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Studies of Cyclodextrin Inclusion Complexes. I. Complex between Cyclodextrins and Bencyclane in Aqueous Solution[1)]

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Bencyclane (Ben) inclusion complexes with α-, β-, and γ-cyclodextrins (CyD's) in aqueous solution were studied by ultraviolet (UV), circular dichroism (CD), and nuclear magnetic resonance (NMR) spectroscopic methods. The composition ratio, the formation constants (*K* values), and the thermodynamic parameters for the three complexes were determined by analysis of the UV and CD spectra. The results indicated that CyD formed a 1 : 1 complex with Ben in all three cases, though the *K* values differed greatly ($\beta > \gamma \gg \alpha$). From the thermodynamic parameters obtained from the temperature dependence, it was concluded that the primary driving force for the complex formation is van der Waals interaction in the case of α- and β-CyD complexes, while it is hydrophobic interaction for the γ-CyD complex. Probable structures of the three complexes are discussed mainly on the basis of NMR data. It was concluded that the aromatic ring of Ben is inserted into the cavity of CyD from the small-diameter side in the case of β- or γ-CyD complexes, and from the opposite side in the case of α-CyD complex. It is highly probable that, in the case of β-CyD complex, binding is tight over the whole aromatic ring; in the case of γ-CyD complex, loose binding takes place over most of the aromatic ring and in the neighborhood of the benzyl carbon, with the *para* position of the aromatic ring extruding from the cavity; in the case of α-CyD complex, only partial inclusion occurs around the *para* position of the aromatic ring.

Keywords——bencyclane; inclusion complex; cyclodextrin; formation constant; chemical shift; UV; CD; NMR; thermodynamic parameter

It is well known that the molecule of cyclodextrin (CyD) has a torus shape with a central void of a few angstroms diameter, as shown in Fig. 1; the internal surface is relatively hydrophobic, and CyD can accomodate various molecules as guests in the non-polar cavity by non-covalent interaction.[2,3)] In many cases, the physical and chemical behavior of guest molecules, including reactivity, is significantly influenced by formation of the inclusion complex with CyD.[4)]

These phenomena have become the object of many application studies in the medicinal field for the improvement of pharmaceutical properties and bioavailability of drugs.[5)] Recently, it has been reported that the inclusion complexation of CyD with bencyclane (Ben),

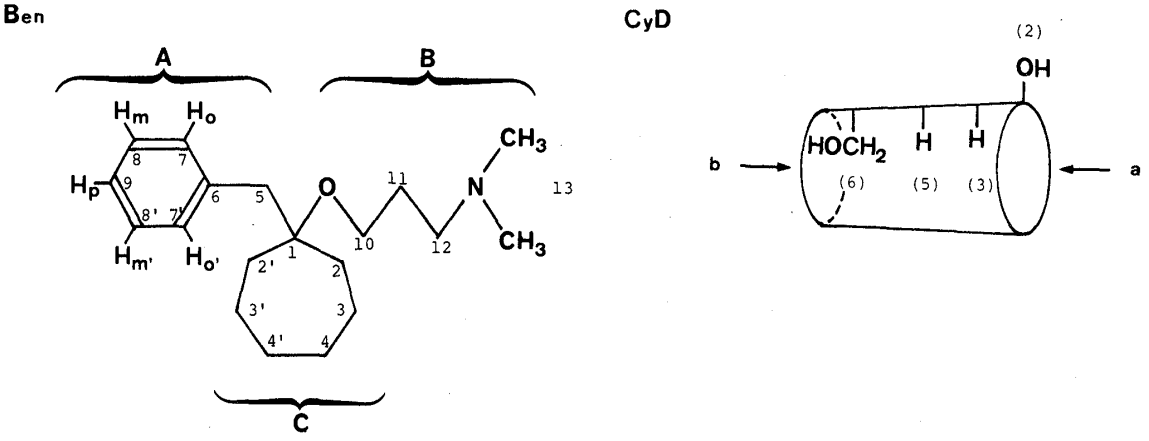

Fig. 1. Structures of Ben and CyD

TABLE I. Molecular Sizes of Ben and CyD's[a]

Ben		CyD's			
Distance between two atoms[b]			α	β	γ
H_m—$H_{m'}$	6.4	Diameter[b]	5—5.5	7—8	8—9
H_{13}—$H_{13'}$	6.0	Depth	7—8	7—8	7—9
H_p—H_5	6.5—7.5	H_6—H_5		3.6	
H_3—$H_{3'}$	7.2	H_5—H_3		2.8	
H_p—H_{13}	15—15.5	H_3—O_2		1.5	

a) Unit: Angstrom.
Molecular size was measured with the aid of CPK models.
b) Numbering of atoms is shown in Fig. 1.

a drug also shown in Fig. 1,[6] mitigates some unfavorable properties of Ben, such as instability in acidic media, low solubility, astringent bitter taste, and so on.[7] In an attempt to clarify the reason for the above improvement upon inclusion complex formation, we have studied this complex in some detail.

The present paper deals with studies of the physicochemical properties of the inclusion complexes of α-, β-, and γ-CyD with Ben by several spectroscopic techniques circular dichroism (CD), ultraviolet (UV), and nuclear magnetic resonance (NMR). The possible structures of these complexes in aqueous solution together with the nature of the binding forces between guest and host molecules are discussed.

Experimental

Materials——Ben fumarate, supplied by Medimpex (Hungary), was converted to free Ben by treatment with aqueous NaOH solution. α-, β-, and γ-CyD were purchased from Tokyo Kasei Ltd. and used without further purification. Deuterium oxide (D_2O), 99.8%, was obtained from Merck & Co., Inc. All other materials and solvents were of analytical reagent grade. *p*-Hydroxybencyclane (OH-Ben) was synthesized by the reported method,[8] and purified by a column chromatographic technique.

Preparation of *N*-[3-(1-Benzyl-cycloheptyl(2,2'-d_2)-oxy)-propyl]-*N*,*N*-dimethylamine (Ben-*d*)——Ben-*d* was synthesized according to Chart 1. A solution of cycloheptanone (2 g, 1.8 mmol) and sodium methoxide (0.5 g, 9 mmol) in D_2O (3.2 g, 160 mmol) and tetrahydrofuran (THF) was refluxed for 0.5 h at 50 °C. The reaction mixture was neutralized under cooling by the addition of deuterated trifluoroacetic acid (1 g, 9 mmol), and the THF was removed *in vacuo*. The resulting aqueous layer was separated and the residue was dried over magnesium sulfate, removal of

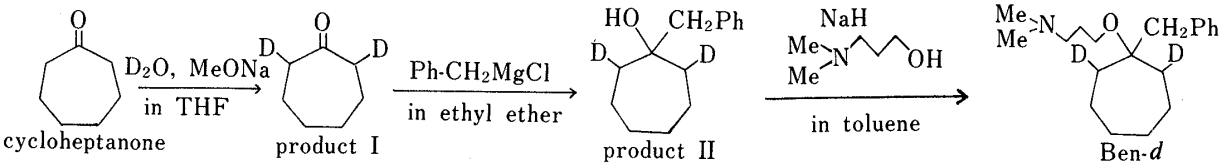

Chart 1. Synthesis of Ben-*d* from Cycloheptanone

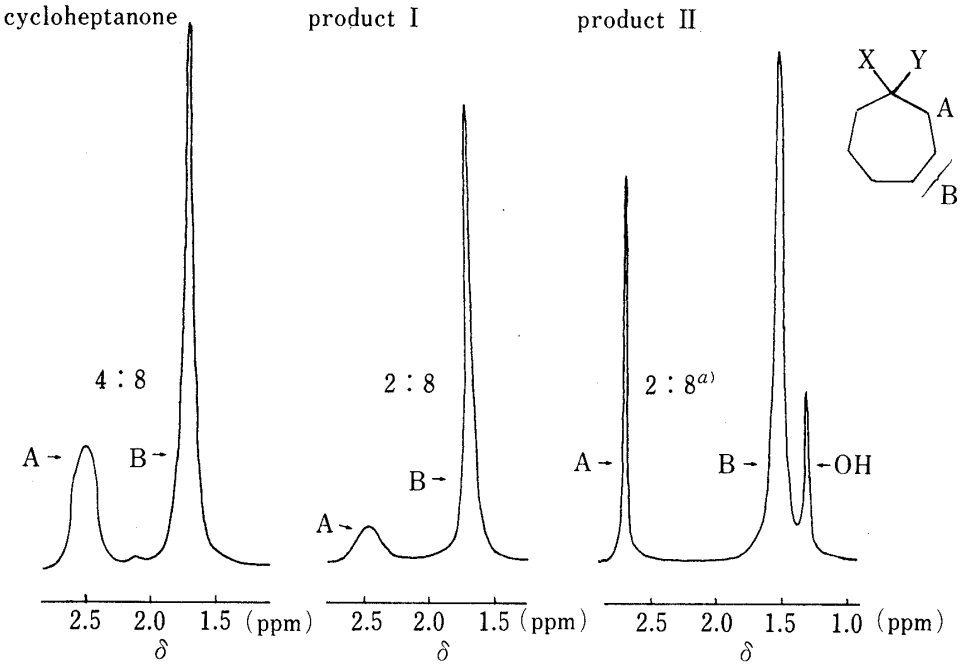

Fig. 2. ^{1}H-NMR Charts of Intermediates and Starting Materials for Ben-*d*

a) Integral ratio A and B.

