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Inclusion Complexation of Barbiturates with β-Cyclodextrin in Aqueous Solution. I. Spectroscopic Study on the Mode of Interaction[1]

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Inclusion complexation of various barbituric and thiobarbituric acid derivatives with β-cyclodextrin in aqueous solution was studied by ultraviolet(UV), circular dichroism(CD), and nuclear magnetic resonance (NMR) spectroscopies. It was found that the induced CD observed for barbituric acids were substantially different from those for thiobarbituric acids. Chemical shifts changes in ^{1}H- and ^{13}C-NMR spectra suggested that not only 5-substituent but also heterocyclic moiety of barbiturate participated in inclusion formation. The stability constant of the inclusion complex was determined by UV method at various pH and temperatures from which the thermodynamic parameters were determined. Free barbituric acids were more favorable to form inclusion complex than ionized form. The relationship between the number of carbon atoms in 5-substituents and the formation constant were sigmoidal but barbituric acids bearing cyclic substituent at 5-C deviate from the correlation curves. Isoequilibrium relationship between ΔH and ΔS values were observed having a compensation temperature of 372° K.

Several studies have been reported on the inclusion complexation of barbiturates.[3,4] However, the detailed mechanism of the inclusion process is still remained speculative, having been mainly investigated by means of circular dichroism (CD) and nuclear magnetic resonance (NMR) studies. Moreover, the decisive method such as X-ray analysis of the crystal has not been provided for the inclusion in solution. For the conformation of the structure, comparative studies including a variety of congeneric derivatives are needed. In this study CD, ultraviolet (UV), ^{1}H-, and ^{13}C-NMR spectra were examined on various barbiturates including those bearing 5-alkyl chain of serial length, N-methyl derivatives and thiobarbiturates. The stability constant of the inclusion complex was determined by UV method at various pH and temperature from which the thermodynamic parameters were determined.

Experimental

Materials——Barbituric acids (BA) and thiobarbituric acids (TBA) used in this study are listen in Table I. Compounds (**1**), (**3**), (**4**), (**5**), (**6**), (**7**), (**15**), (**16**), (**17**), (**18**), (**19**), and (**21**) were synthesized according to the literatures[5] and recrystallized from EtOH–water. Other BA and TBA were obtained commercially and recrystallized from EtOH–water. β-Cyclodextrin (β-CyD) was favored by Teijin Ltd., and α-cyclodextrin (α-CyD) was purchased from Tokyo Kasei Kogyo Co., Ltd., and they were recrystallized from water and dried with P_2O_5 *in vacuo*. Their specific rotatory powers in water were: $[\alpha]_D^{25}=162.0\pm0.5°$ for β-CyD and $[\alpha]_D^{25}=152.0\pm0.5°$ for α-CyD, respectively. All other materials and solvents were of analytical reagent grade.

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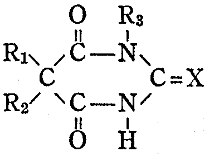
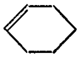
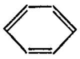
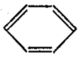
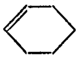
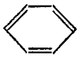
[3] *a*) A.L. Thakkar and P.V. Demarco, *J. Pharm. Sci.*, **60**, 652 (1971); *b*) A.L. Thakkar, P.B. Kuehn, J.H. Perrin, and W.L. Wilham, *ibid.*, **61**, 1841 (1972).

[4] *a*) K. Koizumi and K. Fujimura, *Yakugaku Zasshi*, **92**, 32 (1972); *b*) K. Koizumi, K. Mitsui, and K. Higuchi, *ibid.*, **94**, 1515 (1974).

[5] A.W. Dox, *J. Am. Chem. Soc.*, **46**, 1707 (1927); D.L. Tabern and E.H. Volwiler, *ibid.*, **57**, 1961 (1935).

