[Chem. Pharm. Bull. 23(7)1421—1430(1975)]

UDC 547.586.5'458.6.03

Inclusion Complexes of Cinnamic Acids with Cyclodextrins. Mode of Inclusion in Aqueous Solution[1)]

KANETO UEKAMA,[2a)] MASAKI OTAGIRI, YOUKO KANIE, SHIGEO TANAKA, and KEN IKEDA[2)]

Faculty of Pharmaceutical Sciences, Nagoya City University[2)]

(Received October 2, 1974)

Inclusion complexation of various *trans*- and *cis*-cinnamic acid derivatives with α- and β-cyclodextrins in aqueous solution was studied by circular dichroism (CD), ultraviolet (UV), and nuclear magnetic resonance (NMR) spectroscopies. By the analyses of the induced CD and UV spectral changes, the spatial relationships between guest and host molecules was investigated. To elucidate the inclusion mechanism, stoichiometric ratio, which was found 1:1, formation constant, and thermodynamic parameters for *trans*-cinnamic acid-cyclodextrin systems were determined. Various factors in guest molecule such as ionization, hydrophobic nature, and substituent effect were reflected in inclusion formation. From the evidences of sign of the induced CD and NMR chemical shift, it was proposed that phenyl moiety of cinnamic acid was fixed into (*R*)-configuration within the cavity of cyclodextrin.

The spatial relationship between the guest and host molecules is essential for the inclusion complex formation as has been frequently pointed out.[3)] Lach and his coworkers reported on the complexation of cyclodextrins with various organic compounds including phenyl-substituted carboxylic acids.[4)] These studies were, however, based on the solubility method, and do not provide direct elucidation of the guest molecule included within the cavity of cyclodextrin. We recently reported that circular dichroism (CD) method is useful to examine the mode of interaction of cyclodextrins with drug molecules.[5)]

For the elucidation of inclusion mechanism of phenyl-substituted carboxylic acids, *trans*-cinnamic acids are useful guest molecules because inspite of their simple structure they have extended conjugated resonance system and the effect of inclusion are subtly reflected in various spectra. To investigate the spatial effect on the inclusion, various cinnamic acid derivatives substituted on benzene ring were used as guest molecule. Furthermore, the stereoisomers, *cis*-cinnamic acids are particularly interest for the steric dependency of the inclusion.

Experimental

Materials——*trans*- and *cis*-cinnamic acids used in this study and their physical properties are listed in Table I. Compounds: **1**, **8**, **9**, **10**, **19**, **21**, and **22**, were obtained commercially and recrystallized from

1) A part of this work was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.

2) Location: *Tanabe-dori, Mizuho-ku, Nagoya*; *a*) Present address: *Faculty of Pharmaceutical Sciences, Kumamoto University, 5–1, Oehonmachi, Kumamoto.*

3) *a*) H.M. Powell, *Endeavour*, **9**, 154 (1950); *b*) F. Cramer, W. Saenger, and H-Ch. Spatz, *J. Am. Chem. Soc.*, **89**, 14 (1967); *c*) K. Takeo and T. Kuge, *Die Stärke*, **24**, 281 (1972).

4) *a*) J. Cohen and J.L. Lach, *J. Pharm. Sci.*, **52**, 132 (1963); *b*) J.L. Lach and T-F. Chin, *ibid.*, **53**, 69 (1964); *c*) W.A. Pauli and J.L. Lach, *ibid.*, **54**, 1745 (1965).

5) *a*) K. Ikeda, K. Uekama, M. Otagiri, and M. Hatano, *J. Pharm. Sci.*, **63**, 1168 (1974); *b*) M. Otagiri, K. Ikeda, K. Uekama, O. Ito, and M. Hatano, *Chemistry Letters*, **1974**, 679; *c*) K. Ikeda, K. Uekama, and M. Otagiri, *Chem. Pharm. Bull.* (Tokyo), **23**, 201 (1975); *d*) M. Otagiri, K. Uekama, and K. Ikeda, *ibid.*, **23**, 188 (1975); *e*) M. Otagiri, K. Uekama, T. Miyaji, and K. Ikeda, *Chem, Pharm. Bull.*, (Tokyo) in prepara- tion.