which by filtration gave the product I (1 g, 9 mmol) (note the integral ratio for the 2- and 2′-protons against other protons on product I (2 : 8), compared with that for cycloheptanone (4 : 8) shown in Fig. 2). Product II (0.9 g, 4.4 mmol) was obtained by usual Grignard reaction from product I (1 g, 9 mmol) in ethyl ether and was purified by SiO_2 chromatography. A mixture of NaH (50% oily dispersion, 0.5 g) and product II (0.2 g, 1 mmol) was refluxed in toluene for 1 h and then a toluene solution of 3-dimethylaminopropyl chloride was added in excess. The reaction mixture was refluxed for 5 h. After cooling, the mixture was washed with water and toluene was evaporated off completely to leave an oily residue, which was purified by SiO_2-preparative thin-layer chromatography (TLC) to afford pure Ben-*d* (0.22 g, 0.8 mmol). The composition of the developing solvent for this TLC was *n*-hexane (50) : ethyl ether (40) : MeOH (15) : NH_4OH (0.5). Fumarate of Ben-*d* was obtained as a crystalline material (mp 128—129 °C) from acetone–MeOH in which Ben-*d* and fumaric acid had been dissolved in 1 : 1 molar ratio (the melting point of authentic Ben fumarate, measured at the same time, was 129—130 °C).

CD and UV Measurements——The CD and UV spectra were taken at room temperature on a JASCO model J-500 spectropolarimeter and a Shimadzu model UV-300 spectrophotometer, respectively. The pH of the solution was maintained at the desired value by using HCl–KCl, KH_2PO_4–Na_2HPO_4, or $Na_2B_4O_7$–NaOH buffer systems. The ionic strength of each solution was adjusted to 0.2 by the addition of KCl.

In CD measurements, the spectrum of the mixture containing Ben (OH-Ben) and CyD was corrected by subtracting the spectrum of the blank solution using a JASCO model DP-500 data processor in order to eliminate the effect of turbidity. In UV measurement, the difference absorbance of Ben was measured by using a set of duplicate 1 cm quartz cells: the mixture containing Ben (or OH-Ben) and CyD, and the blank solution were separately placed on the sample cell side; on the reference cell side, the corresponding Ben (or OH-Ben) and CyD solutions were separately placed for comparison.

The composition ratio of the inclusion complex was determined by the continuous variation method.[9]

The formation constants (*K* values) for the β- or γ-CyD complexes with Ben or OH-Ben were determined according to a modified Benesi–Hildebrand procedure (modified B–H formula),[10] by CD and UV measurements,

because the K values of β- and γ-CyD complexes with Ben and OH-Ben were too large to be obtained by use of the normal B–H formula. On the other hand, the K value of Ben·α-CyD complex was determined by use of the normal B–H formula.[11]

For β- and γ-CyD complexes with Ben and OH-Ben, the concentration of the guest molecules was kept equal to that of CyD, the latter being varied between 0.5 and 5.0×10^{-4} M in the case of β-CyD, and between 2 and 10×10^{-4} M in the case of γ-CyD. For Ben·α-CyD complex system, the concentration of Ben was kept constant at 1.5×10^{-4} M in the CD method and 4.0×10^{-4} M in the UV method, and that of α-CyD was varied between 1.0 and 6.0×10^{-2} M in both methods. For the determination of the thermodynamic parameters, the formation constants were measured at appropriate temperatures from 25 to 65 °C.

NMR Measurement——^{1}H-(99.6 MHz) and ^{13}C-NMR spectra (25.05 MHz) were recorded on a JEOL FX-100 machine at 27 °C, and ^{1}H-NMR (200 MHz) on a Varian XL-200 spectrometer at 24 °C. Host and guest molecules were dissolved in D_2O. HDO and dioxane were used as external references for ^{1}H- and ^{13}C-NMR, respectively. Parameters were set in the following range: acquisition time, 4 and 1.2 s; pulse angle, 45 and 30 °; accumulations, 30 and 2500; precision of chemical shift, 0.01 and 0.1 ppm for ^{1}H- and ^{13}C-NMR spectra, respectively.

Results and Discussion

Determination of Composition Ratio and Formation Constant

On addition of CyD to Ben solution, a CD band and difference UV absorbance were obtained at 220—230 nm as shown in Fig. 3. Both Ben and CyD have no intrinsic Cotton effect at 220—230 nm, and no CD band or UV absorption change appeared on addition of maltose (having no cavity) to Ben solution. Therefore, the new CD band and difference absorbance are not simple solvent effects, but represent definite evidence for the formation of the inclusion complex of Ben with CyD.[12]

The composition ratio and K value of the inclusion complex were determined by quantitative analysis of the induced CD and difference absorption spectra. Continuous variation plots of the CD band for the Ben·β-CyD and Ben·γ-CyD systems are illustrated in Fig. 4. Similar stoichiometric relationships were also obtained by use of the difference absorbance. The formation of the 1 : 1 complex in both systems was indicated by the shape of the plots in Fig. 4. It was not possible to determine the molar ratio of Ben·α-CyD complex by

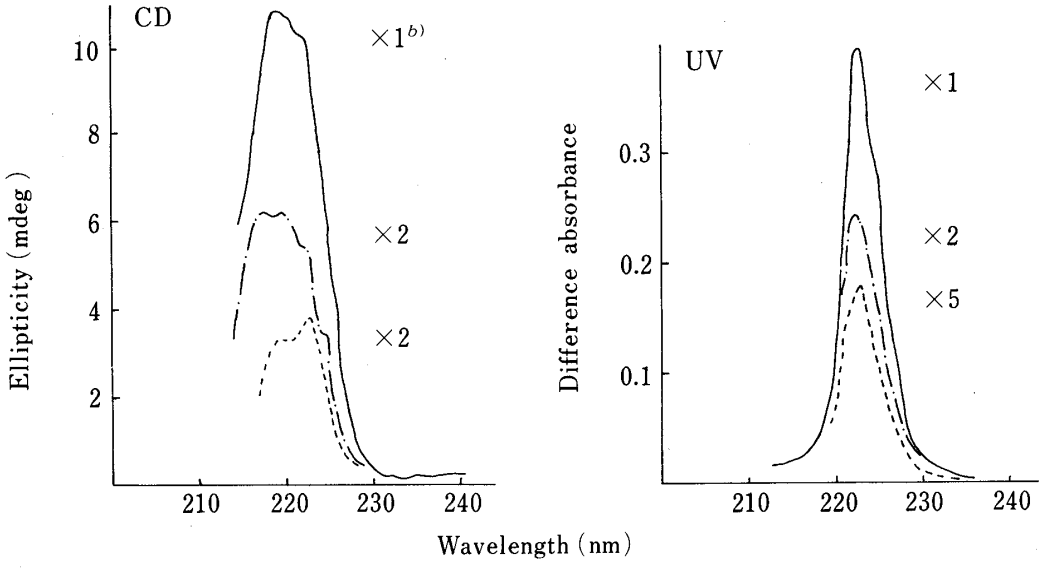

Fig. 3. CD and UV Spectra of Ben·CyD Systems[a)]

a) In phosphate buffer of pH 7.4 (μ=0.2) at 25 °C. *b*) Magnification.
----, α-CyD system; ——, β-CyD system; –·–·–, γ-CyD system.
Concentrations for CD spectral measurement: Ben (2.6×10^{-4} M)·β-CyD (2.6×10^{-4} M); Ben (2.6×10^{-4} M)·γ-CyD (2.6×10^{-4} M); Ben (1.4×10^{-4} M)·α-CyD (6.5×10^{-2} M).
Concentrations for UV spectral measurement: Ben (4.2×10^{-4} M)·β-CyD (4.2×10^{-4} M); Ben (4.0×10^{-4} M)·γ-CyD (4.0×10^{-4} M); Ben (4.0×10^{-4} M)·α-CyD (4.9×10^{-2} M).