TABLE I. Structure of Barbiturates Studied



	Barbiturate	R_1	R_2	R_3	X
1)	CH_3BA	CH_3	C_2H_5	H	O
2)	C_2H_5BA	C_2H_5	C_2H_5	H	O
3)	C_3H_7BA	$CH_3CH_2CH_2$	C_2H_5	H	O
4)	C_4H_9BA	$CH_3CH_2CH_2CH_2$	C_2H_5	H	O
5)	$C_5H_{11}BA$	$CH_3CH_2CH_2CH_2CH_2$	C_2H_5	H	O
6)	$C_6H_{13}BA$	$CH_3CH_2CH_2CH_2CH_2CH_2$	C_2H_5	H	O
7)	$C_7H_{15}BA$	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2$	C_2H_5	H	O
8)	Pentobarbital	$CH_3CH_2CH_2(CH_3)CH$	C_2H_5	H	O
9)	Amobarbital	$(CH_3)_2CHCH_2CH_2$	C_2H_5	H	O
10)	Cyclobarbital		C_2H_5	H	O
11)	Phenobarbital		C_2H_5	H	O
12)	Mephobarbital		C_2H_5	CH_3	O
13)	Hexobarbital		CH_3	CH_3	O
14)	Metharbital	C_2H_5	C_2H_5	CH_3	O
15)	C_2H_5TBA	C_2H_5	C_2H_5	H	S
16)	C_3H_7TBA	$CH_3CH_2CH_2$	C_2H_5	H	S
17)	C_4H_9TBA	$CH_3CH_2CH_2CH_2$	C_2H_5	H	S
18)	$C_5H_{11}TBA$	$CH_3CH_2CH_2CH_2CH_2$	C_2H_5	H	S
19)	$C_6H_{13}TBA$	$CH_3CH_2CH_2CH_2CH_2CH_2$	C_2H_5	H	S
20)	Thiopental	$CH_3CH_2CH_2(CH_3)CH$	C_2H_5	H	S
21)	Thiophenobarbital		C_2H_5	H	S

CD and UV Absorption Studies——The CD and UV spectra were taken with a Jasco 20 A spectropolarimeter and a Hitachi EPS-3T spectrometer, respectively. All measurements were carried out in 0.1 M borate buffer of pH 8.0 at 25° and pH was adjusted to appropriate value by the addition of 0.1 N NaOH or 0.1 N HCl solution. The molar absorption coefficient, ε, and the molar ellipticity, $[\theta]$, were calculated on the basis of total drug concentration. The optical anisotropy factor, g value, which is proportional to the magnitude of the induced Cotton effect, was determined in the same manner as reported previously.[6]

NMR Studies——^{1}H-NMR spectra were measured at an ambient probe temperature of $31 \pm 1°$. Tetramethylsilane (TMS) was used as an external reference for D_2O and no correction was made for susceptibility of the capillary. Proton-noise-decoupled ^{13}C-NMR spectra were taken at $30 \pm 1°$ with a Varian CF-20 NMR spectrometer operating at 20.0 MHz. Dioxane was used as an internal standard and chemical shifts (accurate to ± 0.1 ppm) were referred to TMS.

Determination of Stability Constants——The UV absorption change of a given guest molecule (1.0×10^{-4} M) in the presence of β-CyD (varied from 1.0×10^{-3} to 1.0×10^{-2} M) were quantitatively treated at the maximum wavelength due to the complex formation. The calculation of stability constant, K, value was made in the same manner.[7]

Determination of Partition Coefficients——Apparent partition coefficient, PC, of the drug was determined by the shaking of 10 ml of aqueous barbiturate solution (2×10^{-4} M in phosphate buffer of pH 5.0) with 10 ml $CHCl_3$ for one hour at 25°. Partition coefficient was defined as the ratio of the equilibrium concentration in organic phase to that in aqueous phase, and values obtained are listed in Table III.

Determination of Solubilities——Solubility, S_0, of the drug listed in Table III was determined at 25° in the following manner. Excess amount of barbiturate was added to 0.1 M phosphate buffer of pH 5.0 and was shaken for 48 or 72 hours until the system attained to equilibrium. After equilibration, the solution was pipetted through a cotton filter. Sample solution was then suitably diluted with 0.1 M phosphate buffer of pH 10.0 and analyzed spectrophotometrically.

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6) K. Ikeda, K. Uekama, M. Otagiri, and M. Hatano, *J. Pharm. Sci.*, 63, 1168 (1974).
7) M. Otagiri, K. Uekama, and K. Ikeda, *Chem. Pham. Bull.* (Tokyo), 23, 188 (1975).

