why the K values have such large nucleus dependency. It may be desirous to detect which nucleus reflects more the genuine inclusion phenomen.

TABLE III. Calculated Equilibrium Constants and Chemical Shifts (ppm)

Nuclei and position	Cyclodextrin	$K^{a)}$	δ_{obsd}	δ_{AB}	δ_{AB_2}	$\sigma^{b)}$
C-1	γ	$K_I = 185$	1.73	1.79		0.05
	γ	$K_1 = 74$				
		$K_2 = 5$		1.88	2.15	0.01
H-7	γ	$K_{II} = 18422$	0.79		0.86	0.06
	γ	$K_1 = 270$				
		$K_2 = 103$		0.86	0.77	0.01
C-1	β	$K_I = 2370$	2.37	2.41		0.03
	β	$K_1 = 7950$				
		$K_2 = 69$		2.42	1.19	0.04

a) Unit: $dm^3\,mol^{-1}$.
b) Root-mean-square deviation between the observed and calculated shifts.

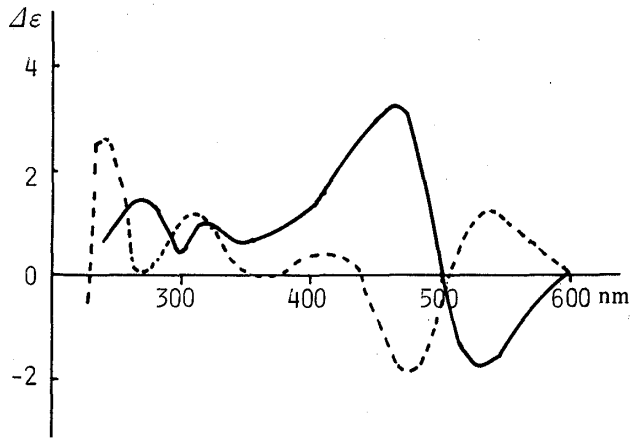

Fig. 3. CD Spectra of β- and γ-Cyclodextrin-induced Orange II

CD spectrum of orange II 2.0×10^{-4} M
+β-cyclodextrin 2.0×10^{-3} M, ------;
+γ-cyclodextrin 2.0×10^{-3} M, ——.
$\Delta\varepsilon$ is the molar circular dichroism coefficient.

TABLE IV. pH Dependency of Induced CD Values for γ-Cyclodextrin–Orange II Complex

pH 2.0		pH 6.0		pH 12.0	
CD_{max} [a]	$(\theta) \times 10^{-3}$ [b]	CD_{max}	$(\theta) \times 10^{-3}$	CD_{max}	$(\theta) \times 10^{-3}$
270	+6.6	272	+4.0		
320	+5.3	318	+3.6	320	+4.0
470	+11.2	466	+9.9	476	+10.6
526	−6.9	528	−5.9	530	−4.3

a) nm. *b*) deg·cm²/dmol⁻¹.

For comparison, orange II–β-cdx complex (for which Job plots suggest only the existence of 1 : 1 complex in both ^{1}H- and ^{13}C-NMR) was considered. The σ value in system III is rather larger than that in system I and the results rule out the coexistence of 2 : 1 complex.

Now we discussed the possibility of system III using NMR method. Furthermore, it would be necessary to discuss above system by the stationary state method.

4) CD Spectra

In the NMR of the cdx complex, it was possible to obtain some information from the host molecule about the direction of inclusion, but difficult from the guest molecule because the inclusion shift spreads over all the protons and carbons. CD spectra can be useful in determining from which direction inclusion occurs.[9b] In Fig. 3, the CD spectrum of γ-cdx complex shows a reversal of the signs in all regions compared to that of β-cdx complex. This may correspond to the behavior of H-6 on the cdx side in the ^{1}H-NMR spectrum which is unaffected in the β-cdx complex[9b] and shifts to high field in the γ-cdx complex. It is possible that these complexes include orange II in different directions.

From a comparison with the CD spectrum of methyl orange–α-cdx complex,[9b] γ-cdx may include mainly orange II at the long axis side. Moreover, the larger $\Delta\varepsilon$ values of γ-cdx complex compared with the β-one suggests the coexistence of the 2 : 1 complex.

Polypeptide,[14] and acridine orange–sodium cellobiose sulfate complex,[15] which form stacking structures, show strong pH dependency, but in this case, electrostatic interaction may be minimal (Table IV).

Experimental

γ-cdx of guaranteed grade was purchased from Nakarai Chemicals Ltd. The other materials and the instruments

used were as described previously.[9] ^{1}H chemical shifts were measured by a frequency counter within an error of ± 0.1 Hz. The resolution of the computer used in ^{13}C-NMR measurement was 0.03 ppm.

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