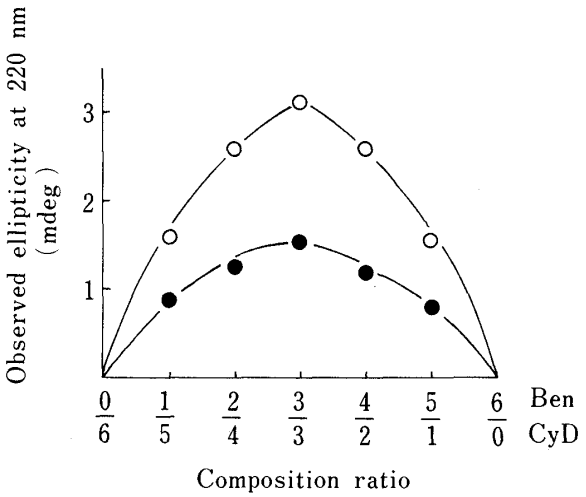

Fig. 4. Continuous Variation Plots of the CD Band for Ben·β-CyD and Ben·γ-CyD Systems[a]

a) CD method in phosphate buffer of pH 7.4 (μ=0.2) at 25 °C.
○, Ben (1.8×10^{-4} M)·β-CyD (1.8×10^{-4} M); ●, Ben (3.0×10^{-4} M)·γ-CyD (3.0×10^{-4} M).

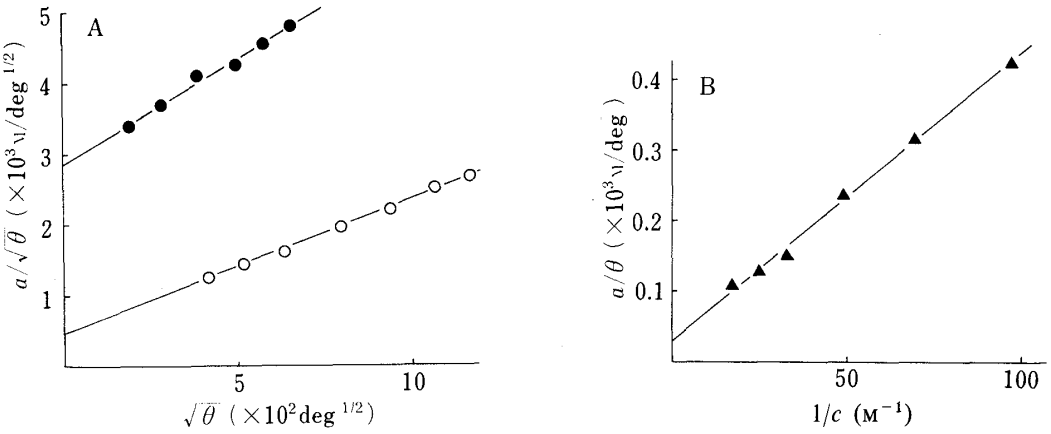

Fig. 5. Benesi–Hildebrand Plots for Ben·α-CyD and Modified Plots for Ben·β-CyD and Ben·γ-CyD Systems[a]

a) CD method in phosphate buffer of pH 7.4 (μ=0.2) at 25 °C.
A, modified B-H plots; B, B-H plots.
▲, Ben (1.5×10^{-4} M)·α-CyD (1.0—6.0×10^{-2} M); ○, Ben (0.5—5.0×10^{-4} M)·β-CyD (0.5—5.0×10^{-4} M); ●, Ben (2.0—10×10^{-4} M)·γ-CyD (2.0—10×10^{-4} M).
In the case of β- and γ-CyD complex systems, the concentration of Ben was kept equal to that of CyD.
a, initial conc. of Ben; c, initial conc. of CyD; θ, observed ellipticity.

the same method, because of its very weak CD band, but a B–H plot calculated on the assumption of a 1 : 1 complex gave a fairly linear relationship as shown in Fig. 5. Thus, the formation of a 1 : 1 complex was also indicated for the Ben·α-CyD system.

The values of K obtained by means of the CD and UV methods are summarized in Table II. In all three complexes, the K values obtained by the CD method were in good agreement with those by the UV method. As can be seen in Table II, the magnitudes of the K values for these three complexes are very different; β (8×10^4 M^{-1}) > γ (4×10^3 M^{-1}) $\gg$ α (3 M^{-1}). In particular, the K value of the complex between Ben and β-CyD is one of the highest among the values for CyD complexes reported in the literature.[2,3] The complex between *p*-hydroxybencyclane (OH-Ben) and β-CyD also showed a high K value, comparable to that of the Ben·β-CyD complex.

At the same time, in order to examine the isotope effect with D_2O, which is usually used as the solvent in studies of complexes by means of NMR spectra,[3] the K value of Ben·β-CyD complex in D_2O was determined from the UV spectra, and a result similar to that in H_2O was obtained. This indicates that the isotope effect with D_2O is negligible in forming Ben·CyD

TABLE II. Formation Constants (K Values)[a]

Complex	Solvent	CD method		UV method	
		K ($\times 10^{-3}$ M^{-1})	θ ($\times 10^{-3}$ M^{-1})	K ($\times 10^{-3}$ M^{-1})	$\Delta\varepsilon$ ($\times 10^{-3}$ M^{-1})
Ben·α-CyD	pH 7.4[b]	0.003±0.005	8±4	0.002±0.003	3.3±0.5
Ben·β-CyD	pH 7.4[b]	80±10	5±1	80±10	1.2±0.1
Ben·γ-CyD	pH 7.4[b]	4±1	3±1	4±0.5	0.6±0.1
OH-Ben·β-CyD	pH 7.4[b]	60±10	7±1	65±10	1.6±0.1
OH-Ben·γ-CyD	pH 7.4[b]	—	—	6±2	1.2±0.2
Ben·β-CyD	H_2O	80±10	5±1	—	—
Ben·γ-CyD	H_2O	4±1	3±1	—	—
Ben·β-CyD	D_2O	—	—	80±10	1.3±0.1
Ben·γ-CyD	D_2O	—	—	3±1	0.8±0.2

a) At 25 °C.
b) In phosphate buffer (μ=0.2).
K, formation constant of inclusion complex; θ, ellipticity; $\Delta\varepsilon$, difference in molar absorbance between guest and complex; —, not measured.

TABLE III. pH Effect on the Formation Constants[a]

pH	K Value (10^{-3} M^{-1})				
	Ben·α-CyD	Ben·β-CyD	Ben·γ-CyD	OH-Ben·β-CyD	OH-Ben·γ-CyD
5	0.003±0.005	75±10	—	—	—
6	0.003±0.005	80±10	4±1	—	—
7.4	0.003±0.005	80±10	4±1	65±10	6±2
9	—	85±10	—	75±20	—
10	0.003±0.005	80±10	3±1	20±5	1±0.4

a) CD method at 25 °C.
—, not measured.

inclusion complexes.

We then investigated the relationships between the inclusion phenomena and the pH of the medium. It was found that the pH of the system does not influence the inclusion phenomena significantly in the case of Ben and CyD's, as shown in Table III. On the other hand, in the case of OH-Ben·β-CyD and OH-Ben·γ-CyD, remarkable changes in the K values were observed around the pH values corresponding to pK_a of the phenolic OH group.

Determination of Thermodynamic Parameters

K and $\Delta\varepsilon$ values at various temperatures between 25 and 65 °C were obtained by measurement of the UV spectra. These data, and the changes of free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) for each inclusion complex calculated from these data, are listed in Table IV.

