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Inclusion Complexes of β-Cyclodextrin with Antiinflammatory Drugs Fenamates in Aqueous Solution[1]

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Inclusion of antiinflammatory drugs fenamates within the cavity of β-cyclodextrin in aqueous solution was confirmed by circular dichroism, ultraviolet absorption, and nuclear magnetic resonance spectroscopies. Solubility and spectral changes were quantitatively investigated and stoichiometric ratio, which was found to be 1 : 1, formation constants, and thermodynamic parameters were obtained for complex formation of β-cyclodextrin with fenamates. Hydrophobic and steric factors were reflected in the values of formation constants. Isoequilibrium relationship between ΔH and ΔS values were observed having a compensation temperature of 284°K. From these evidences mode of inclusion was discussed.

Inclusion complexes of various drugs with cyclodextrins have been successfully applied in pharmaceutical formulations to enhance the solubility,[3] chemical stability,[4] and absorption[5] characteristics of the drugs. The inclusion of optically inactive compounds within a cavity of β-cyclodextrin has been recently known to generate extrinsic Cotton effects.[3c,6] In our preliminary report,[7] it has demonstrated that N-phenylanthranilic acid and its related compounds show the induced circular dichroism (CD) by the formation of inclusion complexes with β-cyclodextrin. The present study deals with the details on the inclusion nature of β-cyclodextrin with antiinflammatory drugs fenamates such as flufenamic, mefenamic, and meclofenamic acids in aqueous solution. With anticipations on the revelation of the mechanism responsible for this interaction, induced CD, ultraviolet (UV) absorption, nuclear magnetic resonance (NMR) chemical shift, and solubility changes were quantitatively investigated. Stoichiometric relationship, formation constants, and thermodynamic parameters for the complex formation are reported. From these observations the mode of inclusion will be discussed.

Experimental

Material——Flufenamic, mefenamic, and meclofenamic acids were favored from Sankyo Co., Ltd. and were recrystallized from EtOH–H_2O. β-Cyclodextrin was favored from Teijin Ltd. α- and β-Cyclodextrins were recrystallized from water and dried with P_2O_5 *in vacuo*. Their specific rotatory powers were; $[\alpha]_D^{25}=152.0\pm0.5°$ for α-cyclodextrin and $[\alpha]_D^{25}=162.0\pm0.5°$ for β-cyclodextrin, respectively. 3-(2,3-Dimethylanilino)-benzoic acid and 4-(2,3-dimethylanilino)-benzoic acid were favored from Grelan Pharmaceutical

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[3] *a*) J.L. Lach and J. Cohen, *J. Pharm. Sci.*, **52**, 137 (1963); *b*) J.L. Lach and W.A. Pauli, *ibid.*, **55**, 32 (1966); *c*) A.L. Thakkar, P.B. Kuehn, J.H. Perrin, and W.L. Wilham, *ibid.*, **61**, 1841 (1972).

[4] H. Schlenk, D.M. Sand, and J.A. Tillotson, *J. Am. Chem. Soc.*, **77**, 3587 (1955); T-F. Chin, P-H. Chung, and J.L. Lach, *J. Pharm. Sci.*, **57**, 44 (1968); J.L. Lach and T-F. Chin, *ibid.*, **53**, 924 (1964); K. Koizumi and K. Fujimura, *Yakugaku Zasshi*, **92**, 32 (1972).

[5] K.H. Frömming and I. Weyerman, *Arzneim-Forsch.*, **23**, 424 (1973).

[6] K. Sensse and F. Cramer, *Chem. Ber.*, **102**, 509 (1969); K. Takeo and T. Kuge, *Die Stärke*, **24**, 281 (1972); M. Otagiri, K. Ikeda, K. Uekama, O. Ito, and M. Hatano, *Chemistry Letters*, **1974**, 679.

[7] K. Ikeda, K. Uekama, M. Otagiri, and M. Hatano, *J. Pharm. Sci.*, **63**, 1168 (1974).

Co., Ltd. 2,2-Iminodibenzoic acid was synthesized referring to the literature.[8] All other materials and solvents were of analytical reagent grade.

