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Studies of Cyclodextrin Inclusion Complexes. II.[1] Application of the Partition Coefficient Method[2]

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The behavior of 1 : 1 inclusion complexes between cyclodextrins (CyD) and bencyclane (Ben) in a two-liquid-phase system of octanol and water was quantitatively investigated by measurement of partition coefficients (PC method). It was found that octanol partially prevents the formation of CyD·Ben complex and the apparent formation constant (K') was calculated from the apparent partition coefficient (P^*). This phenomenon was also observed in general with CyD 1 : 1 inclusion complexes of various drugs. The corrected formation constants (K) of these complexes in the aqueous phase were determined from the K' values by applying the equilibrium equations.

Keywords——partition coefficient; inclusion complex; cyclodextrin; formation constant; octanol; UV; GC; bencyclane; difference absorbance

Considerable attention has been focused on inclusion complexes between cyclodextrin (CyD) and drugs from both fundamental and applications viewpoints.[3] In the preceding paper,[1] we reported the physicochemical properties and structures in the aqueous phase of the inclusion complexes of α-, β-, and γ-CyD with bencyclane (Ben), *N*-[3-(1-benzyl-cycloheptyl-oxy)-propyl]-*N*,*N*-dimethylamine. As an extension of the above study on the Ben·CyD complexes in the aqueous phase, we have investigated their behavior in two-phase systems such as octanol/water. The partition coefficient (PC), especially in the octanol/water system, is an important parameter for describing the transport phenomena of various drugs in biological systems,[4] but, up to the present, there is only a limited number of reports about the PC measurement of CyD inclusion complexes,[5] and no attempt has been made at theoretical analysis of the partition phenomena in a two-liquid-phase system.[6]

In this paper, we describe a study on the inclusion phenomena between CyD and several drugs including Ben, in the octanol/water system. It has been shown that the measurement of PC in octanol/phosphate buffer (pH 7.4) system can be a simple and quantitative analytical method for clarifying inclusion phenomena, and the corrected formation constant (K) of the complex in aqueous phase can be calculated from the apparent formation constant (K'), the latter being determined from the apparent partition coefficient (P^*) by applying a set of equilibrium equations.

Experimental

Materials——Bencyclane fumarate were supplied by Medimpex (Hungary). α-, β-, and γ-CyD's were purchased from Tokyo Kasei Ltd. and used without further purification. The following drugs were extracted from commercial products: levomepromazine (LPZ (Shionogi & Co., Ltd.)); chlorpromazine (CPZ (Shionogi & Co., Ltd.)); imipramine (IPM (Fujisawa Pharmaceutical Co., Ltd.)); hexobarbital (HBT (Teikoku Co., Ltd.)); amobarbital (ABT (Nihon Shinyaku Co., Ltd.)); pentobarbital (PBT (Pitman-Moore, Inc.)), and recrystallized from suitable solvents

Table I. Measurement Conditions for Apparent Partition Coefficient (P^*) of β-CyD·Drug Systems

Drug	D_i ($\times 10^4$ M)	C_i/D_i (Molar ratio)	V_o/V_w (Volume ratio)	Wavelength used for analysis (nm)
Ben	5	1—3	0.5/50	a)
LPZ	5	1—6	0.5/50	303
CPZ	3	1—10	0.2/50	305
IPM	5	1—10	0.2/50	250
HBT	7.5	2.5—10	1/50	240
ABT	2	10—30	1/50	238
IDM	15	1—6	1/10	265
PBT	10	2—6	2/5	238
SA	10	2.5—8	50/10	258

a) Analysis by GC.

(mp 154—156 °C for ABT, 143—145 °C for HBT, 127—128 °C for PBT, 192—193 °C for CPZ hydrochloride, 168—170 °C for IPM hydrochloride, and 185—187 °C for LPZ maleate). Indomethacin was the product of our own company (mp 159—161 °C). All other materials employed were of analytical reagent grade.