Table I. Cinnamic Acid Derivatives Studied and Their Physicochemical Properties

Compound	mp (°C)[a]	pK_a[b]	PC[c]	Analytical wavelength (nm)[d]	
				At pH 1.6	At pH 8.2
trans-isomer					
Cinnamic acid (**1**)	132—133	4.44	0.058	277	268
o-Chlorocinnamic acid (**2**)	208—210	4.23	0.119	274	267
m-Chlorocinnamic acid (**3**)	160—161	4.29	0.132	272	267
p-Chlorocinnamic acid (**4**)	242	4.41	0.138	284	273
o-Methylcinnamic acid (**5**)	164—165	4.50	0.180	278	271
m-Methylcinnamic acid (**6**)	114—115	4.44	0.206	280	272
p-Methylcinnamic acid (**7**)	200	4.56	0.246	286	276
o-Hydroxycinnamic acid (**8**)	210	4.61	0.014	276	268
m-Hydroxycinnamic acid (**9**)	194	4.44	0.017	275	270
p-Hydroxycinnamic acid (**10**)	226	4.67	0.023	307	285
o-Nitrocinnamic acid (**11**)	240—242	4.15	0.022	244	242
m-Nitrocinnamic acid (**12**)	199—200	4.12	0.016	264	260
p-Nitrocinnamic acid (**13**)	293	4.05	0.028	302	312
o-Methoxycinnamic acid (**14**)	186	4.46	0.126	276	268
m-Methoxycinnamic acid (**15**)	120	4.38	0.074	277	270
p-Methoxycinnamic acid (**16**)	175	4.54	0.142	307	285
m-Bromocinnamic acid (**17**)	176—178		0.166	273	286
p-Bromocinnamic acid (**18**)	251—252		0.230	285	275
p-Aminocinnamic acid (**19**)	170		0.052	262	297
p-Dimethylaminocinnamic acid (**20**)	233—235		0.554	268	318
3,4-Dihydroxycinnamic acid (**21**)	200		0.018	322	286
3,4-Dimethoxycinnamic acid (**22**)	172—173		0.139	319	284
cis-Isomer					
Cinnamic acid (**23**)	56—58	3.88	0.036	268	254
p-Chlorocinnamic acid (**24**)	103—104		0.042	270	258
3,4-Dimethoxycinnamic acid (**25**)	100—101		0.057	314	267

a) All melting points were observed on a microscopic hotstage and uncorrected.
b) literary values (ref. 9)
c) apparent partition coefficient of cinnamic acid derivative ($CHCl_3$/0.1 M sodium phosphate buffer (pH 7.0))
d) used for the determination of formation constant, K

EtOH–water. Compounds: **2**, **3**, **4**, **5**, **6**, **7**, **11**, **12**, **13**, **14**, **15**, **16**, **18**, and **20**, were synthesized by the usual method,[6] and recrystallized from EtOH–water. *cis*-Isomers: **23**, **24**, and **25**, were obtained by photoisomerization from the corresponding *trans*-isomers referring to the literature,[7] and recrystallized from ligroin or *n*-hexane. β-Cyclodextrin was favored from Teijin Ltd., and α-cyclodextrin 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-cyclodextrin and $[\alpha]_D^{25}=152.0\pm0.5°$ for α-cyclodextrin, respectively. All other materials and solvents were of analytical reagent grade.

CD and Ultraviolet (UV) Absorption Studies——The CD and UV spectra were obtained with a Jasco 20A spectropolarimeter and a Hitachi EPS-3T spectrometer, respectively. All measurements were carried out in 0.1 M sodium phosphate buffer solution and the pH was adjusted to appropriate value by the addition of 0.05 M NaOH or 0.05 M H_3PO_4 solution. The optical anisotropy factor, g value, which is proportional to the magnitude of the induced Cotton effects was calculated from the equation, $g=\Delta\varepsilon/\varepsilon$, where $\Delta\varepsilon$ is differential dichroic absorption, and ε is the molar absorptivity of guest molecule in the presence of cyclodextrin at the maximum wavelength of CD spectrum.

Nuclear Magnetic Resonance (NMR) Studies——NMR spectra were measured by a JEOL PS-100 spectrometer at ambient probe temperature of 31±1°. Tetramethylsilane was used as an external reference for solvent D_2O and no correction was made for susceptibility.

6) S. Dutt, *Chem. Zentr.*, **96**, 1852 (1925); J.S. Buch and W.S. Ide, "Organic Syntheses," Coll. Vol. II, ed. by A.H. Blatt, John Wiley and Sons, Inc., New York, N.Y., 1943, p. 130.
7) H. Sobbe and F.K. Steinberger, *Chem. Ber.*, **55**, 2225 (1922).

Determination of Formation Constants——The formation constants, K, for α- and β-cyclodextrin complex with cinnamic acids were determined spectrophotometrically according to Scott's equation.[8] The UV absorption changes of given cinnamic acid derivative (constantly 4.0×10^{-5} M) in the presence of cyclodextrin (varied from 1.0×10^{-3} to 1.0×10^{-2} M) were measured at the wavelength showed in Table I. The calculation of K values were essentially same as that of previous paper.[5c]

Determination of Partition Coefficients——Apparent partition coefficients of cinnamic acids were determined by the shaking of 10 ml of aqueous cinnamic acid solution (1.0×10^{-4} M in sodium phosphate buffer of pH 7.0) and 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 I.

Results and Discussion

Induced CD of Cinnamic Acids by the Binding to Cyclodextrins

The CD and UV absorption spectra of *trans*- and *cis*-cinnamic acids in the presence of α- and β-cyclodextrins are shown in Fig. 1. The optical activity of *trans*-cinnamic acid is induced with positive sign by both of α- and β-cyclodextrins in the absorption region of *trans*-isomer, where distinct UV spectral changes are also observed. On the other hand, the optical activity of *cis*-cinnamic acid is induced only by β-cyclodextrin with positive sign, which accompanies with slight change of UV spectrum of *cis*-isomer. The magnitude of the CD of *trans*-isomer induced by α-cyclodextrin is larger than that by β-cyclodextrin. In the presence of β-cyclodextrin, the magnitude of the induced CD for *trans*-isomer is larger than that for *cis*-isomer. The changes in UV spectra are also similar to those in CD spectra.