CD and UV Absorption Studies——The CD and UV spectra were recorded by a Jasco 20A recording spectropolarimeter and a Hitachi EPS-3T spectrometer, respectively. All measurements were carried out in 0.1M sodium phosphate buffer of pH 7.0 at appropriate temperature. The optical anisotropy factor, g value, which is proportional to the magnitude of the induced Cotton effects was calculated from the equation, $g=[\theta]/3300\cdot\varepsilon$, where $[\theta]$ is molar ellipticity (deg/meter·mole/liter), and ε is the molar absorptivity of guest molecule in the presence of β-cyclodextrin at the maximum wavelength of CD spectrum. The formation constant, K_c, was determined according to Scott's equation.[9] UV absorption and ellipticity changes of a given compound (constantly 1×10^{-4}M) in the presence of β-cyclodextrin (varied from 1×10^{-3} to 15×10^{-3}M) were measured at the maximum wavelength due to the complex formation. The calculation of K_c value was essentially same as that of previous paper.[10]

NMR Studies——^{1}H and ^{19}F NMR spectra were measured by a JEOL PS-100 spectrometer at the ambient probe temperature of 31±1°. Tetramethylsilane was used as an external reference for D_2O and no correction was made for susceptibility of the capillary.

Solubility Studies——The solubility studies were carried out according to Higuchi and Lach.[11] Excess amount of drug were added to aqueous β-cyclodextrin solution and was shaken at 25° for 48—62 hr. until the system attain equilibrium. After equilibration, the solutions were pipetted through cotton filter. Sample solution was then suitably diluted with 0.1M sodium phosphate buffer (pH 7.0) and analyzed spectrophotometrically. The presence of β-cyclodextrin in the experimental condition did not interfere on the spectrophotometrical assay. The formation constants for complexes were calculated from phase diagram obtained according to the method of Higuchi and Lach.

Determination of Partition Coefficients——Partition coefficients between aqueous and *n*-octanol phase were determined by the shaking of 10 ml aqueous solution of the compound (2×10^{-5}M in 0.1M sodium phosphate buffer of pH 7.0) and 10 ml of *n*-octanol for one hour at 25°. Partition coefficient was defined as the ratio of the equilibrium concentration in organic phase to that in aqueous solution.

Results and Discussion

CD and UV Absorption Studies

Although β-cyclodextrin has neither CD nor absorption band at longer wavelength than 220 nm, inclusion complexes of β-cyclodextrin with antiinflammatory drugs fenamates showed new CD bands in the wavelength region of the drug chromophores (Fig. 1). The strong fenamate absorption band at 280—300 nm is assigned to the resonance between two phenyl groups across the nitrogen, while the weaker band at about 340 nm is due to electronic transitions occurring in the anthranilic acid portion of the drug molecule.[12] As is seen in Fig. 1, each drug experienced different asymmetric environment of β-cyclodextrin having unique CD characteristics. Flufenamic acid showed positive peaks at 287 and 328 nm indicating that both of electronic transitions in this drug chromophore were perturbed by asymmetric β-cyclodextrin. Meclofenamic acid also showed two positive ellipticities; relatively strong peak at 275 nm and weak peak at 320 nm. According to the symmetry rule,[13] the sign of the induced Cotton effect is governed by the configuration of the asymmetric center and spacial relationship to the perturbed chromophore. In flufenamic and meclofenamic acids, it is assumed that asymmetric center of β-cyclodextrin is located in a region of drug chromophores which made positive contribution to Cotton effects and the resultant complexes are rigid enough to prevent the asymmetric center from entering region of negative contribution.

In the case of mefenamic acid, however, β-cyclodextrin perturbed only longer wavelength region of mefenamic acid to give positive peaks at 315 and 335 nm, although a small negative ellipticity was observable at 280 nm. This may indicate that electronic transition due to

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8) Farbwerke vorm Meister Lucius & Brüning, Ger. Patent 148179 (1902) [*Chem. Zentr.*, **75**, I, 412 (1904)].
9) R.L. Scott, *Rec. Trav. Chim.*, **75**, 787 (1956).
10) M. Otagiri, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **23**, 188 (1975).
11) T. Higuchi and J.L. Lach, *J. Am. Pharm. Ass., Sci. Ed.*, **43**, 349 (1954).
12) C.F. Chignell, *Mol. Pharmacol.*, **5**, 455 (1969).
13) J.A. Schellman, *Accounts Chem. Res.*, **1**, 144 (1968).