The above results may be classified into two types. That is, the data for Ben·γ-CyD complex are different from those of the other complex systems as follows. In the case of Ben·β-CyD complex, the K value decreases rapidly with rise of the temperature, but $\Delta\varepsilon$ is constant between 25—65 °C. Both ΔH and ΔS have large negative values (-11.5 kcal·mol^{-1} and -16.1 cal·mol^{-1}·deg^{-1}, respectively). In the case of OH-Ben·β-CyD complex, the variations of K, $\Delta\varepsilon$, ΔH (-11.3 kcal·mol^{-1}) and ΔS (-16.2 cal·mol^{-1}·deg^{-1}) appear to be nearly the

TABLE IV. Thermodynamic Parameters[a]

Complex	(°C)	K[b] ($\times 10^{-3}$ M^{-1})	$\Delta\varepsilon$[b] ($\times 10^{-3}$ M^{-1})	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (cal/mol·deg)
Ben·α-CyD	25	0.0022	3.3	−0.46		
	35	0.0018	3.3	−0.36	−2.4	−6.4
	45	0.0017	3.3	−0.33		
	55	0.0015	3.3	−0.26		
Ben·β-CyD	25	83	1.2	−6.68		
	35	40	1.2	−6.46		
	45	22	1.2	−6.30	−11.5	−16.1
	55	14	1.2	−6.20		
	65	7.9	1.2	−6.01		
Ben·γ-CyD	25	4.2	0.61	−4.92		
	35	4.0	0.58	−5.06	−0.9	+13.5
	45	3.8	0.55	−5.19		
	55	3.6	0.53	−5.32		
OH-Ben·β-CyD	25	64	1.6	−6.53		
	35	31	1.6	−6.31		
	45	16	1.6	−6.10	−11.3	−16.2
	55	11	1.6	−6.03		
	65	6.3	1.6	−5.85		

a) UV method in phosphate buffer of pH 7.4 (μ=0.2).
b) Accuracy is similar to that in Table II.
K, formation constant of inclusion complex; $\Delta\varepsilon$, difference in molar absorbance between guest and complex; ΔG, free energy change (kcal/mol); ΔH, enthalpy change (kcal/mol); ΔS, entropy change (cal/mol·deg).

same as those of Ben·β-CyD. It is interesting that OH-Ben·β-CyD complex shows thermal behavior similar to that of Ben·β-CyD complex in spite of the substitution with a hydroxyl group. In the case of Ben·α-CyD complex, though a clear decision cannot be made owing to the difficulty of accurate measurement (because of the weak interaction between Ben and α-CyD), the same tendency for independence of temperature was observed as in Ben·β-CyD, and both ΔH and ΔS have small negative values. On the other hand, quite a different tendency was observed in the case of γ-CyD complex. The K value is almost constant between 25—55 °C, but $\Delta\varepsilon$, which is only about one-half that of the β-CyD complex at 25 °C, decreases gradually with increase of temperature. ΔH has a small negative value (-0.9 kcal·mol^{-1}) and ΔS has a large positive value ($+13.5$ cal·mol^{-1}·deg^{-1}).

NMR Study of the Inclusion Complexes

Proton NMR spectra of the host molecule (CyD) and the guest molecule (Ben) were studied to clarify the inclusion phenomena and the structure of the complexes. ^{13}C-NMR spectra of the guest molecule was also studied.

(a) Behavior of the CyD Proton Signals on Inclusion——Upfield shifts of the signals of the H_3 and H_5 protons on the inner surface of CyD were observed in all Ben complex systems, and the magnitudes of the shifts ($\Delta\delta$'s) are proportional to the percentage of CyD bound to Ben, which can be calculated from the K values determined by the CD method (Fig. 6). In the case of the α-CyD complex system, $\Delta\delta$ can be observed only in the region of low percent α-CyD bound to Ben, because of the low K value and/or the limitation of the solubility of α-CyD. Comparing the magnitudes of $\Delta\delta$'s for H_3 and H_5, the shifts for these protons are different in each complex system. That is, $\Delta\delta$ of H_3 is larger than that of H_5 in α-CyD and γ-CyD complexes, while it is smaller in the β-CyD complex. These shifts are considered to be

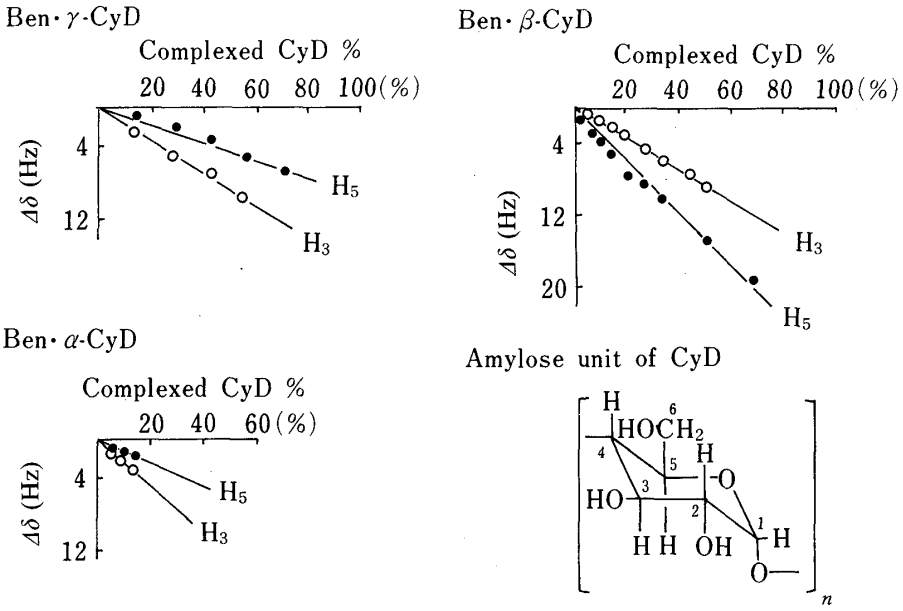

Fig. 6. Induced ^{1}H-Chemical Shifts ($\Delta\delta$'s) of CyD at 100 MHz[a]

a) In D_2O.

TABLE V. Induced ^{1}H-Chemical Shifts ($\Delta\delta$'s) of Ben with 100 MHz[a]

Complex	Complexed Ben (%)	$\Delta\delta$[b] (Hz) Proton of Ben[c]					
		7—9	5	10	11	12	13
Ben·α-CyD	24.1	2.2	6.1	—	3.2	4.9	0
Ben·β-CyD	99.9	−6.6	8.8	—	>10	14.1	8.8
Ben·γ-CyD	99.6	3.4 −7.8	−9.8	—	6.6	4.4	6.4

a) In D_2O.
b) $\Delta\delta = \delta$ (Ben·CyD) $-\delta$ (Ben).
c) Numbering of Ben protons is shown in Fig. 1.
—, not observed.

induced by the diamagnetic anisotropy of the guest molecule (Ben).[13]

(b) Behavior of Ben Proton Signals on Inclusion——On inclusion, the resonance peaks for all protons of Ben were broadened, and most of them were shifted downfield. That is, it was observed that only the signals of aromatic protons of Ben in the case of β-CyD, and the signals of some of the aromatic protons and benzyl protons in the case of γ-CyD were shifted upfield by complex formation, though the signals of all other protons were shifted downfield in the 100 MHz spectra, as shown in Table V. In the case of α-CyD, significant shift of the aromatic proton signals could not be observed in the 100 MHz spectra, although a downfield shift was observed for all other protons.