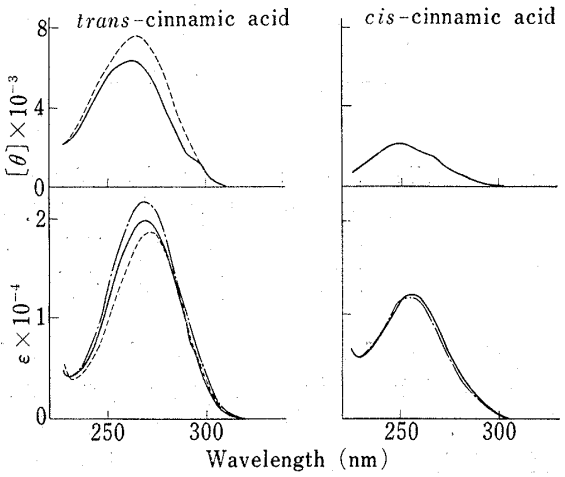

Fig. 1. CD (Upper) and UV Absorption (Lower) Spectra of Cinnamic Acid Isomers in the Presence of Cyclodextrins in 0.1 M Sodium Phosphates Buffer of pH 7.0 at 25°

———: cinnamic acid alone (5.0×10^{-4}M)
– – – – –: cinnamic acid (5.0×10^{-4}M)+α-cyclodextrin (1.0×10^{-2}M)
———: cinnamic acid (5.0×10^{-4}M)+β-cyclodextrin (1.0×10^{-2}M)

Figure 2 shows the induced CD bands (upper) and changes in UV spectra (lower) of methylsubstituted *trans*-cinnamic acids in the presence of α- and β-cyclodextrins, where marked differences are observed between the positional isomers. With the exception of *o*-methylcinnamic acid-α-cyclodextrin system, all systems show new CD bands in the absorption region of the methylcinnamic acids accompanied by UV spectral changes. In *para*-isomer, the induced CD band by β-cyclodextrin is significantly small compared to that by α-cyclodextrin. This indicates that the inclusion in β-cyclodextrin of larger cavity is loose comparing to that of α-cyclodextrin and this is responsible for the magnitudes of the extrinsic optical activity. In *meta*-isomer, the magnitude of induced CD by β-cyclodextrin is almost same as that for α-cyclodextrin, indicating the similar degree of interactions. In *ortho*-isomers, the magnitude of the induced CD was the smallest among three positional isomers. That α-cyclodextrin did not induce the optical activity of *ortho*-isomer indicates that the cavity size of α-cyclodextrin is too small to induce the *o*-methylcinnamic acid.

Table II summarizes λ_{max}, ε, $\Delta\varepsilon$, and g values obtained for various cinnamic acid derivatives bound to α- and β-cyclodextrins, where the differences mentioned above are quantitatively shown. All the induced CD bands show positive Cotton effects and the g values seem to be

8) R.L. Scott, *Rec. Trav. Chim.*, **75**, 787 (1956).

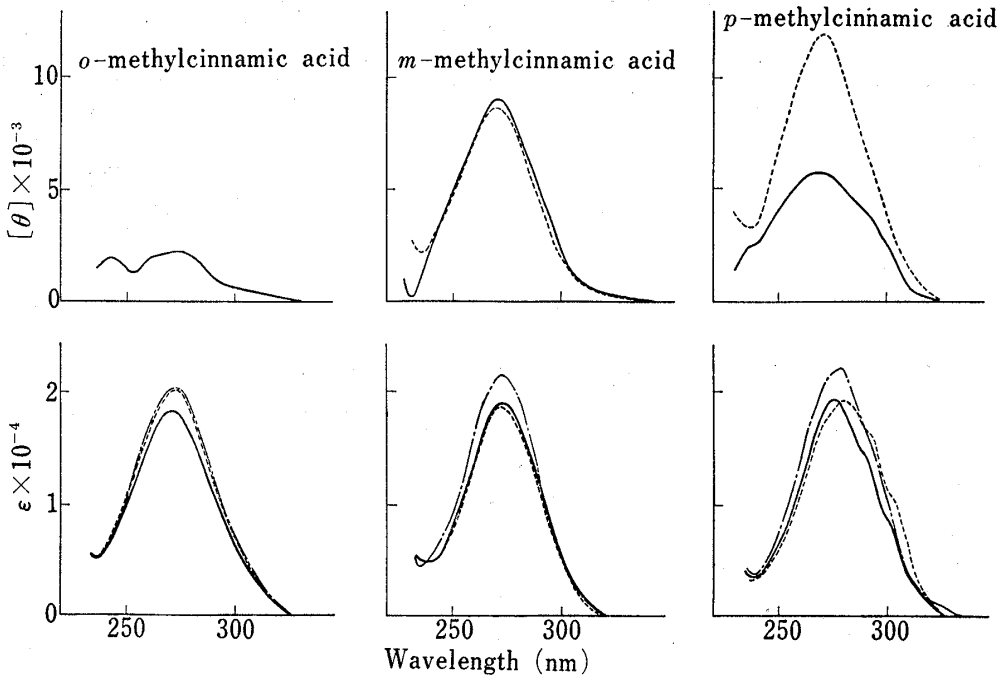

Fig. 2. CD (Upper) and UV Absorption (Lower) Spectra of Methylcinnamic Acids in the Presence of Cyclodextrins in 0.1 M Sodium Phosphate Buffer of pH 7.0 at 25°