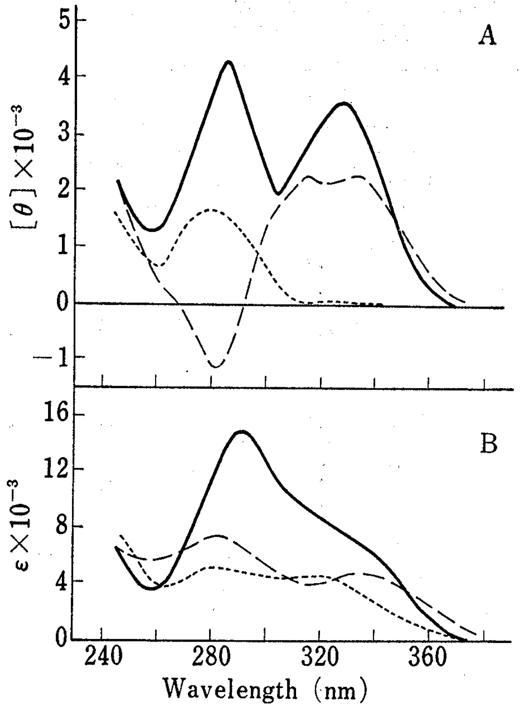

Fig. 1. Circular Dichroism (A) and Absorption (B) Spectra of Fenamates–β-cyclodextrin System

———: flufenamic acid, – – – –: mefenamic acid, - - - - - - -: meclofenamic acid, concentrations: fenamates (5×10^{-5}M), β-cyclodextrin (1×10^{-2}M)
solvent: 0.1M sodium phosphate buffer (pH 7.0)

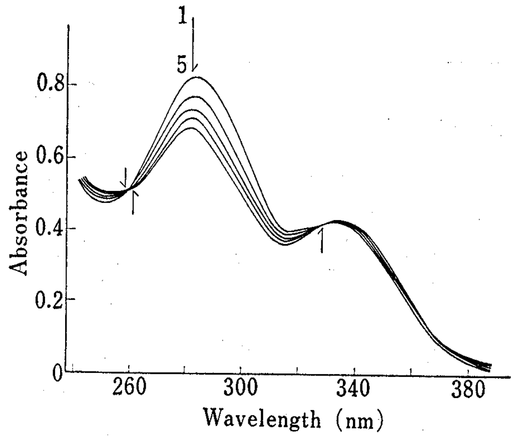

Fig. 2. Effect of β-Cyclodextrin on UV Absorption Spectrum of Mefenamic Acid in 0.1M Phosphate Buffer (pH 7.0)

concentration of mefenamic acid: constantly 6.25×10^{-5}M, concentration of β-cyclodextrin added: curve 1; 0, 2; 0.88×10^{-3}M, 3; 1.76×10^{-3}M, 4; 3.25×10^{-3}M, 5; 8.81×10^{-3}M

anthranilic acid portion of the drug was predominantly perturbed by β-cyclodextrin and configuration of mefenamic acid within a cavity of β-cyclodextrin was somewhat different from that of other fenamates.

Figure 2 shows the effect of β-cyclodextrin on the UV absorption spectrum of mefenamic acid, where absorption maximum (λ_{max}) and intensity changed concomitantly by the increasing amount of β-cyclodextrin. A clear isosbestic point was observed at 328 nm, but two unrelated isosbestic points appeared at 259 and 263 nm, further indicating complicate mechanism of interaction in this system.

In Table I, spectral characteristics such as λ_{max}, ε, $[\theta]$, and g values obtained here and those in the preceding paper[7] were combined to see the structure dependency of inclusion formation. Marked differences between g values of closely related compounds were noted. Stereospecific nature of guest molecule may be responsible for the magnitude and/or sign of induced CD bands. Meclofenamic acid particularly is much less influenced by asymmetric environment than the other compounds. This suggests that the bulky chlorine and methyl substituents prevented a closer interaction with asymmetric center of β-cyclodextrin.