In order to make more detailed analyses of the behavior of each proton signal of the guest molecule (Ben), especially those of the aromatic and benzyl protons, measurement of the 200 MHz NMR spectra was carried out. In the case of uncomplexed Ben (Fig. 7A), the peaks of the aromatic protons between 7.35—7.1 ppm separate at 7.2 ppm into two parts. The ratio of the integral value for the higher field peaks to that for the lower field peaks corresponds to two to three protons. The two higher field protons were assigned as the *ortho* protons (H_o)

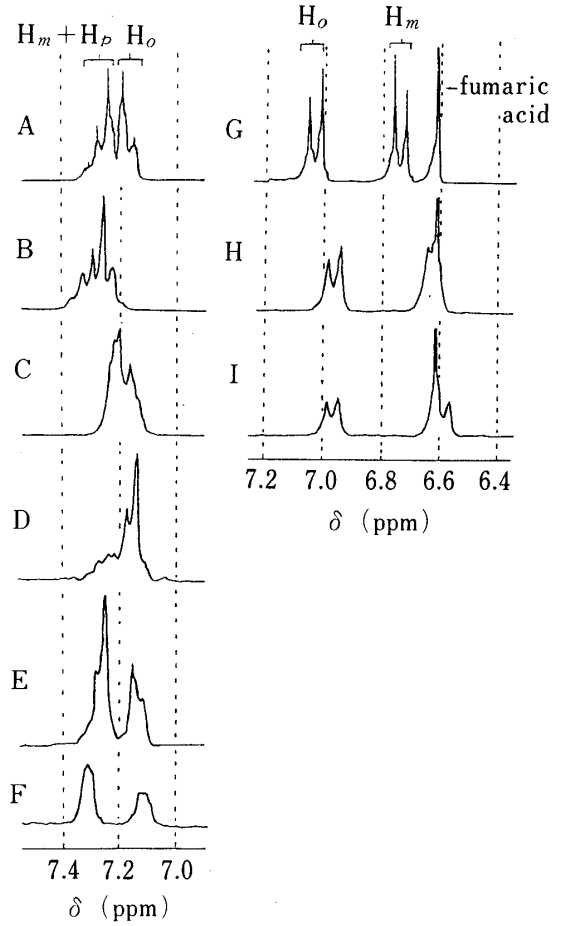

Fig. 7. ^{1}H-NMR Signals of Aromatic Protons at 200 MHz[a)]

a) In D_2O.
A, Ben alone; B, α-CyD·Ben (33%); C, β-CyD·Ben (55%); D, β-CyD·Ben (100%); E, γ-CyD·Ben (54%); F, γ-CyD·Ben (97%); G, OH-Ben alone; H, β-CyD·OH-Ben (56%); I, β-CyD·OH-Ben (99%).
The values in these parentheses represent percentages of the guest bound to CyD.

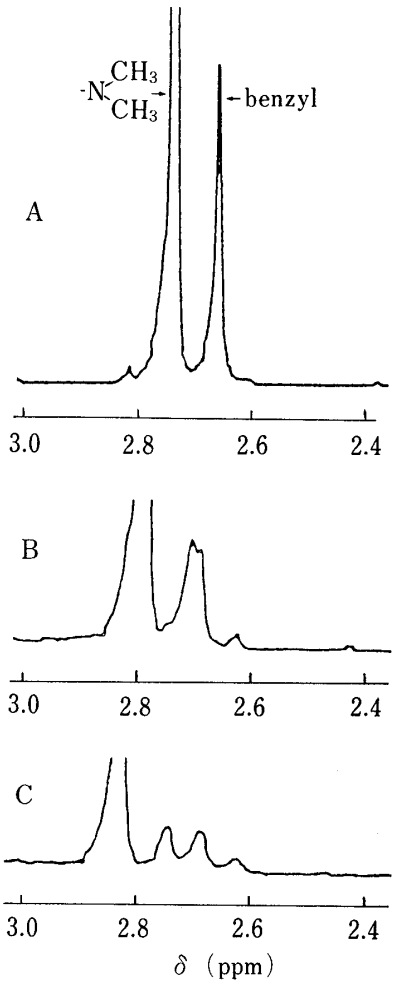

Fig. 8. ^{1}H-NMR Signals of Benzyl Protons in the OH-Ben·CyD System at 200 MHz[a)]

a) In D_2O.
Percentage of OH-Ben bound to β-CyD: A, 0%; B, 56%; C, 99.8%.

from the shape of the peaks. That is, H_o in the phenyl ring of Ben inherently shows a double-doublet, AB-type pattern due to *ortho* (J_{ab} = 7—10 Hz) and *meta* (J_{ab} = 2—3 Hz) coupling. On the other hand, one *para* proton (H_p) is assigned to one of the three protons at lower field. *Meta* protons (H_m) show, in principle, a triplet, AB_2-type pattern due to two *ortho* couplings (J_{ab} = 7—10 Hz). Although the lower field peaks do not appear as a triplet, this can be attributed to overlap of the H_p peak with those of H_m between 7.35—7.2 ppm. In Ben·β-CyD complex, all peaks of aromatic protons were shifted upfield by complex formation. These upfield shifts of all aromatic protons are characteristic in the case of β-CyD, in contrast to the case of α- or γ-CyD. The magnitudes of $\Delta\delta$ for H_m and H_p are larger than that of H_o. This becomes more apparent with increase in the percentage of bound Ben.

In order to confirm the above assignments for the aromatic protons of Ben, the spectra of OH-Ben in the presence and absence of β-CyD were measured at 200 MHz, as shown in Figs. 7G—I. It has been already mentioned that the configuration of OH-Ben·β-CyD should be similar to that of Ben·β-CyD in view of the similar pattern of CD spectra, the similar K values, and the similar thermodynamic parameters (Tables II and IV). In the case of OH-Ben alone, the peaks of the aromatic protons were observed as an AB pattern at 7.1—6.7 ppm. The

peaks at higher field were assigned to H_m and those at lower field to H_o by considering the substituent effect of the *p*-hydroxyl group. The OH-Ben·β-CyD complex system showed the same tendency as did the Ben·CyD complex system, *i.e.*, the magnitude of $\Delta\delta$ for H_m was larger than that for H_o. This consistency in the behavior of $\Delta\delta$ for the aromatic protons in the two complex systems supports the above assignment.

On the other hand, it was observed that the signals of the benzyl protons as a whole were shifted downfield by inclusion, both in Ben· and OH-Ben·β-CyD complexes. Additionally, though the signal pattern of the benzyl protons cannot be observed directly in Ben complex systems because of overlapping with dimethyl peaks, the equivalent two benzyl protons in OH-Ben were split to AB-type signals by the addition of β-CyD, and the separation of the inner pair of this peak increased with increase in the percentage of OH-Ben bound to β-CyD, as shown in Fig. 8. This phenomenon probably also occurs in Ben·β-CyD complex, which has nearly the same structure as OH-Ben·β-CyD complex. In Ben·γ-CyD complex, the H_o and benzyl proton signals are shifted downfield by the complex formation. In Ben·α-CyD complex, almost all of the aromatic proton signals were shifted downfield except one, which retained its original position. This unshifted proton was assigned to H_p, because both *ortho* and *para* protons should have an integral value corresponding to two protons. In this complex system, the signals of the benzyl protons were also shifted downfield by inclusion.

Concerning the behavior of the other protons in the 100 MHz spectra, it was found that on inclusion the signals of the propylamine protons and the cycloheptyl ring protons were shifted downfield as a whole, accompanied with line-broadening. In the 200 MHz NMR spectra, the protons of the propylamine moiety showed a tendency similar to that in the 100 MHz NMR, but some phenomena relating to the protons in the cycloheptyl ring were observed in more detail. That is, some of the signals were shifted upfield in the 200 MHz NMR of the β-CyD and γ-CyD complex systems, as shown in Fig. 9. In order to assign the upfield-shifted protons, the NMR spectra of Ben-*d* were measured both in the absence and presence of CyD, and were compared with those of Ben·CyD complex. By comparison of the spectra

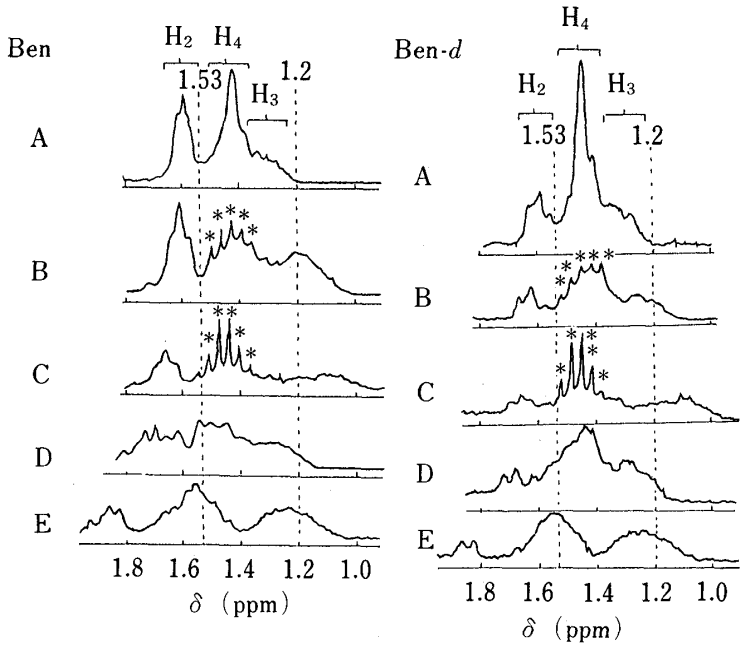

Fig. 9. ^{1}H-NMR Signals of Cycloheptyl Ring Protons at 200 MHz[a)]

a) In D_2O.
A, guest alone; B, γ-CyD·guest (59%); C, γ-CyD·guest (97%); D, β-CyD·guest (50%); E, β-CyD·guest (100%).
The values in these parentheses represent percentages of the guest bound to CyD.
* Peaks due to impurities in the γ-CyD sample.