———: methylcinnamic acid alone (5.0×10^{-4}M)
– – – –: methylcinnamic acid (5.0×10^{-4}M)+α-cyclodextrin (1.0×10^{-2}M)
———: methylcinnamic acid (5.0×10^{-4}M)+β-cyclodextrin (1.0×10^{-2}M)

largely dependent upon the steric factors in the guest and the host molecules. In β-cyclodextrin-*trans*-cinnamic acid systems, *g* values decreased in the order of *meta*, *para*, and *ortho*-isomers. In the cases of α-cyclodextrin, however, *g* value of *para*-isomer is larger than that of *meta*-isomer and that of *ortho*-isomers are smallest and for some *ortho*-isomers induced CD was not observed. *cis*-Cinnamic acids showed smaller CD band by the binding to β-cyclodextrin, and neither CD nor UV changes were observed in the presence of α-cyclodextrin. *cis*-3,4-Dimethoxycinnamic acid, which has the largest size among the guest molecule studied, did not show any optical activity by both of α- and β-cyclodextrins. Similar tendency was also observed in their UV absorption changes. These results clearly indicate that the bulkiness of the guest molecule and the cavity size of the host is particularly responsible for the generation of induced CD band. Since intrinsic Cotton effects of cyclodextrins are observed below 220 nm, the observed CD bands above 220 nm can be assigned to the extrinsic optical activity of cinnamic acids due to the inclusion.

To determine the stoichiometric ratio of the inclusion complex, the induced optical activity was quantitatively measured. Figure 3 shows the continuous variation plots of the ellipticity change at 270 nm for *trans*-cinnamic acid-α-cyclodextrin system as an example, which indicate 1:1 complex formation. Although continuous variation analysis was not carried out on all systems, similar stoichiometric relationship can be expected for all other systems from the linear Scott's plots obtained as will be later shown.

Formation Constants and Thermodynamic Parameters for Inclusion Complex Formation

Figure 4 shows the effect of α-cyclodextrin on UV spectrum of *trans*-cinnamic acid in sodium phosphate buffer of pH 1.6 at 25°. By the increasing of α-cyclodextrin, the absorption maximum (λ_{max}) and the intensity of *trans*-cinnamic acid change concomitantly with isobestic points at 230 and 300 nm. Accompanying UV spectral changes are observed for *trans*-cinnamic acid-β-cyclodextrin system. The presence of isobestic points and the result of continuous variation plots (Fig. 3) indicate that cinnamic acids and cyclodextrins form 1:1 complex in

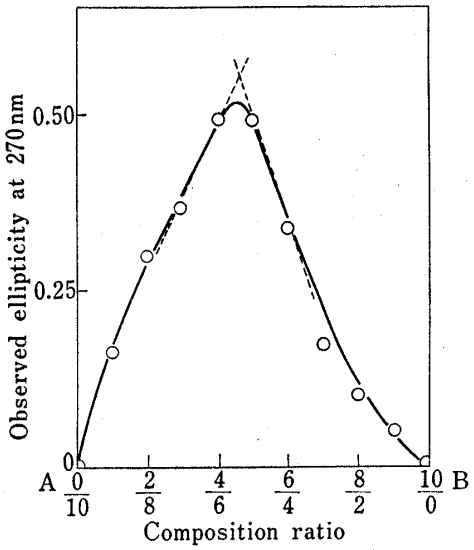

Fig. 3. Continuous Variation Plots for α-Cyclodextrin (A)-*trans*-Cinnamic Acid (B) System in 0.1 M Sodium Phosphate Buffer of pH 7.0 at 25°

A: α-cyclodextrin (1×10^{-3}M)
B: *trans*-cinnamic acid (1×10^{-3}M)

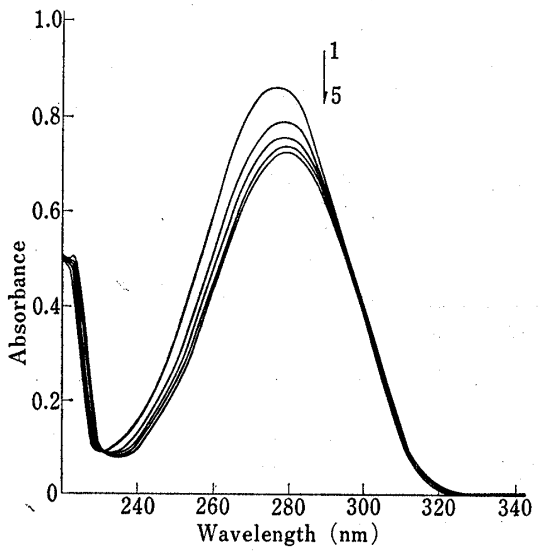

Fig. 4. Effect of α-Cyclodextrin on UV Absorption Spectrum of *trans*-Cinnamic Acid in 0.1 M Sodium Phosphate Buffer of pH 1.6 at 25°

concentration of *trans*-cinnamic acid: constantly 4.0×10^{-5}M
concentration of α-cyclodextrin: curve 1; 0, 2; 5×10^{-4} 3; 1×10^{-3}, 4; 2×10^{-3}, 5; 6×10^{-3}M