In contrast to β-cyclodextrin, α-cyclodextrin showed no appreciable complex formation with all the compounds studied, indicating that the cavity size of α-cyclodextrin is not large enough to include the bulky guest molecules.

NMR Studies

Since NMR technique has been known to give useful information for inclusion nature of β-cyclodextrin complexes with aromatic guest molecules,[10,14] chemical shift changes following

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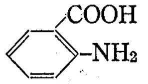
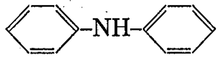
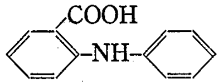
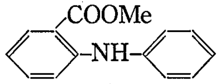
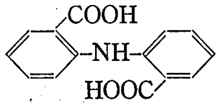
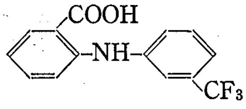
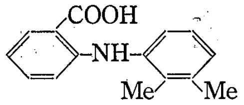
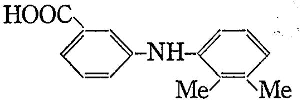
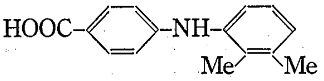
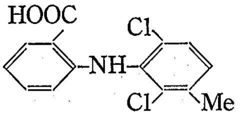
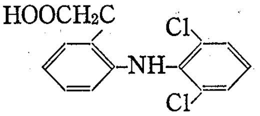
[14] *a*) P.V. Demarco and A.L. Thakkar, *Chem. Commun.*, **1970**, 2; *b*) A.L. Thakkar and P.V. Demarco, *J. Pharm. Sci.*, **60**, 652 (1971).

Table I. UV Absorption and CD Spectra of N-Phenylanthranilic Acids and Their Related Compounds Bound to β-Cyclodextrin[a)]

Compound		UV		CD		
		λ_{max} (nm)	$\varepsilon^{b)}$ ($\times 10^{-4}$)	λ_{max} (nm)	$\Delta\varepsilon^{c)}$	$g^{d)}$ ($\times 10^{4}$)
Anthranilic acid (I)		240 310	0.74 0.30			
Diphenylamine (II)		283	1.40	275	1.21	1.03
N-Phenylanthranilic acid (III)		291 332	1.26 0.53	285 332	2.36 0.61	1.90 1.15
Methyl N-phenylanthranilate (IV)		287 343	1.40 0.64	293 335	2.06 0.78	1.62 1.28
2,2-Iminodibenzoic acid (V)		289 338	1.17 0.73	233 242 292 345	1.00 −0.58 −1.45 −0.23	0.81 −0.73 −1.27 −0.32
Flufenamic acid (VI)		292 320	1.46 0.86	287 328	1.30 1.09	0.93 1.36
Mefenamic acid (VII)		283 336	0.70 0.46	280 315 335	−0.33 0.61 0.73	−0.29 1.13 1.16
3-(2,3-Dimethylanilino)-benzoic acid (VIII)		265 315	0.92 0.30	250 290 315	1.94 −0.18 0.09	2.00 −0.30 0.30
4-(2,3-Dimethylanilino)-benzoic acid (IX)		295	1.79	230 293	2.06 2.06	2.10 1.15
Meclofenamic acid (X)		285 320	0.48 0.43	275 320	0.43 0.03	0.91 0.07
Diclofenac acid (XI)		279	0.96	280	1.12	0.40

a) Concentrations of N-phenylanthranilic acids and β-cyclodextrin were of 5×10^{-5}M and 1×10^{-2}M, respectively.
b) apparent molar absorption coefficient c) differential dichroic absorption d) optical anisotropy factor (see text)

the interaction between β-cyclodextrin and fenamates were examined by high resolution NMR.