Procedure for the Determination of PC——The inclusion complex between drug and CyD was partitioned between 1-octanol and phosphate buffer of pH 7.4 (volume ratio, V_o/V_w). Both drug and CyD were dissolved first in the aqueous phase to give concentrations D_i and C_i, respectively. The mixture of the two phases was shaken at 180 rpm for 1 h at room temperature (24 ± 1 °C). Then the two phases were separated by centrifugation at 10000 rpm for 10 min, and the aqueous phase was withdrawn. The concentration of the drug (D_w) in the aqueous phase was determined spectroscopically at an appropriate wavelength, except for Ben which was determined by means of gas-chromatography (GC) (conditions of GC analysis for Ben: apparatus, Hitachi model 163 gas-chromatograph; glass column, 1 m × 3 mm i.d. packed with 3% APGL coated on Chromosorb W. AW. DMCS (60—80 mesh); temperature, column temperature, 220 °C; injector temperature, 250 °C; flow rate of N_2, 50 ml/min; hydrogen flame ionization detector (FID); internal standard, carbetapentane citrate; peak area, calculated with a Shimadzu model E1A integrator). The apparent PC (P^*) was calculated by use of the following equation:

$$P^* = (D_i - D_w) \cdot V_w \cdot D_w^{-1} \cdot V_o^{-1}$$

The conditions for the measurement of the P^* values for β-CyD complexed with various drugs are shown in Table I. The concentrations of CyD and octanol were determined by colorimetry using the phenol–sulfuric acid method[7] and by GC (conditions of GC analysis for octanol: column, glass column 1 m × 3 mm i.d. packed with 2% PEG-20M coated on Chromosorb W. AW. DMCS (60—80 mesh); column temperature, 80 °C; injector temperature, 110 °C; internal standard, nonanol; other conditions as in the case of Ben), respectively.

Measurement of Ultraviolet (UV) Absorption Spectra——Measurement was done with a Shimadzu model 300 spectrophotometer. The K_i of the complex between octanol and CyD was determined by using the changes of difference absorbance of the Ben·β-CyD complex system on the addition of various amounts of octanol (0, 0.82, 1.63, and 3.25 mM). The difference absorbances of Ben·CyD complexes were measured by a method similar to that described in the previous paper.[1]

Theoretical

In the present paper, the effect of octanol, which is soluble in the aqueous phase, on partitioning of the CyD·drug complex between the octanol and aqueous phases, was considered to be as shown in Chart 1. This is an extended treatment of the partitioning of drug·CyD complex in the octanol/water system.

In the multiple equilibria represented in Chart 1, we have set up the following two hypotheses. First, neither free CyD nor inclusion complex can move into the octanol phase from the aqueous phase because of their high hydrophilicity. Second, the concentration of free octanol in the aqueous phase is kept constant during the measurement process by the supply of free octanol from the octanol phase in spite of its consumption due to the formation of the

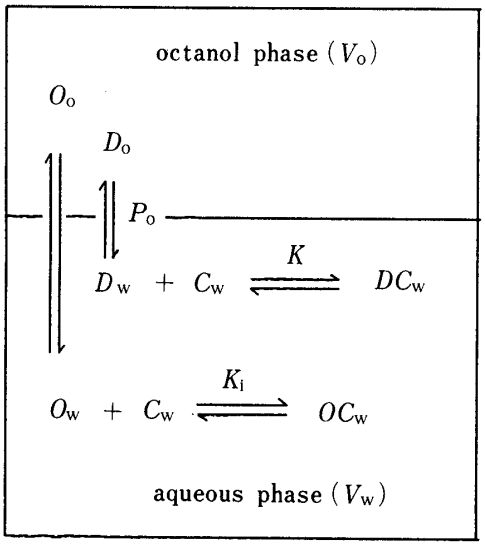

Chart 1. Possible Equilibria Involved in the Partitioning of the Drug·CyD (1 : 1) Complex System between Octanol and Aqueous Phase

D, drug; *C*, CyD; *O*, octanol; *DC*, drug·CyD (1 : 1) complex; *OC*, octanol·CyD (1 : 1) complex; *K*, formation constant of *DC* in aqueous phase; K_i, formation constant of *OC* in aqueous phase; P_0, apparent PC of free drug between octanol and aqueous phase; V_o, volume of octanol phase; V_w, volume of aqueous phase. Suffixes o and w stand for octanol and aqueous phases, respectively.

octanol·CyD complex.