TABLE VI. Induced ^{13}C-Chemical Shifts ($\Delta\delta$) of Ben[a]

Carbon of Ben[d]	$\Delta\delta$[b] (ppm) Complex[c]		
	Ben·α-CyD	Ben·β-CyD	Ben·γ-CyD
1	−0.30	−0.85	−0.88
2	0.17	−0.06	−0.12
3	0.05	−0.65	0.66
4	0.10	−0.37	0.58
5	−0.11	3.58	3.36
6	−0.05	−1.05	−1.51
7	0.02	−0.58	0.20
8	0.02	−0.15	0.09
9	−0.10	0.58	0.31
10	0.17	0.02	−0.37
11	−0.02	0.44	0.46
12	0.05	−0.39	−0.46
13	−0.01	0.09	0.05

a) In D_2O.
b) $\Delta\delta$ (ppm) = δ (Ben·CyD) − δ (Ben).
c) Percentage of Ben bound to CyD: α-CyD, 37%; β-CyD, 99%; γ-CyD, 98%.
d) Numbering of carbons is shown in Fig. 1.

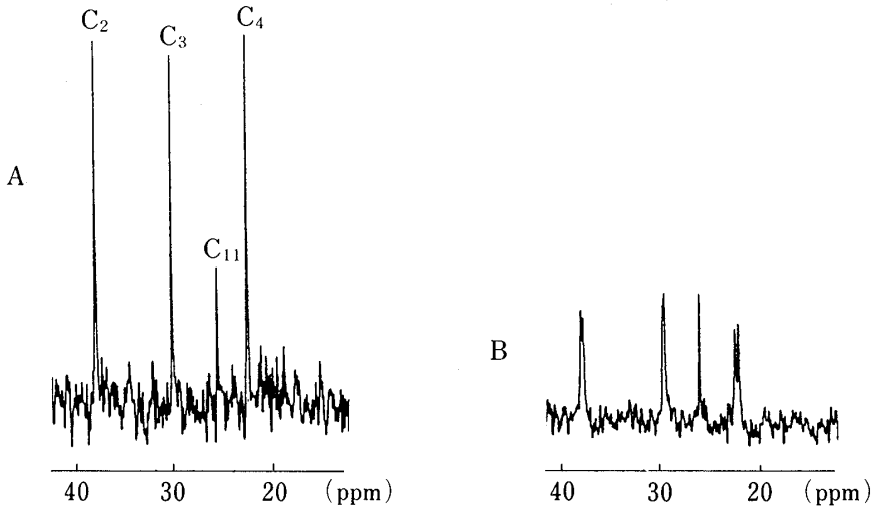

Fig. 10. ^{13}C-NMR Signals of Cycloheptyl Carbons[a]

a) In D_2O.
A, Ben alone; B, β-CyD·Ben (99%).
The values in these parentheses represent percentages of Ben bound to CyD.

of the guest molecules alone (Ben and Ben-*d*), the peaks between 1.53—1.8 ppm were assigned to the protons at the 2-position (H_2) on the cycloheptyl ring, because only about two protons are observed at 1.53—1.8 ppm in the NMR spectra of Ben-*d*. On the other hand, the remainder of the peaks of the cycloheptyl protons consists of two parts, sharp low-field and broad high-field, in the guest molecules alone. It is expected that the peak shape of H_3, which is influenced directly by four protons (H_2 and H_4), should be broader than that of H_4, which is influenced by only two protons (H_3). Thus, the broad high-field peak and sharp low-field peak were assigned to H_3 and H_4, respectively. In the complex state, the behavior of the cycloheptyl protons was as follows. The signals of the H_2 and H_4 protons shift downfield in Ben·β-CyD complex, and scarcely move in Ben·γ-CyD complex. However,

TABLE VII. Induced ^{13}C-Chemical Shifts ($\Delta\delta$) of CyD[a]

Carbon of CyD	$\Delta\delta$[b] (ppm) Complex[c]		
	α-CyD·Ben	β-CyD·Ben	γ-CyD·Ben
1	−0.01	0.23	0.82
2	0.28	−0.13	0.04
3	−0.06	0.28	0.06
4	0.13	0.16	0.79
5	0.08	−0.13	0.38
6	0.27	−0.32	−0.03

a) In D_2O.
b) $\Delta\delta$ (ppm) = δ (CyD·Ben) − δ (CyD).
c) Percentage of CyD bound to Ben: α-CyD, 37%; β-CyD, 99%; γ-CyD, 98%.

that of H_3 shifts upfield in both complexes, and the magnitude of $\Delta\delta$ in γ-CyD complex is larger than that in β-CyD complex.

^{13}C-NMR spectroscopy is known to be useful for analyzing CyD inclusion phenomena.[14] In the present studies also, ^{13}C-NMR spectra were measured. Although meaningful behavior of the carbon signals of Ben upon inclusion could not be observed in ^{13}C-NMR studies, as shown in Table VI, it was found that signal peaks of the intrinsically equivalent carbons on the cycloheptyl ring appeared as two split signals of equal integral value in the Ben·β-CyD complex, or as a shouldered signal in the Ben·γ-CyD complex (Fig. 10). On the other hand, the chemical shift changes for carbons of CyD's upon addition of Ben are shown in Table VII.

Probable Structure of the Inclusion Complex

The probable structure of the inclusion complex in each system was studied by using Corey–Pauling–Koltun (CPK) models, and by considering the CD, UV, NMR data, molecular size and structure of the guest molecules, and cavity size of the host molecules.

The structure of Ben consists of three portions: benzyl part (A), aminopropyl part (B), and cycloheptyl part (C) as shown in Fig. 1. It can be concluded from considerations of size, that not the whole molecule, but only one of the three portions of Ben can be included within the cavity of CyD (Table I). Furthermore, the whole molecule of Ben cannot pass through the cavity owing to the steric hindrance of the other two parts. It will be discussed below which of the parts of Ben is actually included, and from which direction, the large diameter side (a-direction) or the small-diameter side (b-direction).

From the similarity in the appearance of the CD and difference UV spectra shown in Fig. 3, it seems highly probable that the same part of Ben is included in all three types of complexes.

In order to compare quantitatively the sizes of the cavity and the guest molecule, the diameter of each part of the Ben molecule and that of the cavity in each CyD were measured with the aid of CPK models, with the results shown in Table I. From a comparison of the molecular sizes of CyD and Ben, the following conclusions were reached. In the case of α-CyD complex, part C cannot be included at all, and neither part A nor B can penetrate deeply into the cavity, if it is included at all. In the case of β-CyD complex, the diameters of parts A and B fit the cavity with van der Waals radii, but part C is a poorer fit. In the case of γ-CyD complex, neither part A nor B can form a tight inclusion, because the cavity size is too large, but part C could fit the cavity of γ-CyD.