Results and Discussion

CD and UV Studies

Figure 1 shows CD and UV spectra of phenobarbital (11) in the absence and the presence of β-CyD as a typical example of BA, where positive peak at 219 nm and shoulders at 240 and 270 nm were observed in the induced CD band and marked change in UV absorptivity was accompanied. Other BA also showed positive ellipticity and similar UV absorption changes in the presence of β-CyD. Figure 2 shows CD and UV spectra of thiophenobarbital (**21**)–β-CyD system. All TBA studied exhibited negative peak around 260 nm and positive peaks around 240 and 300 nm, in general. According to the symmetry rule of Schellman,[8] the sign of the Cotton effect depends on the spatial relationship between the asymmetric center and perturbed chromophore. The different patterns of the induced CD suggest that the configuration of TBA within the cavity of β-CyD is different from that of BA.

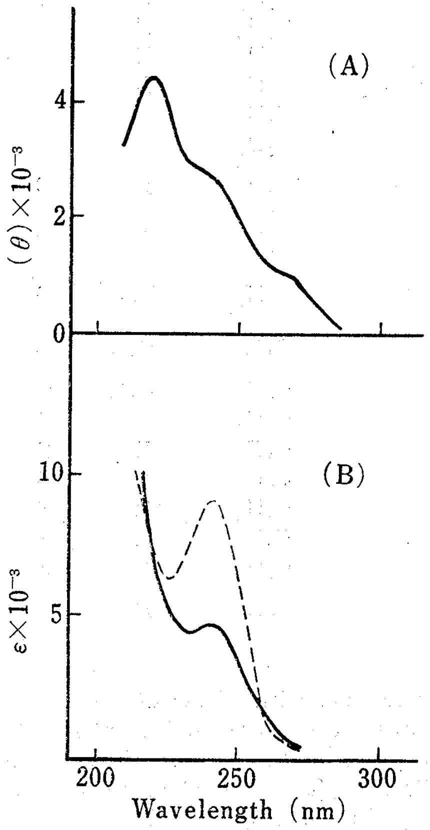

Fig. 1. Circular Dichroism (A) and UV Absorption Spectra (B) of Phenobarbital–β-Cyclodextrin System

------: phenobarbital (1×10^{-4}M) alone
——: phenobarbital (1×10^{-4}M)+β-cyclodextrin (1×10^{-2}M)
solvent: 0.1 M borate buffer (pH 8.0)

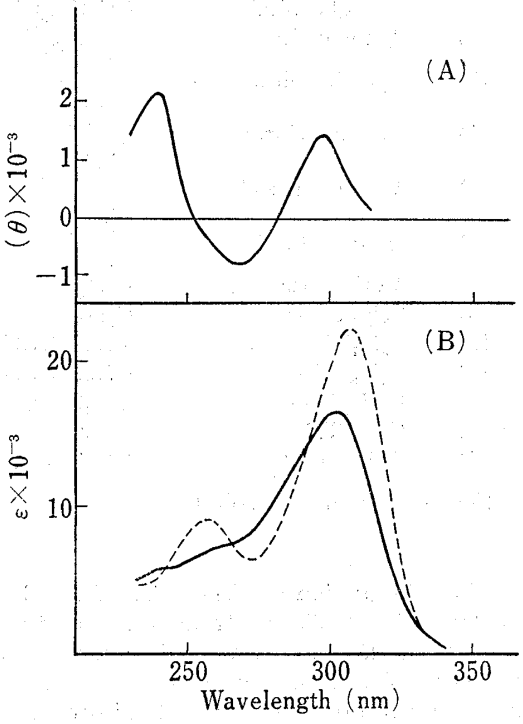

Fig. 2. Circular Dichroism (A) and UV Absorption Spectra (B) of Thiophenobarbital–β-Cyclodextrin System

------: thiophenobarbital (1×10^{-4}M) alone
——: thiophenobarbital (1×10^{-4}M)+β-cyclodextrin (1×10^{-2}M)
solvent: 0.1 M borate buffer (pH 8.0)

Table II summarizes λ_{max}, ε, $[\theta]$, and g values obtained. According to the CD studies on 5-alkyl-5-(2′-pentyl)barbituric acid three ellipticity bands at 212, 240, and 260 nm were assigned to π–π^*, n–σ^* (or second n–π^*), and n–π^* transitions, respectively, where the molar ellipticity of n–π^* band was significantly larger compared with that of π–π^* band.[9] The

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[8] J.A. Schellman, *Accounts Chem. Res.*, **1**, 144 (1968).