Figure 3 shows the effect of flufenamic acid on ^{1}H–NMR spectrum of β-cyclodextrin in D_2O. Protons located within the cavity of β-cyclodextrin such as H-3 and H-5[14a)] showed higher field shift and progressive broadening by the increasing amount of flufenamic acid. On the other hand, no anisotropic shielding was detected for the protons located exterior of the cavity[14a)] such as H-1 and H-2. Figure 4 shows effect of β-cyclodextrin on the ^{1}H–NMR spectrum of flufenamic acid in D_2O. It is apparent that phenyl signals at δ 9—7 (ppm) were remarkably broadened in the presence of β-cyclodextrin, suggesting that free rotation of phenyl groups in drug was restricted by the binding to β-cyclodextrin. Similar ^{1}H–NMR shifts changes were observed for other fenamates. These chemical shifts behaviors obviously indicate that aromatic moiety of the drug was included within the cavity of β-cyclodextrin, which was consistent with the results obtained from CD spectra. Moreover, ^{19}F–NMR signal

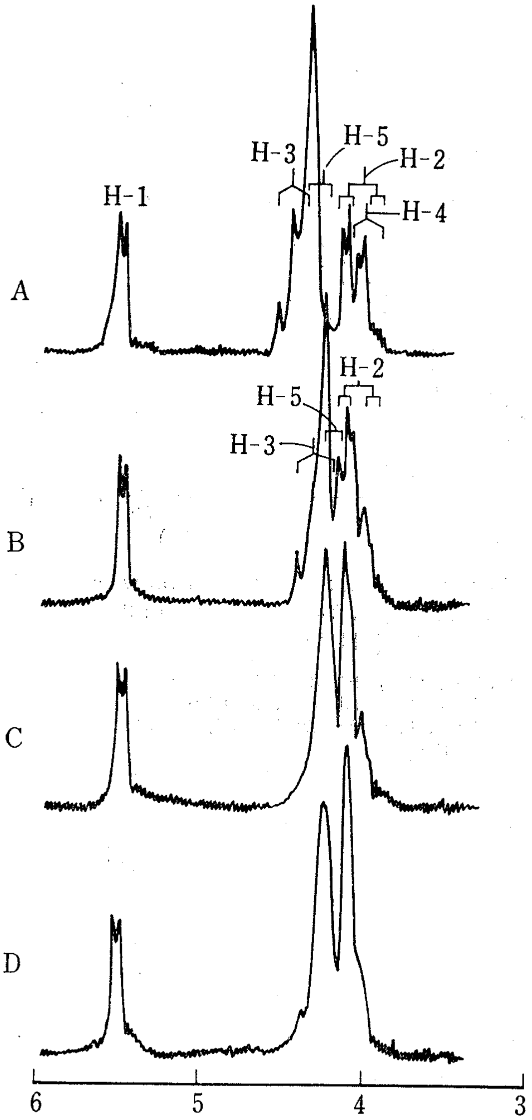


Fig. 3. ^{1}H-NMR Spectra of β-Cyclodextrin Containing Various Amounts of Flufenamic Acid

molar ratio; flufenamic acid/β-cyclodextrin; (A) 0.00, (B) 0.25, (C) 0.50, (D) 1.00, solvent: 0.1 M NaOH in D_2O

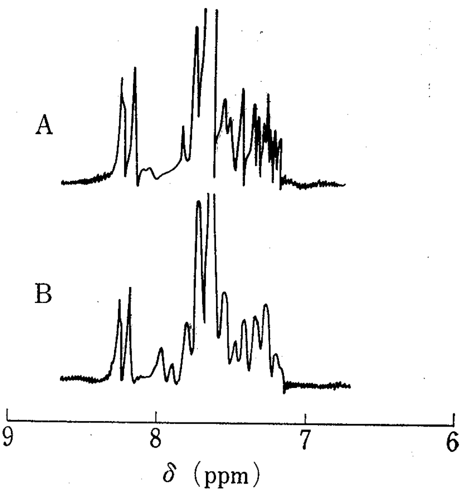


Fig. 4. Effect of β-Cyclodextrin on NMR Spectra of Flufenamic Acid

A: flufenamic acid (0.05 M) alone B: flufenamic acid (0.05 M)+β-cyclodextrin (0.05 M) solvent: 0.1 M NaOH in D_2O

of flufenamic acid located at δ 646 Hz shifted to δ 661 Hz in the presence of 0.15 M β-cyclodextrin in dimethyl sulfoxide (DMSO). The lower field shift could be attributed to the hydrogen bonding association of CF_3 substituent of flufenamic acid with interior protons of β-cyclodextrin cavity.