The possibility of the aminopropyl part (B) of Ben being included may be ruled out in all

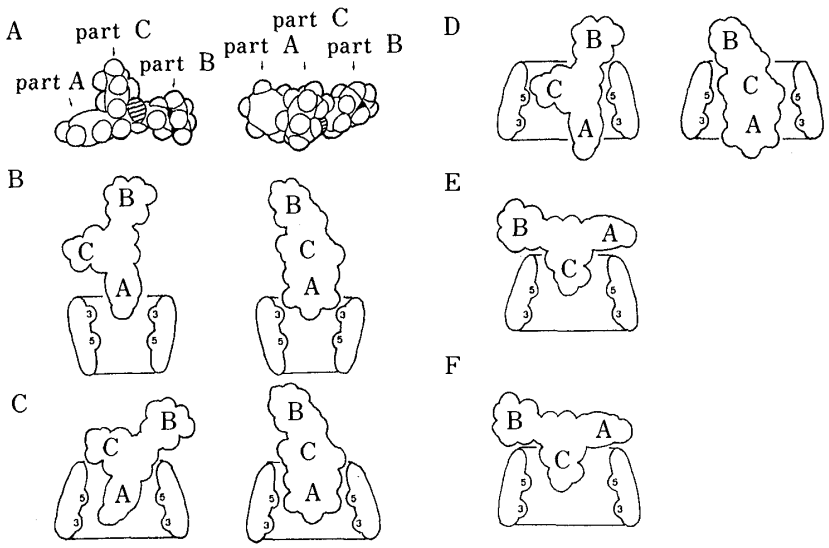

Fig. 11. CPK Space-filling Model of Guest Molecules and Various Possible Orientations of Ben in the CyD Cavity

A, Ben; B, α-CyD·Ben (part A); C, β-CyD·Ben (part A); D, γ-CyD·Ben (part A); E, β-CyD·Ben (part C); F, γ-CyD·Ben (part C).

three complex systems, considering the independence of the K value on pH change of solvent, as shown in Table III, because, if part B is included, the K values should change substantially around pH 9.5[15] corresponding to the pK_a of this part.[16] Thus, the part of the Ben molecule included in the cavity is thought to be A or C, to which the above considerations on size apply.

Furthermore, taking into account the position of each part in Ben, the following assumption can be made. In Ben having a molecular shape like the letter "T," part A is situated at the level bar portion, while part C is at the perpendicular portion as shown in Fig. 11A. Then, as regards the manner of inclusion into the cavity of CyD, it is assumed that part A can penetrate not only in parallel, but also obliquely with respect to the molecular axis of the guest as shown in Figs. 11C and D. However, part C can be included only in parallel because both parts A and B are obstructed by the rim of CyD as shown in Figs. 11E and F. Therefore, part C, even if included, could penetrate only shallowly, compared with part A. In fact, while there is no positive evidence for the inclusion of part C, it can be assumed that the benzyl part (A) is included within the cavity of CyD on the basis of the following three facts. First, only one CD band and difference UV band at 220—230 nm attributable to phenyl π–π^* transition appeared on the addition of every CyD (Fig. 3)[12]; second, the magnitude of the shifts for H_3 and H_5 in the case of β-CyD is of similar degree to that of β-CyD complex with aromatic substances reported by Demarco (Fig. 6)[13]; third, the changes of the chemical shifts (upfield shifts) of the aromatic protons and of the benzyl protons showed different behavior from those (downfield shifts) of all other protons of Ben as listed in Table V (100 MHz). These upfield shifts of the signals of the aromatic protons and/or benzyl protons on inclusion might be caused by C–C bond anisotropy of CyD and so on.[17] As additional evidence, the included part was determined conclusively by the result obtained with OH-Ben. The K values of OH-Ben·CyD complexes decreased markedly above pH 10, corresponding to the pK_a of the phenolic hydroxyl group, as shown in Table III.[18] These phenomena indicate that complexation between OH-Ben and CyD was depressed by increase in the amount of the ionized form of OH-Ben, and this is direct evidence that the OH-containing part is included.[16]

Next, we proceed a step further and conclude that the inclusion takes place by the approach of Ben from the large-diameter side (a-direction) in the case of α-CyD complex and from the small-diameter side (b-direction) in the case of β- and γ-CyD complexes. The reason

is as follows. In the case of α-CyD complex system, it was observed in the 200 MHz NMR spectra that the signal of the *para* proton remains at its original position, although all other aromatic proton signals shift downfield on inclusion (Fig. 7B). From the above observations, two possibilities can be considered for the guest position: the *para* hydrogen alone enters into or is extruded from the cavity. But, in view of the results obtained using CPK models as mentioned above, and the behavior of $\Delta\delta$ of the aromatic protons in the case of β-CyD complex, the former case seems more plausible, that is, Ben is included only around the *para* position of the phenyl ring. Combining this result with the observation that the magnitude of $\Delta\delta$ for H_3 is larger than that for H_5 in the host molecule (α-CyD), it seems reasonable to assume that the inclusion takes place from the large-diameter side of α-CyD.

In the β-CyD complex of Ben, it has been concluded that the aromatic ring entered from the opposite side, that is, from the small-diameter side, since the relative magnitude of $\Delta\delta$ for H_3 and H_5 is contrary to the case of α-CyD; $\Delta\delta(H_5) > \Delta\delta(H_3)$. This behavior of H_3 and H_5 is the same as reported by Thakkar, who assumed that association took place by approach of the guest molecule from the primary hydroxyl side of CyD (b-direction), based on a comparison of the relative magnitude of $\Delta\delta$ for H_5 and H_3 in the barbiturate·β-CyD complex system.[19] By considering the induced chemical shift change ($\Delta\delta$) of the guest molecule (Ben), the possible structure of Ben complex may be further defined. As mentioned before, the signals of all aromatic protons of Ben shift upfield on inclusion in β-CyD, although those of almost all other protons, except for some on the cycloheptyl ring, shift downfield. Considering the upfield shift of the signals of all aromatic protons in Ben, the large magnitude of $\Delta\delta$ for H_3 and H_5 in CyD, the extremely large K value and the induced strong CD and UV band, it may be concluded that tight inclusion occurs over the whole aromatic ring of Ben. The structure of Ben·β-CyD complex obtained on the basis of the above reasoning is shown in Fig. 12, which is also supported by the consideration of the phenobarbital·β-CyD complex reported by Thakkar.[19] In the phenobarbital·β-CyD complex, the signals of H_5 shows a large upfield shift, similar in extent to that in Ben·β-CyD complex, though the H_3 signal does not.

In the γ-CyD complex, the relative magnitude of $\Delta\delta$ for H_5 and H_3 is reversed as compared to the case of β-CyD (Fig. 6): $\Delta\delta(H_3) > \Delta\delta(H_5)$. This might be taken to indicate that the guest molecule enters from the opposite side. However, from the viewpoint of energy, the inclusion should occur from the small-diameter side which provides a larger driving force to release high-energy water from the cavity as in β-CyD. The larger magnitude of $\Delta\delta$ for H_3 than H_5 could be explained by assuming that the phenyl ring of Ben is introduced from the b-

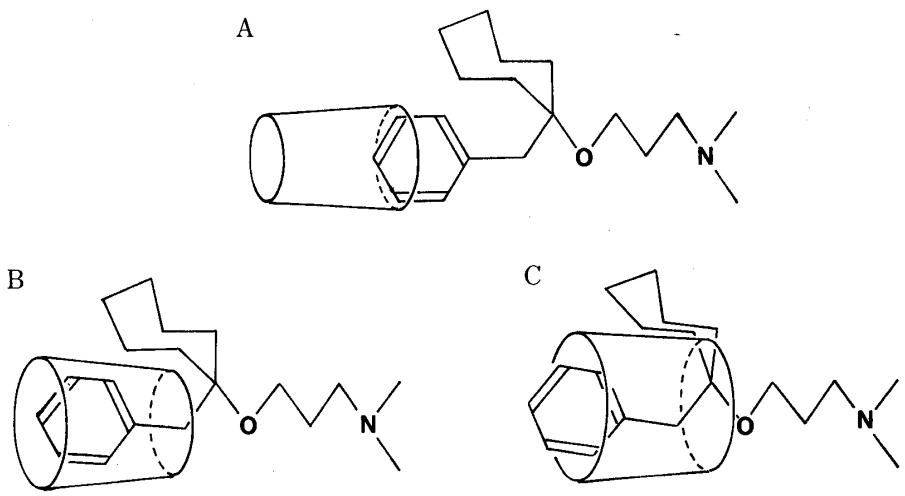

Fig. 12. Schematic Drawing of Structures of Ben with CyD's in the Aqueous Phase
A, α-CyD·Ben; B, β-CyD·Ben; C, γ-CyD·Ben.

direction, but passes the neighborhood of H_5 and is situated around H_3. It was also observed from the 200 MHz NMR spectra that upfield signal shifts for H_o and benzyl protons, and downfield signal shifts for H_m and H_p are induced by inclusion. These features of $\Delta\delta$'s may result from a situation such that H_m and H_p extrude from the large-diameter side of γ-CyD, and only H_o and benzyl protons are located within the cavity.