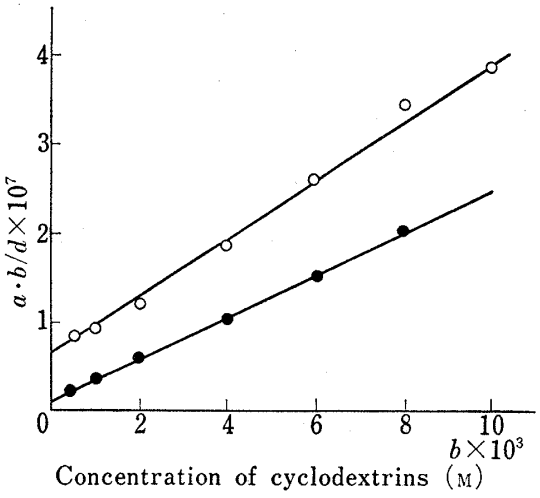

Fig. 5. Typical Scott's Plots for Interaction between *trans*-Cinnamic Acid and Cyclodextrins in 0.1 M Sodium Phosphate Buffer of pH 1.6 at 25°

—●—: α-cyclodextrin-*trans*-cinnamic acid system
—○—: β-cyclodextrin-*trans*-cinnamic acid system
a: concentration of *trans*-cinnamic acid (4×10^{-5}M)
b: concentration of α- and β-cyclodextrin (0.5×10^{-3}—10×10^{-3}M)
d: change in absorbance of *trans*-cinnamic acid on adding cyclodextrins

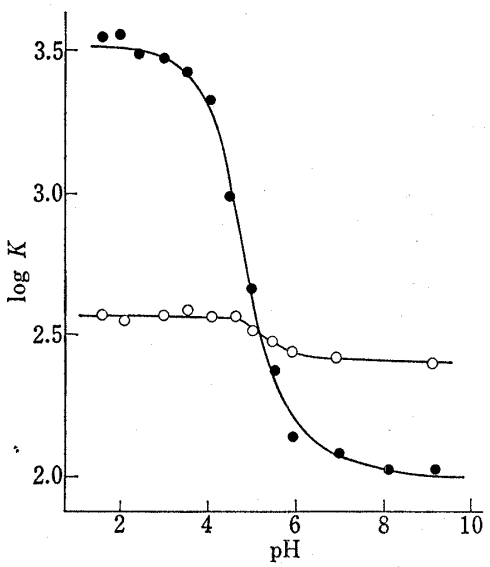

Fig. 6. pH Profile for Formation Constants of *trans*-Cinnamic Acid-Cyclodextrins Complexes

—●—: α-cyclodextrin-cinnamic acid system
—○—: β-cyclodextrin-cinnamic acid system

relatively dilute solution. However, Lach and coworker reported previously[4c] that *trans*-cinnamic acid and cyclodextrin form 2:1 complex on the basis of phase solubility analysis. The higher order complexation detected by their solubility study may become from the high concentration of the interactant, where the dimerization of the substrate is suspicious. That

Table II. Induced CD and UV Spectra of Cinnamic Acid Derivatives Bound to α- and β-Cyclodextrin[a]

Substituent	α-Cyclodextrin system					β-Cyclodextrin system				
	UV maximum		DC maximum		$g^{d)}$ ($\times 10^4$)	UV maximum		CD maximum		$g^{d)}$ ($\times 10^4$)
	Wavelength (nm)	$\varepsilon^{b)}$ ($\times 10^{-4}$)	Wavelength (nm)	$\Delta\varepsilon^{c)}$		Wavelength (nm)	$\varepsilon^{b)}$ ($\times 10^{-4}$)	Wavelength (nm)	$\Delta\varepsilon^{c)}$	
trans-Isomer										
H	271	1.86	265	2.27	1.22	269	1.99	264	1.89	0.95
o-Cl	270	1.87	260	0.91	0.49	268	1.74	270	1.21	0.70
m-Cl	269	1.94	260	1.45	0.75	269	1.87	266	2.95	1.58
p-Cl	275	2.79	269	4.06	1.46	274	2.85	265	2.00	0.70
o-CH_3	272	2.00	—e)	—e)		272	1.82	270	0.61	0.34
m-CH_3	274	1.88	270	2.64	1.40	274	1.88	270	2.76	1.47
p-CH_3	281	1.90	272	3.64	1.92	277	1.93	270	1.73	0.89
o-OH	270	1.72	270	0.52	0.30	269	1.64	267	0.73	0.45
	314	0.84	315	0.21	0.25	315	0.84	320	0.61	0.73
m-OH	272	1.74	270	1.33	0.76	273	1.72	267	1.85	1.08
p-OH	287	2.19	280	2.21	1.01	287	0.14	280	2.30	1.07
o-NO_2	244	1.94	—e)	—e)		243	1.68	230	0.94	0.56
m-NO_2	261	2.92	260	0.88	0.30	261	2.89	247	1.58	0.55
p-NO_2	321	1.64	304	1.83	1.12	315	1.57	318	0.76	0.48
p-OCH_3	290	2.00	284	3.21	1.61	287	1.94	280	1.94	1.00
3,4-di-OH	288	1.34	280	1.03	0.77	288	1.30	290	1.15	0.88
	313	1.26	310	0.79	0.63	314	1.23	315	1.06	0.86
3,4-di-OCH_3	288	1.45	284	0.80	0.55	287	1.58	285	1.00	0.63
	310	1.50	300	0.85	0.57	309	1.50	303	0.85	0.57
cis-Isomer										
H	254	1.26	—e)	—e)		255	1.24	250	0.67	0.54
3,4-di-OCH_3	269	1.26	—e)	—e)		269	1.20	—e)	—e)	
	298	0.80	—e)	—e)		300	0.74	—e)	—e)	