The following set of equations may be derived from the assumptions given above.

$$K=\frac{(DC_w)}{(D_i-V_o\cdot D_o\cdot V_w^{-1}-DC_w)(C_i-DC_w-OC_w)} \tag{1}$$

$$K'=\frac{(DC_w)}{(D_i-V_o\cdot D_o\cdot V_w^{-1}-DC_w)(C_i-DC_w)} \tag{2}$$

$$K_i=\frac{(OC_w)}{(O_w)(C_i-DC_w-OC_w)} \tag{3}$$

$$P_0=\frac{(D_o)}{(D_i-V_o\cdot D_o\cdot V_w^{-1}-DC_w)} \tag{4}$$

$$P^*=\frac{(D_o)}{(D_i-V_o\cdot D_o\cdot V_w^{-1})} \tag{5}$$

where D_i : initial concentration of drug
C_i : initial concentration of CyD
K' : apparent formation constant for DC
P^* : apparent PC of drug in the presence of CyD.

In Eq. (5), as P^* is measurable experimentally, and (D_i), V_w, and V_o are all known, (D_o) is determined as follows:

$$(D_o)=(D_i)/(P^{*-1}+V_o\cdot V_w^{-1}) \tag{6}$$

Substitution of the value of (D_o) into Eq. (4) gives (DC_w) as follows:

$$(DC_w)=(D_i)-(P_0^{-1}+V_o\cdot V_w^{-1})(D_o) \tag{7}$$

where P_0 is equal to the PC of the drug in the absence of CyD and is observable. Next, (OC_w) is given by the following equation by substitution of (DC_w) into Eq. (3), where (O_w) may be obtained gas-chromatographically in the absence of CyD, and K_i may be determined spectroscopically:

$$(OC_w)=(O_w)(C_i-DC_w)/(O_w+K_i^{-1}) \tag{8}$$

Finally, K and K' values are determined by substituting the values of (D_o), (DC_w) and (OC_w) obtained above into Eqs. (1) and (2), respectively.

The PC method developed here may be applied in the same manner even if octanol is replaced by another organic solvent, which is able to form a two-phase system with the aqueous phase.

Results and Discussion

Effect of CyD on Apparent PC of Drug

In order to study the behavior of Ben·CyD inclusion complexes in the two-phase system, PC measurement of Ben only, CyD only or the 1:1 mixture of them was carried out in octanol/buffer (pH 7.4) system. The result is shown as P^* of Ben and that of CyD in Table II. It can be seen that the P^* values of Ben decreased remarkably upon addition of β- and γ-CyD. In contrast, no appreciable change in P^* was observed on the addition of α-CyD or maltose as shown in Table II.

The formation constant of the inclusion complex between Ben and each CyD has already been determined by circular dichroism (CD) and UV methods,[1] the values (K_s) being 0.002—0.003×10^3, 80×10^3, and 4×10^3 M^{-1} for α-, β-, and γ-CyD complexes, respectively, and no significant interaction was observed between Ben and maltose.[1]

From the above facts, it is expected that the changes of P^* of Ben upon the addition of CyD reflect the degree of complex formation. Then, K' values were calculated according to Eq. (2), and the results are listed in Table II. These K' values are remarkably lower than the formation constants (K_s's) determined by the spectroscopic method. It is expected that the competitive inhibiting action of octanol, which is slightly soluble in the aqueous phase, as shown in Chart 1, induced the significant difference between K' and K_s values.

On the other hand, no change of concentration was observed for each CyD in the aqueous phase before and after distribution, whether Ben was present or not. This result supports the first assumption in Chart 1, that neither free nor complexed CyD can move across the phase boundary and pass into the octanol phase.