Formation Constnats of Inclusion Complexes

Induced optical activities, UV absorption, and solubility changes due to the complex formation between β-cyclodextrin and N-phenylanthranilates were quantitatively treated to obtain stoichiometric relationship and formation constants, K_c. Figure 5 shows a continuous variation plot of the ellipticity changes for N-phenylanthranilic acid–β-cyclodextrin system, which indicate 1:1 complex formation. Similar stoichiometric relationship can be expected for β-cyclodextrin complexes of the other compounds. Figure 6 shows Scott's plots of ellipticity changes(10) for flufenamic acid-β-cyclodextrin system. Application of UV absorption changes to Scott's equation also showed linear plots, indicating 1:1 stoichiometry. Formation constants of inclusion complexes were calculated from slopes and intercepts of the plots. Figure 7 shows the solubility of flufenamic acid as a function of β-cyclodextrin concentration in water. The plateau observed in this plot indicates that the complex of limited solubility is formed. Solubilizing effect of β-cyclodextrin on the other drugs were similar to that of flufenamic acid. Formation constants were then calculated on the basis of 1:1 from the initial straight line portion of solubility diagram.

Table II summarizes K_c values obtained by the three different method along with partition coefficients of the guest molecules studied. In all cases the K_c values obtained by solubility method fairly agreed with those obtained by spectroscopic methods. The large values obtained for these formation constants indicate a higher degree of stability presumably due to an

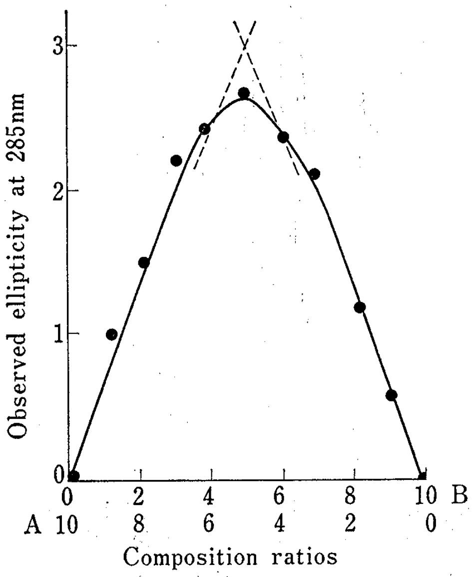


Fig. 5. Continuous Variation Plots for N-Phenylanthranilic Acid–β-cyclodextrin System in 0.1M Phosphate Buffer (pH 7.0)

A: N-phenylanthranilic acid (1×10^{-3}M)
B: β-cyclodextrin (1×10^{-3}M)

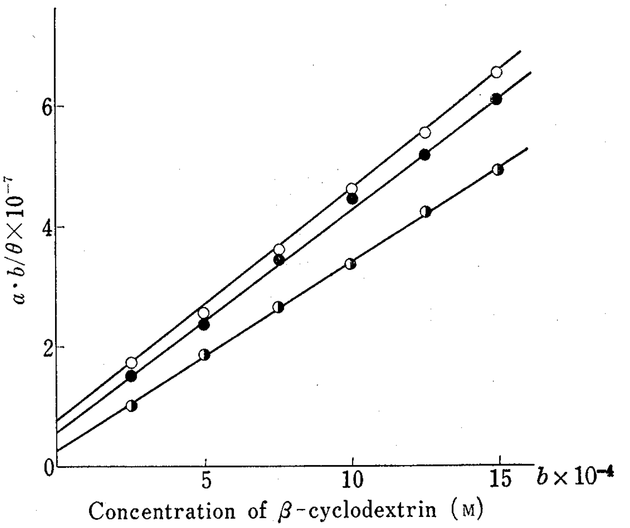


Fig. 6. Scott's Plots for Interaction between Flufenamic Acid and β-Cyclodextrin in 0.1M Phosphate Buffer (pH 7.0)

temperature: ◐—◐; 25°, ●—●; 40°, ○—○; 55°
a ; concentration of flufenamic acid (1×10^{-4}M)
b ; concentration of β-cyclodextrin (5×10^{-4}, 2.5×10^{-3}M)
θ: observed ellipticity

