[Chem. Pharm. Bull. 24(11)2825—2831(1976)]

UDC 547.586.5'26.03.04.547.458.6.04

Hydrolysis Rate of Ethyl Cinnamates in the Presence of Cyclodextrin[1]

Shigeo Tanaka, Kaneto Uekama,[2a] and Ken Ikeda

Faculty of Pharmaceutical Sciences, Nagoya City University[2]

(Received March 15, 1976)

The effect of cyclodextrin (CyD) on the alkaline hydrolysis of various ethyl cinnamates was investigated. The inclusion by α-CyD retarded the hydrolysis in all cases but that by β-CyD accelerated for esters bearing electron withdrawing substituents. Stability constant, K_c, and rate constant, k_c, of the complexes were determined kinetically on the basis of 1:1 complexation. To elucidate the reaction mechanism the hydrolysis rates of *para* substituted ethyl benzoates, ethyl phenylacetate, ethyl phenylpropionate, and ethyl phenylbutyrate in the presence of β-CyD were examined. Results showed that the effect of the inclusion primarily could be attributed to the magnification of electrostatic effect in hydrophobic cavity of CyD, *i.e.* "microsolvent effect", rather than complex stability.

Many studies have been reported on the use of cyclodextrin (CyD) inclusion complexation for pharmaceutical purpose.[3] The CyD inclusion has been known to affect the stability of drugs.[4] Generally the included drug is supposed to be protected from the attack of reactive species, but in some cases the chemical reaction is accelerated by the inclusion. The effect of CyD on reaction rate has been studied for the model elucidation of enzyme mechanism and various accelerating factors have been reported, *i.e.* catalysis by dextrin alkoxide ion, microsolvent effect and steric specificity.[5] However the effect of CyD on reaction rate is manifested variously case by case depending upon the kind of reaction and chemical structure of substrate.

The authors have recently studied on the CyD inclusion of substituted cinnamic acids and reported on the relationship between physicochemical properties of the guest molecule and complexation stability.[6] In this study the hydrolysis rate of various ethyl cinnamates in the presence of CyD was examined and the results show that the effect of the inclusion primarily could be attributed to the magnification of substituent electrostatic effect in the hydrophobic cavity of CyD, *i.e.* "microsolvent effect."

Experimental

Materials——Ethyl esters used in this study are as follow; ethyl cinnamate (**1**) bp 144° (15 mmHg), ethyl *o*-methylcinnamate (**2**) bp 148.4° (12 mmHg), ethyl *m*-methylcinnamate (**3**) bp 146° (23 mmHg), ethyl *p*-methylcinnamate (**4**) bp 147° (11.5 mmHg), ethyl *o*-nitrocinnamate (**5**) mp 41.5—42°, ethyl *m*-nitrocinnamate (**6**) mp 75°, ethyl *p*-nitrocinnamate (**7**) mp 138—139°, ethyl *m*-methoxycinnamate (**8**) bp 160° (7 mmHg), ethyl *p*-methoxycinnamate (**9**) mp 47—48°, ethyl *m*-chlorocinnamate (**10**) mp 28°, ethyl *p*-chlorocinnamate (**11**) bp 155° (11 mmHg), ethyl *p*-cyanocinnamate (**12**) mp 70.5—71.5°, ethyl *cis*-cinnamate (**13**) bp 118—120° (10 mmHg), ethyl benzoate (**14**) bp 87.2° (10 mmHg), ethyl *p*-nitrobenzoate (**15**) mp 56.5—57°, ethyl *p*-chlorobenzoate (**16**) bp 110—111° (11 mmHg), ethyl *p*-methylbenzoate (**17**) bp 97° (6 mmHg), ethyl phenylacetate (**18**) bp 120—125° (17 mmHg), ethyl 3-phenylpropionate (**19**) bp 123—124° (20 mmHg), ethyl phenylbutyrate

1) A part of this work was presented at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, April 1975.