Determination of K_i of Complex between Octanol and CyD

The competitive inhibition effect of octanol on the formation of Ben·CyD complex was confirmed in the aqueous phase by measurement of the UV spectra. Figure 1 illustrates the relationships between the difference absorbance of Ben and the initial concentration of Ben (equal to β-CyD) in buffers of pH 7.4 which contain four different concentrations of octanol, and Fig. 2 shows the modified Benesi–Hildebrand plots (modified B–H plots)[1] for the same system. From the above data, K' values were determined and the results are listed in the

Table II. Apparent Partition Coefficients (P^*) of Ben, CyD, and Ben·CyD Complex[a]

Species mixed	Molar ratio	P^* of Ben	P^* of CyD	K' ($\times 10^{-3}$ M^{-1}) (PC method)	K_s ($\times 10^{-3}$ M^{-1}) (CD method)
Ben	1:0	400	—	—	—
Ben + maltose	1:7	400	—	—	—
Ben + α-CyD	1:1	400	0	—	0.003
Ben + β-CyD	1:1	160	0	4	80
Ben + γ-CyD	1:1	240	0	1.6	4

a) In octanol/buffer (pH 7.4) system at 25 °C.
K', apparent formation constant of Ben·CyD inclusion complex; K_s, formation constant of Ben·CyD inclusion complex determined by the CD method.

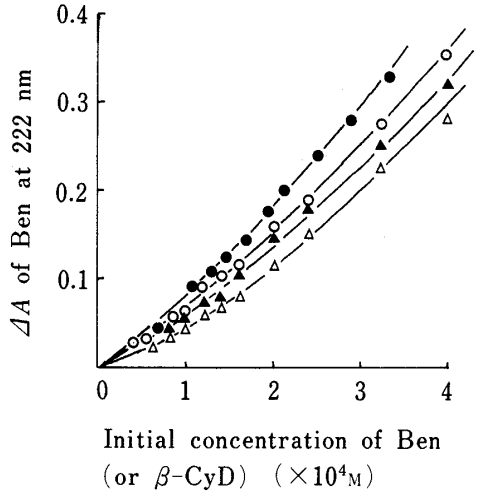

Fig. 1. Effect of Octanol[a] on the Difference Absorbance[b] of the Inclusion Complex between Ben and β-CyD at Various Initial Concentrations of Ben[c]

a) Concentration of octanol: ●, 0 mM; ○, 0.82 mM; ▲, 1.63 mM; △, 3.25 mM.
b) At 222 nm.
c) In phosphate buffer of pH 7.4.

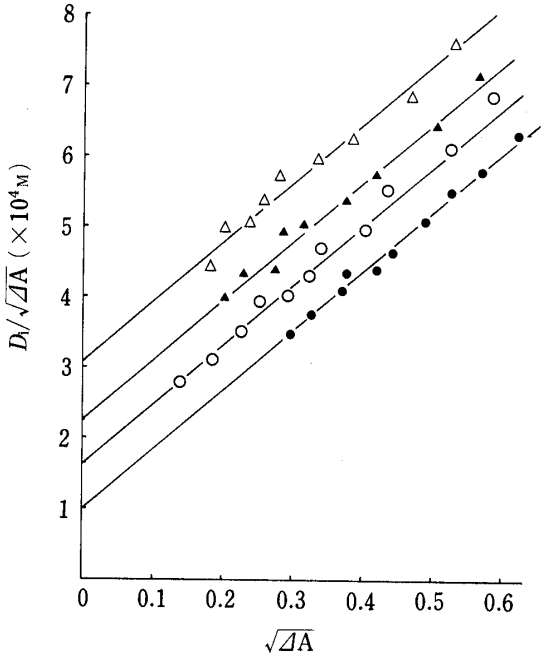

Fig. 2. Modified Benesi–Hildebrand Plots for the Ben·β-CyD Complex System in Buffer (pH 7.4) Containing Various Amounts of Octanol[a]

a) Concentration of octanol: ●, 0 mM; ○, 0.82 mM; ▲, 1.63 mM; △, 3.25 mM.
D_i, initial conc. of Ben or CyD; ΔA, difference absorbance of Ben at 222 nm.