a) Concentrations of cinnamic acids and cyclodextrins were of 5×10^{-4}M and 1×10^{-2}M, respectively, in 0.1M sodium phosphate buffer of pH 7.0.
b) apparent molar absorption coefficient
c) differential dichroic absorption
d) optical anisotropy factor (see text)
e) could not be observed

the continuous variation plots (Fig. 3) is unsymmetrically concave at higher B/A value may be attributed to the same cause.

Figure 5 shows the Scott's plots of UV absorption changes for *trans*-cinnamic acid-α- and β-cyclodextrin systems. The formation constant, K, was calculated from the slope and intercept of the linear plots assuming 1:1 inclusion. Figure 6 shows the pH profile of log K for *trans*-cinnamic acid-α- and β-cyclodextrin systems, where typical sigmoid curves were obtained with an inflection point at pK_a of *trans*-cinnamic acid. Therefore, the formation constants of the free and ionized forms of cinnamic acids were determined at pH 1.6 and 8.2, respectively. Cinnamic acids are of free form at pH 1.6 and are completely ionized at pH 8.2 as is expected from their pK_a values[9] (see Table I).

Table III summarizes the K values obtained at various conditions, where the steric hindrance and the nature of the substituents are seemed to be reflected in the inclusion. These values are consistent with the results of the CD studies (Table II). In all cases, the free

9) G. Kortüm, W. Vogel, and K. Andrussow, "Dissociation Constants of Organic Acids in Aqueous Solution," Butterworths, London, 1961.

Table III. Formation Constants (M^{-1}) for Inclusion Complexes of *trans*-Cinnamic Acids with α- and β-Cyclodextrins at 25°

Substituent	*K* value at pH 1.6[a]		*K* value at pH 8.2[b]	
	α-CyD[c] system	β-CyD[c] system	α-CyD[c] system	β-CyD[c] system
H	3460	371	109	313
o-Cl	563	761	283	501
m-Cl	2620	1110	423	684
p-Cl	4640	595	286	689
o-CH_3	277	417	—[d]	—[d]
m-CH_3	3070	948	132	335
p-CH_3	13600	439	353	466
o-OH	1110	380	—[d]	—[d]
m-OH	1320	425	90	232
p-OH	1990	570	110	412
o-NO_2	103	480	—[d]	—[d]
m-NO_2	510	57	—[d]	—[d]
p-NO_2	578	242	263	333
o-OCH_3	557	61	—[d]	—[d]
m-OCH_3	2490	451	71	392
p-OCH_3	10300	658	277	341
m-Br	1390	687	295	541
p-Br	12300	1250	580	1120
p-NH_2	807	184	—[d]	—[d]
p-$N(CH_3)_2$	1290	177	181	1190
3,4-di-OH	2190	372	246	366
3,4-di-OCH_3	4190	1100	—[d]	—[d]

a) At this pH cinnamic acids are substantially in free form.
b) At this pH cinnamic acids are substantially ionized.
c) α-CyD=α-cyclodextrin, β-CyD=β-cyclodextrin
d) could not be determined with accuracy due to the small changes of UV spectra

form of cinnamic acids are favorable to form inclusion complex compared to the ionized form. This trend is particularly significant in α-cyclodextrin complex as is seen in Fig. 6. This may be due to the hydration of the ionized cinnamic acids, which is assumed to be greater than that of free and is unfavorable for the smaller cavity of α-cyclodextrin.

Figure 7 shows the relationship between log *K* and log partition coefficient (*PC*) of the guest molecules, where cinnamic acids with larger *PC* show greater *K* value. This indicates that hydrophobic environment of cyclodextrin cavity is more attractive to highly hydrophobic guest molecule. It is interesting that the spatial relationship between guest and host molecules are also seen in log *K*—log *PC* correlations. In *para*-isomers, *K* values for α-cyclodextrin are more dependent on the hydrophobic property of guest molecule comparing to those for β-cyclodextrin. In *meta*-isomers, however, the above trend is reversed. From these correlations shown in Fig. 7, following suggestions may be deduced on the bulkiness of the guest molecule and the cavity size of cyclodextrin: (I) The bulky *meta*-isomers are compact in the narrower α-cyclodextrin cavity and the hydrophobicity of the guest molecule does not effect so significantly on the inclusion, (2) the linear molecule of *para*-isomers are not so tight in the larger β-cyclodextrin cavity and the hydrophobicity of the guest is not effective, (3) in the systems of *meta*-isomers-β-cyclodextrin and *para*-isomers-α-cyclodextrin the fitness of the guest in the cavity is medial between above two cases and *K* is largely depend upon the hydrophobicity. The attempt to correlate the activity of mono-substituted *para*- and *meta*-cinnamic acids with the Hammett sigma constants was unsuccessful. *K* values for *meta*- and *para*-isomers of hydroxycinnamic acid are significantly large in spite of its low *PC*, which suggest that hydrogen bonding is also operative in this interaction.