[9] F.I. Carroll and R. Meck, *J. Org. Chem.*, **34**, 2676 (1969); F.I. Carroll and A. Sobti, *J. Am. Chem. Soc.*, **95**, 8512 (1973).

TABLE II. Induced CD and UV Spectra of Barbiturates Bound to β-Cyclodextrin[a]

Barbiturate	CD			UV	
	λ_{max} (nm)	$[\theta]$ ($\times 10^{-3}$)	g[b] ($\times 10^{4}$)	λ_{max} (nm)	ε ($\times 10^{-4}$)
$\mathrm{CH_3BA}$	215	1.02	0.48	216	0.60
	240	0.15	0.11	237	0.43
$\mathrm{C_2H_5BA}$	215	3.64	1.90	216	0.54
	245	1.32	0.91	237	0.51
$\mathrm{C_3H_7BA}$	215	1.90	0.93	215	0.62
	240	0.61	0.40	238	0.46
$\mathrm{C_4H_9BA}$	215	1.35	0.64	215	0.64
	240	0.65	0.53	238	0.38
$\mathrm{C_5H_{11}BA}$	215	0.92	0.43	215	0.58
	240	0.21	0.17	238	0.36
$\mathrm{C_6H_{13}BA}$	212	2.60	1.17	215	0.59
	250	0.83	1.07	240	0.42
Pentobarbital	215	3.10	1.36	215	0.69
	245	0.70	0.90	240	0.27
Amobarbital	215	2.68	1.17	215	0.70
	250	0.75	0.81	240	0.27
Cyclobarbital	215	3.40	0.93	215	1.11
	240	1.42	0.61	238	0.71
	260	1.01	1.35	270	0.14
Phenobarbital	219	4.55	1.73	220	0.70
	240	2.74	1.74	240	0.48
	267	0.90	3.41	265	0.10
Mephobarbital	220	5.98	2.75	225	0.59
	250	2.02	2.72	247	0.26
	270	0.95	5.30	267	0.07
Hexobarbital	215	3.16	1.26	221	0.76
	240	1.30	1.97	245	0.20
Metharbital	220	2.27	1.12	221	0.62
$\mathrm{C_2H_5TBA}$	245	3.58	2.13	240	0.45
	263	−1.86	0.91	257	0.68
	303	6.70	1.58	303	1.29
$\mathrm{C_3H_7TBA}$	245	2.94	1.37	238	0.62
	264	−1.84	0.93	253	0.75
	305	4.84	0.78	300	1.96
$\mathrm{C_4H_9TBA}$	240	2.06	1.00	238	0.63
	265	−1.10	0.53	255	0.68
	305	1.10	0.20	298	1.82
$\mathrm{C_5H_{11}TBA}$	237	1.06	0.53	238	0.60
	265	−1.20	0.54	256	0.69
	305	−1.80	0.32	297	1.70
Thiopental	240	2.45	1.25	240	0.60
	300	−2.25	0.51	259	0.60
				296	1.35
Thiophenobarbital	240	2.20	1.19	240	0.56
	270	−0.90	0.36	261	0.74
	298	1.34	0.24	301	1.65

a) Concentrations of barbiturates and β-cyclodextrin were of 1×10^{-4} M and 1×10^{-2} M.

b) optical anisotropy factor (see text) three peaks (or shoulder) found in phenobarbital–β-CyD system may be attributed to the above corresponding transitions, respectively. However, contrary to the above, the found band attributable to π–π^* transition is significantly larger than that attributable to n–π^* transition as shown in Fig. 1. Since the electronic dipole moment of π–π^* transition is generally larger than that of n–π^*, the obtained results indicate that dipole-dipole interaction plays important role for the generation of the induced CD band. Further it is noteworthy that g values of BA at shorter λ_{max} are larger than those at longer λ_{max} and this trend is particularly significant in N-methyl BA.

Experiments were carried out also on α-CyD. However, no significant induced CD and UV changes were observed for all BA and TBA. This indicates that the smaller cavity of α-CyD is insufficient in size for the inclusion.

NMR Studies

It is conceivable that when an aromatic moiety of guest molecule is included in a cavity of β-CyD, protons located within a cavity of β-CyD are susceptible to anisotropic shielding of aromatic moiety, and protons located on the exterior of the cavity are relatively unaffected.