TABLE III. Formation Constant (K_i) of Octanol·β-CyD Complex[a]

Conc. of octanol (mM)	K' ($\times 10^{-3}$ M^{-1})	$\Delta\varepsilon$ ($\times 10^{-3}$ M^{-1} cm^{-1})	K_i ($\times 10^{-3}$ M^{-1})
0.0	80±10	1.2±0.1	
0.82	33±5	1.2±0.1	2.4±0.7
1.63	15±3	1.2±0.1	2.5±0.7
3.25	9.4±2	1.2±0.1	2.6±0.5

a) Analysis by the UV method in phosphate buffer (pH 7.4) at 25 °C.
K', apparent formation constant of Ben·β-CyD complex; $\Delta\varepsilon$, difference in molar absorbance between Ben and Ben·β-CyD complex; K_i, formation constant of octanol·β-CyD complex.

second column of Table III. As the amount of octanol added was increased, the K' values decreased (in the second column of Table III). From this observation, it is clear that octanol largely prevents the formation of the inclusion complex between Ben and β-CyD. The difference between the absorbances of Ben in the presence and absence of CyD, ΔA, is expressed in Eq. (9):

$$\Delta A=(\varepsilon_3-\varepsilon_1-\varepsilon_2)(DC)+(\varepsilon_2+\varepsilon_4-\varepsilon_5)((OC)'-(OC)) \quad (9)$$

where ε_1, ε_2, ε_3, ε_4, and ε_5 are the molar extinction coefficients of Ben, CyD, Ben·CyD complex, octanol and octanol·CyD complex, respectively, and $(OC)'$ represents the equilibrium concentration of octanol·CyD complex in the absence of Ben. As the second term in the right-hand side of Eq. (9) is negligible at 222 nm, compared with the first term, Eq. (9) is reduced to Eq. (10):

$$(DC)=\Delta A/\Delta\varepsilon \tag{10}$$

where $\Delta\varepsilon$ represents $(\varepsilon_3-\varepsilon_1-\varepsilon_2)$ and is determined from the slope of a modified B–H plot as shown in Fig. 2. Substitution of (DC) into Eq. (1), gives (OC), defined by the following equation:

$$(OC)=(C_i)-(DC)-K_s^{-1}(DC)((C_i)-(DC))^{-1} \tag{11}$$

where K_s has been previously determined to be $80\times10^3\,\mathrm{M}^{-1}$, in the absence of octanol.

K_i values determined by substituting (OC) and (DC) into Eq. (12), are listed in the fourth column of Table III.

$$K_i=\frac{(OC)}{(O_i-OC)(C_i-DC-OC)} \tag{12}$$

where (O_i) represents the initial concentration of octanol added to the Ben·CyD complex system. In Eq. (12), the concentration of free octanol has been changed to (O_i-OC) from (O_w) of Eq. (3), because the octanol phase (which has supplied free octanol to the aqueous phase in the measurement process of the partition coefficient) does not exist in this measurement. K_i values should be constant regardless of the amount of octanol contained in the aqueous solution, if the assumption of Chart 1 that octanol competitively inhibits the formation of Ben·CyD complex holds. In fact, K_i values, which were calculated from the experimental data, were constant irrespective of the concentration of octanol contained in the solvent, and the mean of these K_i values is $2.5\times10^3\,\mathrm{M}^{-1}$. The $\Delta\varepsilon$ values were also found to be constant, and further, the patterns of the difference absorbance are similar as shown in Fig. 3. The above three results indicate that the mode of interaction between Ben and β-CyD is probably not altered by the presence of octanol. Therefore, it can be assumed that the formation of the inclusion complex between octanol and β-CyD partially prevents the formation of Ben·β-CyD inclusion complex, and the decrease of ΔA was induced by this phenomena. Thus, the above considerations support the basic assumption of Chart 1, that equilibrium between octanol and β-CyD·octanol inclusion complex exists in the aqueous phase.