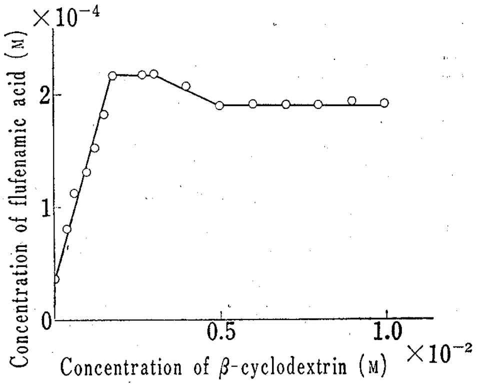


Fig. 7. Solubilizing Effect of β-Cyclodextrin on Flufenamic Acid in Water at 25°

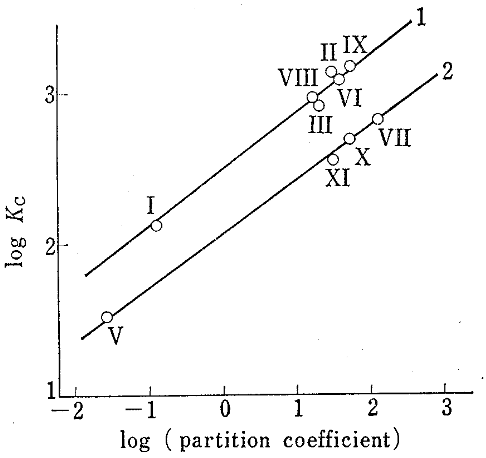


Fig. 8. Relationship between Formation Constants of β-Cyclodextrin Complexes and Partition Coefficients of Their Guest Molecules

Numbers refer to compounds in Table I.

interaction involving inclusion formation and other attractive forces. There found correlation between formation constant and partition coefficient, where the compounds with large partition coefficients showed greater tendency to form inclusion complex (Fig. 8). This indicates that hydrophobic environment of β-cyclodextrin cavity is more attractive to less water soluble guest molecules. However, it is particularly noteworthy that the formation constants of bulky guest molecule such as mefenamic, meclofenamic, and diclofenac acids were much lower than those would be expected from their partition coefficients (line 2 in Fig. 8). This may reflect steric hindrance due to *ortho* substituent, methyl and/or chlorine, in these drug molecules.

Table II. Formation Constants of β-Cyclodextrin Complexes with N-Phenylanthranilic Acids and Their Related Compounds, and Their Partition Coefficients at 25°

Compound	Partition coefficient	Formation constant (M^{-1}) Solubility method	UV method	CD method
Anthranilic acid (I)	0.11	130	—[a]	—[a]
Diphenylamine (II)	32.3	—	800	1330
N-Phenylanthranilic acid (III)	22.0	460	920	790
2,2′-Iminodibenzoic acid (V)	0.02	44	90	33
Flufenamic acid (VI)	39.0	1380	1330	1270
Mefenamic acid (VII)	123	570	630	620
3-(2,3-Dimethylanilino)-benzoic acid (VIII)	16.4	—	—	880
4-(2,3-Dimethylanilino)-benzoic acid (IX)	51.9	—	—	1490
Meclofenamic acid (X)	60.0	470	640	490
Diclofenac acid (XI)	34.1	150	510	340

a) could not be determined with accuracy

Thermodynamics of Inclusion Complex Formation

Thermodynamic parameters for complex formation were determined from the temperature dependency of formation constants and were summarized in Table III. In general, van't Hoff plots fell fairly on straight lines over the temperature range 25—55°. As is seen in Table III, all ΔH values were negative, while ΔS values were considerably different in each case. Positive ΔS values obtained for flufenamic and N-phenylanthranilic acids indicate favorable entropy changes, while negative ΔS values obtained for mefenamic and meclofenamic acids show unfavorable entropy changes. These effects may be interpreted on the basis of hydrophobic bond formation which is principally responsible for the nature of solvent water.[15] It is proposed that entropy changes in the course of the binding process are solely due to the disordering, partial or total, of the iceberg,[16] which accompany the hydrophobic interactants; the complexed molecule will thus be accompanied either by a less ordered iceberg or by an iceberg containing a smaller number of water molecules than icebergs of two separate