In order to obtain further evidence on the approach direction of Ben to CyD, ^{13}C-NMR spectra of the three kinds of CyD's were measured in the presence and absence of Ben (Table VII). The C_1 and C_2 atoms of CyD are omitted from consideration, because C_1 has been reported not to be influenced by direct interaction with the guest molecule[20] and C_2 is situated at the outermost position of the CyD cavity. Thus, the magnitudes of $\Delta\delta$'s for C_3, C_4, C_5, and C_6, which are situated in that order along the a-direction (Fig. 1), were compared. Although, in the case of γ-CyD complex, a definite correlation between the relative magnitudes and the shielding effect of Ben was not observed, the carbon nearer to the small-diameter side was shielded more strongly in β-CyD complex, while the opposite tendency was found in the α-CyD complex. These results seem to support the conclusion based on ^{1}H-NMR data, that Ben enters β-CyD from the small-diameter side, and α-CyD from the large-diameter side.

Turning to the protons on the part of Ben which is always located outside the CyD cavity, it has been described previously that most of them show downfield signal shifts. However, the signal of H_3 on the cycloheptyl ring in Ben·β-CyD and Ben·γ-CyD complexes was shifted upfield by inclusion, and the magnitude of $\Delta\delta$ for this proton is larger in γ-CyD complex than in β-CyD complex, as shown in Fig. 9. On the other hand, the H_2 and H_4 signals are shifted downfield in the β-CyD complex, and are scarcely shifted in the γ-CyD complex. These properties of the cycloheptyl protons can be understood by considering that H_2 is little affected by the screening environment inside the CyD cavity,[21] because it is located at the end of the cavity when inclusion takes place, while H_3 is fixed fairly well outside CyD. Moreover, in the β-CyD complex, it is observed that the signal peak of the intrinsically equivalent protons H_2 and $H_{2'}$ becomes split upon fixation owing to the formation of the inclusion complex. In the γ-CyD complex, this phenomenon cannot be observed. This difference can be attributed to the difference in the magnitude of the complex formation constant.

The phenomena that the single ^{1}H-NMR peak of equivalent benzyl protons in OH-Ben and the single ^{13}C-NMR peak of equivalent cycloheptyl carbons in Ben split upon addition of β-CyD seem to arise from the considerable fixation of the guest molecule inside or outside β-CyD, upon inclusion. These split signals could not result from two peaks due to free and complexed forms, but reflect two different environments caused by the inclusion. Hence, the observation of the split peaks suggests that the benzyl part and some part of the cycloheptyl ring could be complexed randomly in the direction perpendicular to the symmetry axis of CyD, but might be located with a limited orientation against one amylose unit in CyD on the microscopic scale.

Thus, it has become apparent that the Ben inclusion complexes with three kinds of CyD all have different structures, as shown in Fig. 12. The orientation of Ben inside and/or probably outside the cavity of CyD seems to be limited considerably in the β-CyD complex.

Driving Force of Inclusion Complexation

Driving forces for inclusion complexation between CyD and a guest molecule may include van der Waals–London dispersion force, hydrogen bonding, hydrophobic interaction, release of high-energy water molecules from the cavity of CyD, and release of strain energy in the macromolecular ring of CyD.[22] In order to define the driving force, we analyzed the thermodynamic parameters as follows.

In Ben·β-CyD complex, $\Delta\varepsilon$ values are constant regardless of temperature. Similar phenomena were observed but not discussed in the study of the molecular ellipticity of *p*-

nitrophenyl-*N*-acetyl-β-D-glucosamide complexes with α- and β-CyD reported by Harata.[23] We interpret these phenomena as follows. The independence of $\Delta\varepsilon$ values from temperature means that the thermal motion of β-CyD is depressed by the tight packing and the deep penetration of Ben within the cavity of β-CyD. This tight inclusion will produce not only a large favorable ΔH ($-11.5\,\mathrm{kcal\cdot mol^{-1}}$) due to the contribution of van der Waals–London dispersion froce, but also a large unfavorable ΔS ($-16.1\,\mathrm{cal\cdot mol^{-1}\cdot deg^{-1}}$) due to the loss of the conformational freedom of β-CyD and/or the translational and rotational freedom of Ben.[12a,23,24]

In the case of the α-CyD complex, it is assumed that the included aromatic ring of Ben cannot penetrate deeply into the comparatively small cavity of α-CyD, because of the intricate structure of Ben, but merely sits on top of the cavity as suggested for the complex of bulky adamantanecarboxylate with α-CyD.[23] This shallow penetration of Ben does not sufficiently restrict the conformational freedom of α-CyD, and is probably responsible for the small negative values of ΔH and ΔS.

In Ben·γ-CyD, in contrast to the case of α- and β-CyD complexes, the reduction of $\Delta\varepsilon$ with increase of temperature may be due to the increase of the mean distance between the chromophor of Ben and the perturbing dipoles of γ-CyD, owing to increase of the thermal vibration of γ-CyD, even if Ben is included within the cavity of γ-CyD. That is to say, this complex is loosely packed, because there is an appreciable space between the guest and the cavity walls of γ-CyD. The weak interaction owing to this loose inclusion between Ben and γ-CyD is supported by the small negative value of ΔH ($-0.9\,\mathrm{kcal\cdot mol^{-1}}$). On the other hand, the very large value of ΔS ($+13.5\,\mathrm{cal\cdot mol^{-1}\cdot deg^{-1}}$), in contrast to the case of α-CyD or β-CyD complex, may be attributed mainly to the following factors: (1) there will be very little loss of conformational freedom of γ-CyD because of the loose fit[24]; (2) since Ben will presumably penetrate more deeply into the larger cavity of γ-CyD than into the smaller cavity of α-CyD or β-CyD, and since the cavity of γ-CyD includes more water (sixteen mol)[25] than that of α-CyD (two mol)[26] or β-CyD (eight mol)[27] in the uncomplexed state, the included Ben will release a larger number of high-energy water molecules from the cavity of γ-CyD to the bulk solvent, compared to the case of α- or β-CyD.[24] Thus, the γ-CyD complex formation seems to be governed by hydrophobic interaction.[22,28]

Thus, it appears that not only the complex stability, but also the driving force of complexation are influenced by the molecular size of the guest and the cavity size of CyD.

Conclusion

In the present paper, dealing with a series of Ben·CyD inclusion complexes, the following conclusions have been reached.

(1) The molar ratio of Ben to CyD is 1 : 1 in each complex.

(2) The K values of Ben inclusion complexes with α-, β-, and γ-CyD are very different (2—3, 8×10^4 and $4\times10^3\,\mathrm{M^{-1}}$, respectively).

(3) The isotope effect of D_2O is negligible in the formation of Ben·CyD inclusion complexes.

(4) The part of Ben included within the cavity of CyD is the aromatic ring in each complex.

(5) The aromatic ring of Ben is introduced into the cavity of CyD from the large-diameter side in α-CyD complex, and from the small-diameter side in β- and γ-CyD complexes.

(6) The relative disposition between Ben and CyD may be as follows: in α-CyD complex, only the neighborhood of the *para* position of the aromatic ring of Ben is included within the cavity of α-CyD; in β-CyD complex, Ben is bound tighty over the whole aromatic

ring inside the cavity, and moreover, some other parts of Ben are fixed significantly outside the cavity; in γ-CyD complex, Ben is bound loosely to γ-CyD at the aromatic ring and around the neighborhood of the benzyl position, with the *para* position of the aromatic ring extruding from the large-diameter side of γ-CyD.

(7) The primary driving force for formation of the inclusion complex seems to be van der Waals interaction for α- and β-CyD complexes, while it is hydrophobic interaction for γ-CyD complex.

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