Figure 3 shows the effect of phenobarbital on ^{1}H-NMR spectrum of β-CyD in D_2O. Protons located within the cavity of β-CyD, H-3 and H-5 showed higher field shift, while no anisotropic shielding was detected for the exterior H-2 and H-4. These observations are in good agreement with the explanation of Thakkar, *et al.*[3] who suggested that the shift was due to the inclusion of phenyl group of phenobarbital in the cavity of β-CyD. However, the following results are against their suggestion. Figure 4 shows the effect of β-CyD on the ^{1}H-NMR spectrum of mephobarbital (**12**) in D_2O. Protons assigned to N-methyl and C-5 ethyl showed lower field shift while no appreciable shift of phenyl protons was observed. This implies that both heterocyclic moiety of phenobarbital and 5-ethyl are included in the cavity.

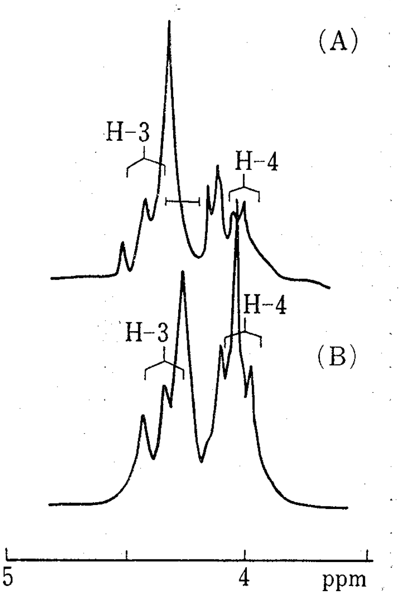

Fig. 3. Effect of Phenobarbital-Na on ^{1}H-NMR Spectra of β-Cyclodextrin in D_2O

(A): β-cyclodextrin (0.02M) alone
(B): β-cyclodextrin (0.02M)+phenobarbital-Na (0.05M)

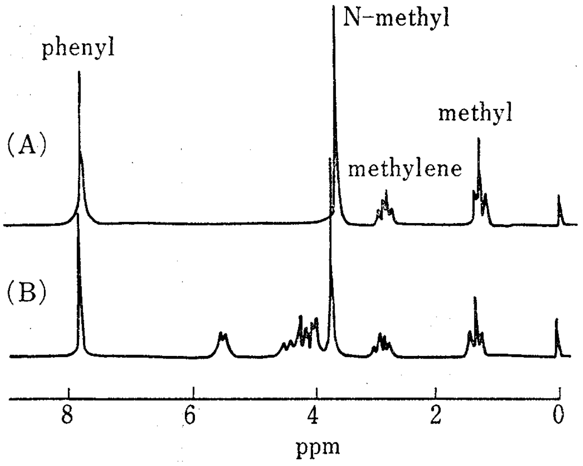

Fig. 4. Effect of β-Cyclodextrin on ^{1}H-NMR Spectra of Mephobarbital-Na in D_2O

(A): mephobarbital-Na (0.1M) alone
(B): mephobarbital-Na (0.1M)+β-cyclodextrin (0.05M)

Figure 5 shows the effect of β-CyD on ^{13}C-NMR spectrum of phenobarbital in D_2O. The shifts of C-4 and C-6 signals to higher field and the shift of C-5 signal to lower field are detectable while other signals, *e.g.* those of C-2 and phenyl carbons, are hardly shifted. Accord-

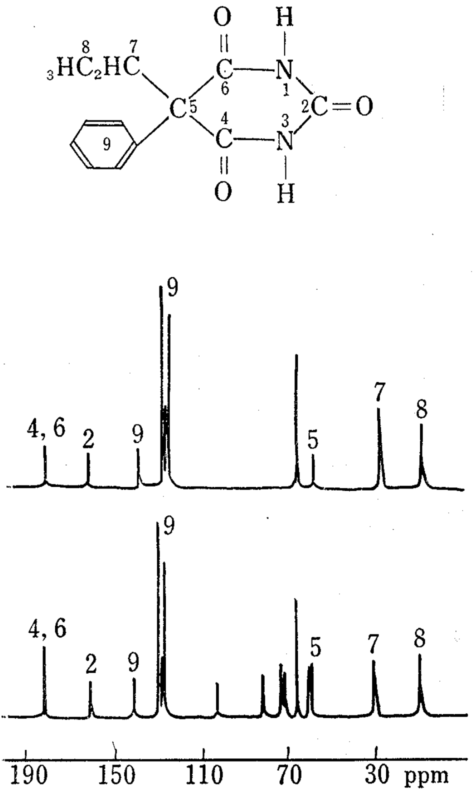

Fig. 5. Effect of β-Cyclodextrin on ^{13}C-NMR Spectra of Phenobarbital-Na in D_2O