In the same manner, the K_i values were determined to be $1.7\times10^3\,\mathrm{M}^{-1}$ between octanol and γ-CyD, and $0.7\,\mathrm{M}^{-1}$ between methanol and β-CyD. These values are consistent with the reported values determined by the UV method.[8]

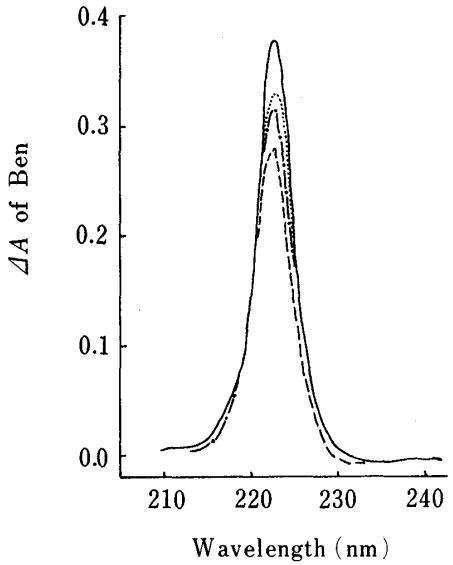

Fig. 3. Difference Absorbance Patterns of the Ben·β-CyD Complex System in Buffer (pH 7.4) Containing Various Amounts of Octanol[a]

a) Concentration of octanol: ——, 0 mM; ------, 0.82 mM; – – – –, 1.63 mM; ————, 3.25 mM.

TABLE IV. Formation Constants of β-CyD 1:1 Inclusion Complexes with Various Drugs as Determined by the PC Method[a]

Drug	log P_0	K' ($\times 10^{-3}$ M^{-1}) (PC method)	K ($\times 10^{-3}$ M^{-1}) (PC method)	K ($\times 10^{-3}$ M^{-1}) (Other methods)
Ben	2.6±0.2	5.0±1.0	40±10	80 (CD) (UV)[1]
LPZ	2.8±0.2	2.4±0.2	19±3	16 (CD) (HPLC)[9] 25 (UV)[9]
CPZ	3.3±0.1	1.2±0.2	11±2	8.3 (HPLC)[9] 12 (UV)[9] 7.9 (CD)[9]
IPM	2.5±0.3	1.0±0.2	7±1	
HBT	1.6±0.1	0.15±0.05	1.1±0.1	1.3[b] (HPLC)[9] 1.1[b] (UV)[10]
ABT	2.1±0.1	0.06±0.04	0.5±0.3	0.9[b] (POT)[11] 1.0[b] (UV)[11] 1.5[b] (HPLC)[9]
IDM	1.0±0.1	0.06±0.01	0.5±0.1	0.53 (SB)[12]
PBT	2.1±0.1	0.06±0.02	0.4±0.1	1.3[b] (HPLC)[9] 0.9[b] (POT) (UV) (CD)[11]
SA	−0.8±0.1	0.02±0.01	0.16±0.1	0.35 (UV)[13] 0.6 (SB)[13]

a) In phosphate buffer (pH 7.4) at 25 °C.
b) These values, at pH 7.4, were calculated from data in the literature.
P_0, apparent PC of drug between octanol and buffer (pH 7.4); K', apparent formation constant of β-CyD·drug inclusion complex; K, corrected formation constant of β-CyD·drug inclusion complex.

Determination of the Formation Constants of β-CyD 1:1 Inclusion Complexes with Various Drugs by the PC Method

As the phenomena described above seem to be general, the present PC method may be applied to β-CyD 1:1 inclusion complexes with various compounds. The K values of the CyD inclusion complexes with various drugs were determined by this method, and the results are given in Table IV. These values are in good agreement with those determined by other methods. Accordingly, the interactions between the drugs given in Table IV and β-CyD can also be analyzed quantitatively even in two-phase systems by the present PC method.

Conclusion

Inclusion phenomena between Ben and CyD were analyzed in detail in a two-phase system, and it was found that the PC method developed here is applicable to determine the formation constant of the CyD inclusion complexes in aqueous solution. The distinctive characteristics of this method are as follows.

(1) The measurement process is easy. Even if the concentration of free octanol in the water phase is not measured every time, a formation constant (K) close to K_s determined by the spectroscopic method can be obtained in most cases by using the mean concentration of octanol (3×10^{-3} M).

(2) This method is applicable even to a complex which shows no change in the spectra.

(3) It can be applied to a wide range of complexes by selecting a suitable organic phase in accordance with the lipophilicity of the compound measured.

(4) The octanol/water system shown in Chart 1 can be assumed to be a very simple model for interpretation of the behavior of inclusion complexes in biological systems.

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