2) Location: *3-1, Tanabe-dori, Mizuho-ku, Nagoya*; *a*) Present address: *Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto.*

3) J.L. Lach and W.A. Pauli, *J. Pharm. Sci.*, **55**, 32 (1966); Y. Hamada, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **23**, 1205 (1975); M. Kurozumi, N. Nambu, and T. Nagai, *ibid.*, **23**, 3062 (1975).

4) T.-F. Chin, P.-H. Chung, and J.L. Lach, *J. Pharm. Sci.*, **57**, 44 (1968); K. Koizumi, J. Tatsumi, M. Ohae, H. Kumagai, and T. Hayata, *Yakugaku Zasshi*, **89**, 1594 (1969).

5) D.W. Griffiths and M.L. Bender, *Advance in Catalysis*, **23**, 209 (1973).

6) K. Uekama, M. Otagiri, Y. Kanie, S. Tanaka, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **23**, 1421 (1975).

(**20**) bp 129° (9 mmHg). Compounds **1**, **14**, and **18** were commercially obtained and other compounds were synthesized and purified by distillation *in vacuo* or recrystalization by EtOH. Compound **13** was obtained by photoisomerization from compound **1** according to the literature[7] and distilled *in vacuo* three times. α- and β-CyD were prepared as previously.[6] All other materials and solvents were of analytical grade. Acetonitrile was redistilled immediately before use.

Analytical Procedure——The separation of remaining ester from the hydrolyzate was carried out by ether extraction of the reaction solution. Most of esters extracted were determined by gas chromatography using JEOL JGC-1100. The determination of other esters such as **5**, **6**, **7**, and **12** was carried out by spectrophotometry. The gas chromatographic specifications are as follow: sample volume, 5 μl; column, 5% silicone GE SE-30 on Diasolid M (60—80 mesh) in glass column (3 mm × 1 m); carrier gas, N_2 (115 ml/min); injection temp., 330°; column temp., from 100° to 160° according to boiling points of substance; FID detector temp., 225°; internal standard, dialkyl phthalates.

Kinetic Procedure——The hydrolyses were carried out mainly in pH 10.5 phosphate buffer (0.1 M, ionic strength=0.3). Ester dissolved in acetonitrile was added to buffer solution containing various amount of CyD. The starting concentrations of ester, CyD and acetonitrile were 2 to 5×10^{-4}, 2 to 10×10^{-3}, and 0.767M (4% (v/v)), respectively.

Hydrolysis rate of the included ester, k_c, and stability constant of inclusion complex, K_c, were determined by the following equation[5] based on the scheme shown. The concentration of CyD, (CyD), can be supposed to be constant as it is exceedingly larger than the ester concentration.

$$\begin{array}{ccc} & & K_c \\ \text{ester} + \text{CyD} & \rightleftharpoons & \text{ester-CyD} \\ k_o \downarrow & & \downarrow k_c \\ \text{product} & & \text{product} \end{array}$$

$$\frac{(\text{CyD})}{k_o-k_{obs}} = \frac{1}{k_o-k_c}(\text{CyD}) + \frac{1}{K_c(k_o-k_c)} \qquad (1)$$

In Eq. 1 k_o and k_{obs} are apparent first-order rate constants in simple aqueous buffer solution and that in the presence of CyD, respectively. Values k_c and K_c were estimated by the least-squares analysis of the linear relationship between (CyD)/(k_o–k_{obs}) and (CyD). On the plotting to determine k_c and K_c, the added concentration of CyD was used for (CyD). Value K_c determined is then an apparent constant for the complexation. The effect of acetonitrile on above scheme will be discussed quantitatively in Appendix.

Results and Discussion

The hydrolysis of ethyl cinnamates proceeds in pseudo first-order and the effect of CyD on the observed rate constant, k_{obs}, of ethyl cinnamate is shown in Fig. 1, as an example.