Thermodynamic parameters for the inclusion were determined from the temperature dependency of formation constants at pH 1.6 and 8.2, respectively. In general, van't Hoff plots fell fairly on straight line over the temperature range 15—45° (Fig. 8). As is seen in Table IV, the nature of substituent and ionization effect of guest molecule are again reflected

Table IV. Thermodynamic Parameters for the Complex Formation of *trans*-Cinnamic Acids with α- and β-Cyclodextrins at 25°

Substituent	Ionic species	α-Cyclodextrin system			β-Cyclodextrin system		
		ΔG (kcal/mole)	ΔH (kcal/mole)	ΔS (e.u.)	ΔG (kcal/mole)	ΔH (kcal/mole)	ΔS (e.u.)
o-CH_3	free form[a]	−3.33	−5.2	−6.7	−3.57	−2.9	+2.8
m-CH_3		−4.76	−7.1	−8.2	−4.06	−6.0	−6.7
p-CH_3		−5.64	−12.5	−23.0	−3.60	−11.9	−27.8
o-OH		−4.15	−7.5	−11.2	−3.51	−5.5	−7.1
m-OH		−4.26	−7.6	−11.5	−3.58	−4.6	−3.7
p-OH		−4.50	−7.2	−8.7	−3.76	−5.2	−4.5
m-CH_3	ionized form[b]	−2.89	−5.2	−7.8	−3.44	−4.5	−3.7
p-CH_3		−3.47	−9.6	−20.1	−3.64	−4.2	−1.5
m-OH		−2.67	−7.1	−15.1	−3.23	−5.1	−6.4
p-OH		−2.78	−6.5	−12.4	−3.57	−4.9	−4.5

a) determined at pH 1.6
b) determined at pH 8.2

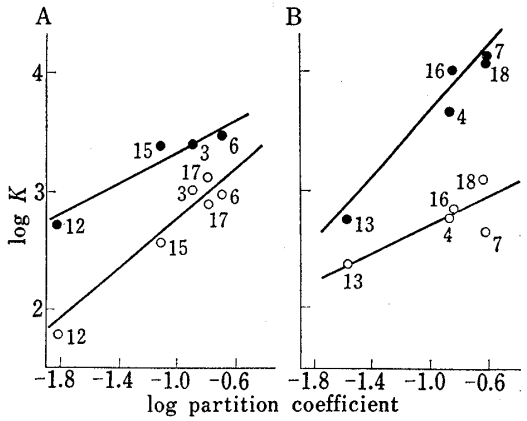

Fig. 7. Relationships between Formation Constants of Cyclodextrin Complexes and Partition Coefficients of Their Guest Molecules

A: *meta*-substituted *trans*-cinnamic acid system
B: *para*-substituted *trans*-cinnamic acid system
● and ○ are α- and β-cyclodextrin complexes, respectively. numbers refer to cinnamic acids in Table I

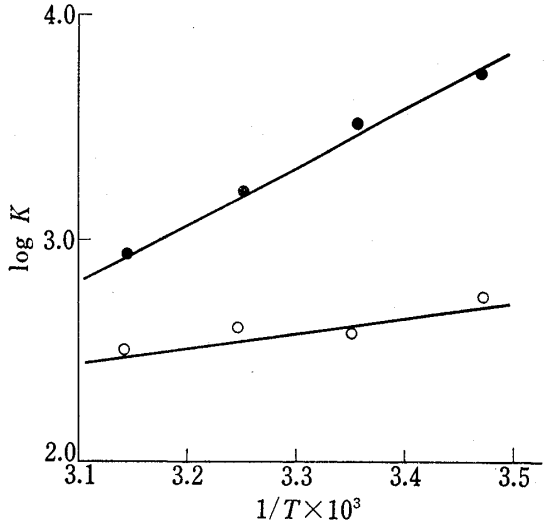

Fig. 8. Typical van't Hoff Plots for Formation Constants of *trans*-Cinnamic Acid-Cyclodextrin Complexes

—●—: α-cyclodextrin-*trans*-cinnamic acid system
—○—: β-cyclodextrin-*trans*-cinnamic acid system

in the values of ΔH and ΔS. In a series of *o*-, *m*-, and *p*-isomers of methylcinnamic acid, ΔH become favorable in this order, while ΔS shows unfavorable changes in the same order. On the other hand, any significant changes in both values of ΔH and ΔS were not observed for the series of hydroxycinnamic acids. As has been pointed out by Cramer, *et al.*[3b] methyl group is surrounded by ice-berg structure and around hydroxy group the water is tightly solvated by hydrogen bonding. This difference seems to be related to the different feature of thermodynamic parameters. In the ionized form of guest molecule also thermodynamic behavior similar to the free form of acids was observed. For the full interpretation on these thermo-

dynamic parameters, the other factors such as salt effect may be necessary.[10] Figure 9 shows the linear relationships between ΔH and ΔS for inclusion formations of *para*- and *meta*-isomers with cyclodextrins, where unfavorable entropy changes are largely compensated by