Table III. Thermodynamic Parameters for the Complex Formation of N-Phenylanthranilates with β-Cyclodextrin

Compound	Temp. (°K)	ΔG (kcal/mole)	ΔH (kcal/mole)	ΔS (e.u.)	ΔSu[b] (e.u.)
N-Phenylanthranilic acid (III)	298 313 328	−3.95 −4.02 −4.14	−2.08	6.27	14.3
Flufenamic acid (VI)	298 313 328	−4.23 −4.27 −4.31	−3.48	2.43	10.4
Mefenamic acid (VII)	298 313 328	−3.80 −3.67 −3.60	−5.77	−6.64	1.34
Meclofenamic acid (X)	298 313 328	−3.65 −3.29 −3.00	−10.0	−21.5	−13.5

a) For the calculation of these values, formation constants were determined by CD method at various temperature.
b) unitary entropy change (see references 17, 18)

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15) W.P. Jenks, "Catalysis in Chemistry and Enzymology," McGraw-Hill Book Co., New York, N.Y., 1969, p. 393.
16) H.S. Frank and M.W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).

interactants.[17] The removal of water molecules from the ordered structure should result in a favorable entropy change. Thus, positive entropy values observed for flufenamic and N-phenylanthranilic acids is attributed to the formation of hydrophobic bonding. On the other hand, the unfavorable entropy changes observed for mefenamic and meclofenamic acids may be due to the smaller disordering of displaced water molecules which are probably released from drug molecules as well as β-cyclodextrin cavity, because formation constants of mefenamic and meclofenamic acids were smaller than those of flufenamic and N-phenylanthranilic acids (Table II). The unitary entropy change[17,18] for meclofenamic acid again suggested an unfavorable entropy change (Table III). Since unitary function depends only on the factors that involve the interaction of drug and β-cyclodextrin with the solvent and with each other, the contribution due to randomness of mixing with solvent are excluded. Thus, in the case of meclofenamic acid hydrophobic interaction seems to be less important for the formation of inclusion complex with β-cyclodextrin, and general trend of complex formation such as hydrogen bonding may be reflected in the thermodynamic parameters.

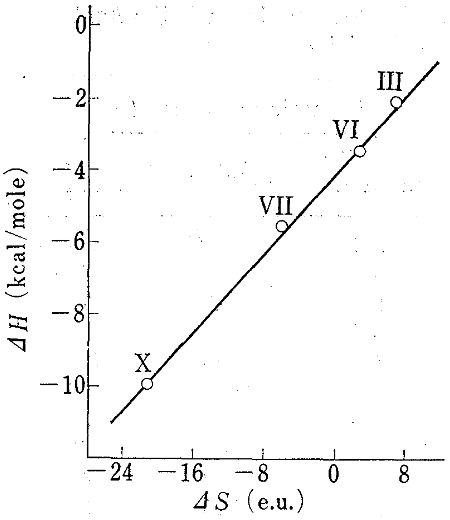

Fig. 9. Plot of ΔH *vs.* ΔS for Complex Formation of β-Cyclodextrin with N-Phenylanthranilates

Numbers refer to compounds in Table I.

The solvent ordering should be accompanied by the increasing number of solvent-solvent bonds (in this case water-water hydrogen bonding) and consequently results in a favorable enthalpy changes. In fact, unfavorable entropy changes were largely compensated by favorable enthalpy changes, as is seen in Fig. 9. This compensation effect has been observed frequently in water, where their compensation temperatures (T_c)[19] lie in a relatively narrow range from 250 to 315°K.[20] The slope of the line in this plot corresponds to T_c and has a value of 284°K. Recently, similar results were reported for inclusion formation of cyclodextrins with various organic and inorganic guest molecules.[21]

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