(A): phenobarbital-Na (0.2M) alone
(B): phenobarbital-Na (0.2M)+β-cyclodextrin (0.05M)

	(A)	(B)
C-4,6	182.8	182.2
C-2	162.0	161.9
C-9	140.3	140.4
	130.0	129.9
	129.0	128.9
	127.3	127.2
C-5	60.1	60.8
C-7	29.6	30.0
C-8	9.4	9.6

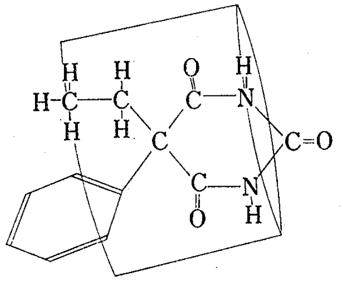

Fig. 6. Possible Structure of Inclusion Complex of β-Cyclodextrin with Phenobarbital

ing to the recent studies on the effect of solvent polarity on ^{13}C-NMR shift of BA, C-2, C-4, and C-6 signals in organic solvents appear at higher field compared with those in aqueous solution and C-5 signal in organic solvents appears at lower field than that in water.[10] The results shown in Fig. 5 may again support reasonably that C-4, C-5, and C-6 of phenobarbital locate in the hydrophobic cavity.

Referring to the above observation a possible structure of phenobarbital–β-CyD complex may be illustrated as shown in Fig. 6. This depiction is also admissible to the examination with the CPK[11] model.

Physicochemical Properties and Stability Constants

The molar ratio of BA–β-CyD complex[3b,4b] has been severally known to be 1:1, from which stability constant, K, was determined by Scott method[12] from UV changes. Figure 7 shows the pH dependencies of the stability constant for phenobarbital and pentobarbital. The inflections are observed around their pK_a values and ionized form of the barbiturates are also complexed with β-CyD and they showed smaller stability constant. This also supports that pyrimidine moiety is accommodated in the cavity of β-CyD. The detailed stoichiometric relationship will be discussed in the following studies.[13]

In Table III the K values for BA, K_f and K_i, are shown, where K_f and K_i are the stability constants of the inclusion complexes of unionized and ionized guest molecules, respectively. For all compounds K_f values are larger than K_i and the ratio of K_f/K_i is especially larger for BA bearing phenyl or cyclohexenyl substituents. Figure 8 shows the relationship between the partition coefficient and stability constant. Sigmoidal correlation was found for BA and TBA having aliphatic substituent at 5-C. BA and TBA bearing cyclic substituent at 5-C, (**10**), (**11**), and (**21**), deviate from the correlation curves. While N-methyl BA (**12** and **13**) has K values lower than the general tendency although these compounds have ring 5-substituent. This may be accounted from the decrease of NH which is capable of H-bonding with 0 in

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10) A. Fratiello, M. Mardirossian, and E. Chauez, *J. Mag. Res.*, **12**, 221 (1973).
11) Abbreviation of Corey-Pauling-Koltum molecular model.
12) R.L. Scott, *Rec. Trav. Chim.*, **75**, 787 (1956).
13) T. Miyaji, Y. Kurono, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **24**, 1155 (1976).

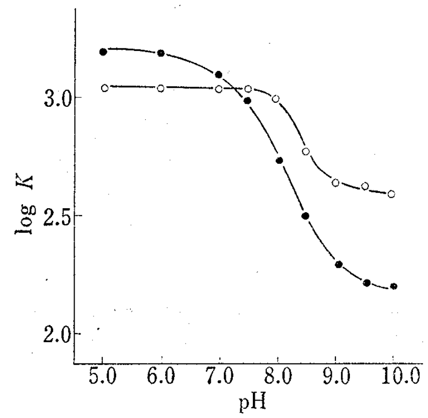

Fig. 7. pH Profile for Stability Constants of Barbiturates–β-Cyclodextrin Complexes