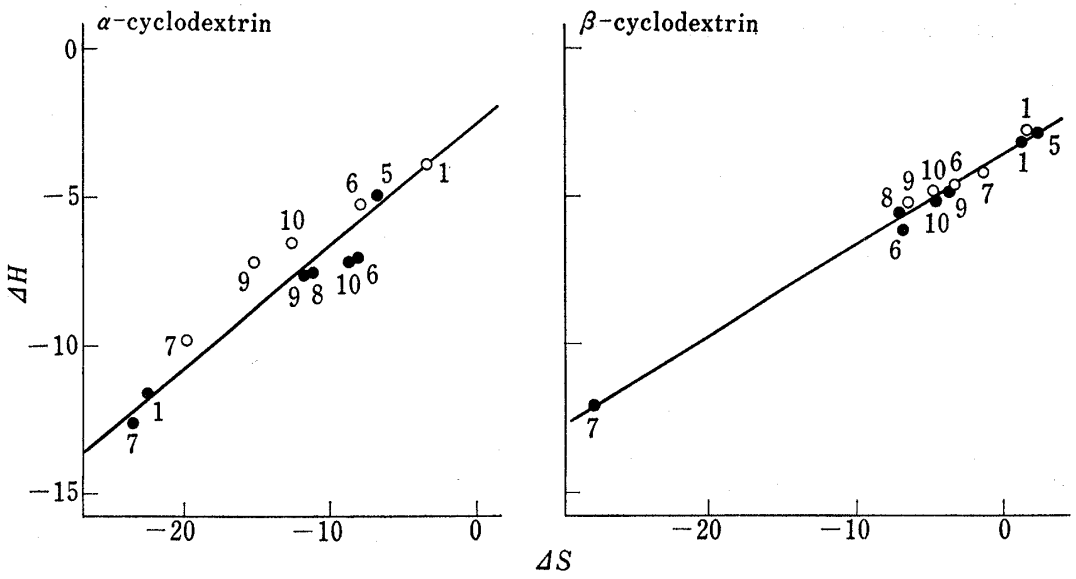

Fig. 9. Plots of ΔH *vs.* ΔS for Complex Formation of *trans*-Cinnamic Acids with Cyclodextrins

—●—: free form —○—: ionized form
numbers refer to compounds in Table I

favorable enthalpy changes. The slope of the linear plots, compensation temperatures (T_c)[11,12] is 427°K for α-cyclodextrin and 302°K for β-cyclodextrin, respectively.

Presumed Structure of Inclusion Complex

In the NMR study appreciable changes of chemical shifts which are assigned to inner hydrogen of cyclodextrin were observed. In the case of *trans*-cinnamic acid-β-cyclodextrin system, for example, protons located within the cavity of β-cyclodextrin such as H-3 and H-5[13] showed shifts to higher field, while no anisotropic shielding was detected for protons at exterior of the cavity,[13] such as H-1 and H-2. These chemical shift behaviors suggest that aromatic moiety of cinnamic acid was predominantly included within the cavity of β-cyclodextrin. It has been known that an optical active (*R*)-β-(3-bromo-2,4,6-trimethyl) pheny-β-chlro-α-methylacrylic acid shows a positive CD band.[14] Since all the induced CD bands of cinnamic acid derivatives exhibited positive sign peaks (Table II), it is reasonable to assume that cinnamic acids are fixed into (*R*)-configuration by the binding to cyclodextrin.

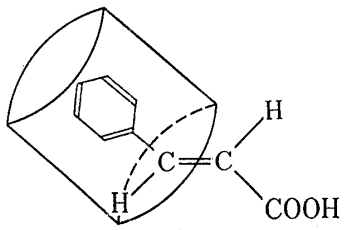

Fig. 10. Proposed Structure of Inclusion Complexes of Cyclodextrins with Cinnamic Acids

10) K. Mochida, A. Kagita, Y. Matsui, and Y. Date, *Bull. Chem. Soc. Japan*, **46**, 3703 (1973).
11) J.E. Leffler and E. Grundwald, "Rates and Equilibria of Organic Reactions," John Wiley and Sons, Inc., New York, N.Y., 1963; E.A. Lewis and L.D. Hansen, *J. Chem. Soc.*, Perkin II, 1973, 2081.
12) R. Lumry and S. Rajender, *Biopolymers*, **9**, 1125 (1970); J.B. Nagwekar and N. Muangnoicharoen, *J. Pharm. Sci.*, **62**, 1439 (1973).
13) P.V. Demarco and A.L. Thakkar, *Chem. Commun.*, **1970**, 2.
14) R. Adams and N.W. Miller, *J. Am. Chem. Soc.*, **62**, 53 (1940).

From the CD and NMR studies the possible structure of the inclusion complex is proposed as is illustrated in Fig. 10, where phenyl moiety of cinnamic acid is included within the cavity of cyclodextrin and free rotation of phenyl ring is restricted consequently.

Aknowledgement The authors are grateful to Prof. M. Hatano and Dr. O. Ito, Tohoku University, for their helpful discussions throughout this work.