—●—: phenobarbital-β-cyclodextrin system
—○—: pentobarbital-β-cyclodextrin system

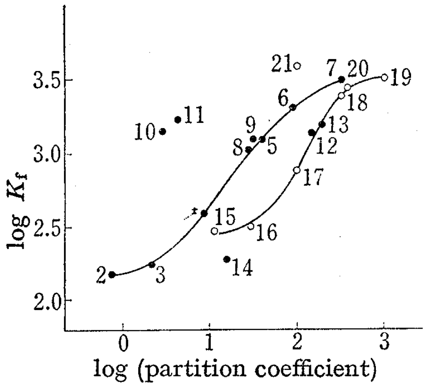

Fig. 8. Relationships between Stability Constants of β-Cyclodextrin Complexes and Partition Coefficients of Guest Molecules

Table III. Stability Constants of β-Cyclodextrin with Barbiturates, and Their Partition Coefficients and Solubilities at 25°

	Barbiturate	K_f[a] (M^{-1})	K_i[b] (M^{-1})	Partition coefficient[c]	Solubility[d] (M)
1)	CH_3BA	—[e]	—[e]	0.19	7.48×10^{-2}
2)	C_2H_5BA	150	—[e]	0.75	3.74×10^{-2}
3)	C_3H_7BA	180	70	2.00	4.35×10^{-2}
4)	C_4H_9BA	390	130	9.67	1.63×10^{-2}
5)	$C_5H_{11}BA$	1200	350	38.6	3.08×10^{-3}
6)	$C_6H_{13}BA$	2150	820	78.5	9.65×10^{-4}
7)	$C_7H_{15}BA$	2950	960	334	3.74×10^{-4}
8)	Pentobarbital	1040	390	28.0	4.17×10^{-3}
9)	Amobarbital	1190	410	28.3	2.35×10^{-3}
10)	Cyclobarbital	1400	200	2.95	5.33×10^{-3}
11)	Phenobarbital	1650	160	4.40	4.39×10^{-3}
12)	Mephobarbital	1500	130	191	2.07×10^{-4}
13)	Hexobarbital	1280	380	153	1.02×10^{-3}
14)	Metharbital	190	100	16.0	8.00×10^{-3}
15)	C_2H_5TBA	290	120	11.1	5.28×10^{-3}
16)	C_3H_7TBA	300	120	27.9	1.60×10^{-3}
17)	C_4H_9TBA	760	210	103	7.33×10^{-4}
18)	$C_5H_{11}TBA$	2380	520	306	1.96×10^{-4}
19)	$C_6H_{13}TBA$	3150	1430	926	8.39×10^{-5}
20)	Thiopental	2700	390	326	1.80×10^{-4}
21)	Thiophenobarbital	3960	900	63.9	2.64×10^{-4}

a) At pH 5.0 barbiturates are substantially in free form.
b) At pH 10.0 barbiturates are substantially ionized.
c) between $CHCl_3$ and aqueous pH 5.0 buffer
d) in 0.1M phosphate buffer of pH 5.0
e) could not be determined with accuracy due to the small changes of UV spectra

β-CyD and also the steric hindrance of methyl group. The relationships between the solubility and the stability constant are also sigmoidal for BA and TBA series. Figure 9 shows the relationships between the summed number of carbon atoms in 5-substituents and the stability constant. Cyclobarbital, phenobarbital and N-methyl BA deviate from the general sigmoidal relationship also here. Sigmoidal correlation observed in Fig. 8 and 9 may indicate that spatial relationship between host and guest molecules is important in inclusion complexation; *i.e.* smaller or larger size of the guest molecule, in particular substituent at 5-C in BA and TBA,

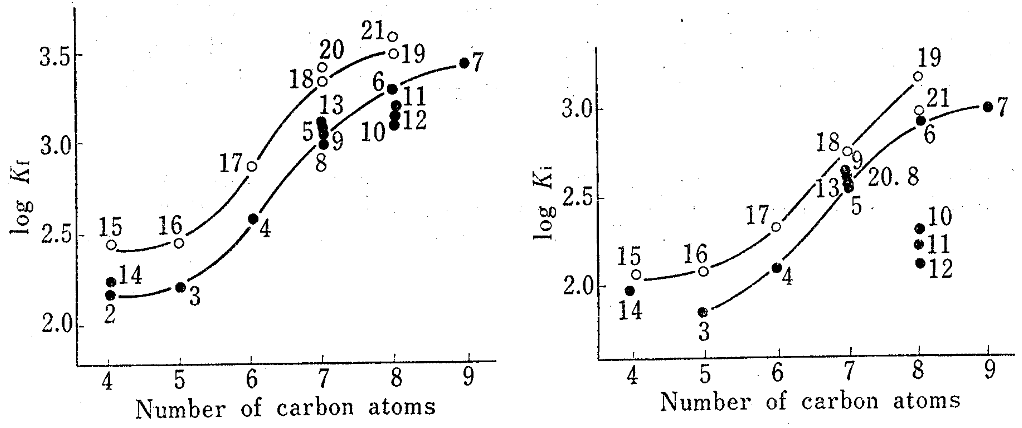

Fig. 9. Relationships between Stability Constants and Number of Carbon Atoms in 5,5-Substituents

●: barbituric acid derivatives ○: thiobarbituric acid derivatives

is no longer effective toward β-CyD cavity. BA bearing bulky substituents (**10, 11, 12**) has significantly smaller K_i values which indicates that those anions are especially suppressed for the inclusion.

Thermodynamic Parameters

Thermodynamic parameters were determined from the dependency of the stability constant on the temperature at pH 5.0 (Table IV). In general, van't Hoff plots fell fairly on the straight line over the temperature range 15—45°. As seen in Table IV the inclusion complexations are predominantly due to favorable enthalpy changes and the entropy change contradictorily contributes in general. This indicates that hydrophobic interaction is not predominant for the inclusions of BA and TBA but other intermolecular forces such as H-bonding and dipole-dipole interactions should be considered. The compensatory relation-

TABLE IV. Thermodynamic Parameters for the Complex Formation of β-Cyclodextrin with Barbituric Acid and Thiobarbituric Acid Derivatives at pH 5.0 and at 25°

Barbiturate	ΔG (kcal/mole)	ΔH (kcal/mole)	ΔS (e.u.)
3) C_3H_7BA	−3.07	−2.77	+1.08
4) C_4H_9BA	−3.53	−3.77	−0.77
5) $C_5H_{11}BA$	−4.20	−5.02	−2.93
6) $C_6H_{13}BA$	−4.58	−6.18	−5.45
7) $C_7H_{15}BA$	−4.23	−7.65	−9.75
8) Pentobarbital	−4.11	−4.84	−2.43
9) Amobarbital	−4.19	−6.31	−6.23
10) Cyclobarbital	−4.29	−4.83	−3.80
11) Phenobarbital	−4.39	−10.3	−19.9
12) Mephobarbital	−4.33	−9.48	−17.3
13) Hexobarbital	−4.22	−5.61	−4.71
15) C_2H_5TBA	−3.35	−3.71	−0.22
16) C_3H_7TBA	−3.38	−3.93	−1.77
17) C_4H_9TBA	−3.93	−4.87	−3.16
18) $C_5H_{11}TBA$	−4.60	−5.64	−5.33
19) $C_6H_{13}TBA$	−4.77	−7.07	−8.17
20) Thiopental	−4.68	−6.15	−5.02
21) Thiophenobarbital	−4.89	−8.22	−11.2

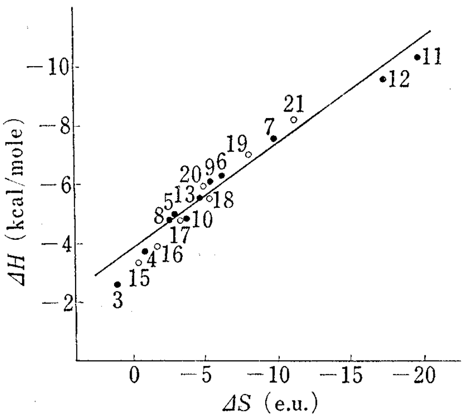

Fig. 10. Plots of ΔH vs. ΔS for Complex Formation of Barbiturates with β-Cyclodextrin

●: barbituric acid derivatives
○: thiobarbituric acid derivatives

ship between ΔH and ΔS is shown in Fig. 10. The compensatory temperature[14] calculated by least squares method is 372°K. The plots of phenobarbital and mephobarbital, which have largely negative ΔS, deviate slightly from the linear relation with deficient compensation of ΔH.

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