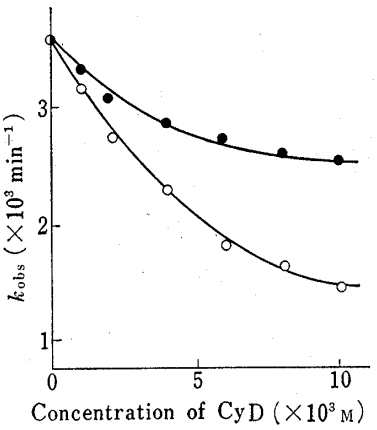

Fig. 1. Observed Rate Constants fo the Hydrolysis of Ethyl Cinnamate with Varying Concentration of CyD at pH 10.50 and 40°

○: α-CyD-ethyl cinnamate system
●: β-CyD-ethyl cinnamate system

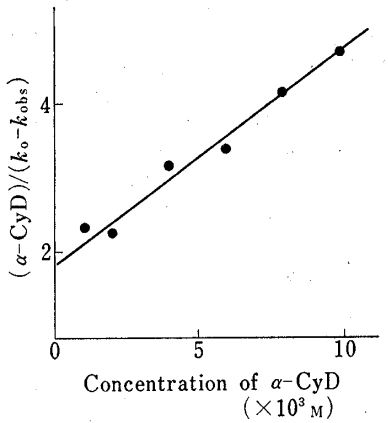

Fig. 2. Determinations of K_c and k_c from Kinetic Data (Fig. 1) of Ethyl Cinnamate-α-CyD Complex According to Eq. 1

7) H. Sobbe and F.K. Steinberger, *Chem. Ber.*, **55**, 2225 (1922).

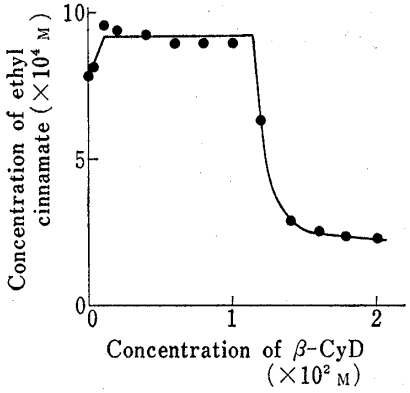

Fig. 3. Phase-solubility Diagram of Ethyl Cinnamate-β-CyD System in Water at 25°

Solubility studies were employed according to the literature (ref. 8). The molar ratio was estimated to be 1:1 from the analysis of B_s type diagram and isolated complex.

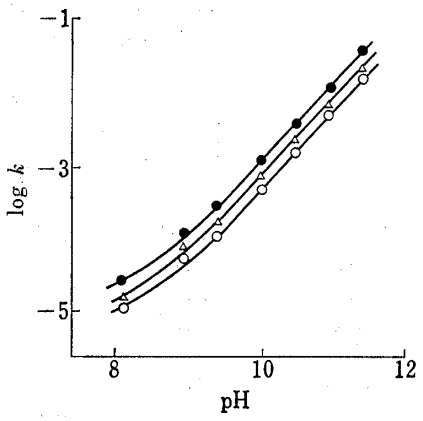

Fig. 4. pH Profile for Hydrolysis Rate of Ethyl Cinnamate in the Absence and Presence of CyD at 40°

●: in the absence of CyD
○: in the presence of α-CyD (1×10^{-2}M)
△: in the presence of β-CyD(1×10^{-2}M)

In this example the presence of CyD retarded the reaction but in some esters bearing electron withdrawing substituents accerelative effect was observed. As is shown in Fig. 2 the plots from the data on α-CyD in Fig. 1 are linear according to Eq. 1, which support the 1:1 complexation scheme. The 1:1 molar ratio can be supported from the analysis of isolated complex and solubility studies[8]; an example is shown in Fig. 3. As is seen in Fig. 4 pH-profile of log k_{obs} is linear even at higher pH, which is different from the results on phenyl esters by Bender, *et al.*[9] and this indicates that alkoxide ion catalysis is not operative. The presence of alkoxide ion catalysis may also be excluded because presumable tetrahedral intermediate following scheme may be apt to revert to native ester, even if it was formed. In other words, the formation of EtO^- ion may be much less admissible comparing to alkoxide ion formation, which could be presumed from the lower protolytic activity of EtOH compared to that of CyD.

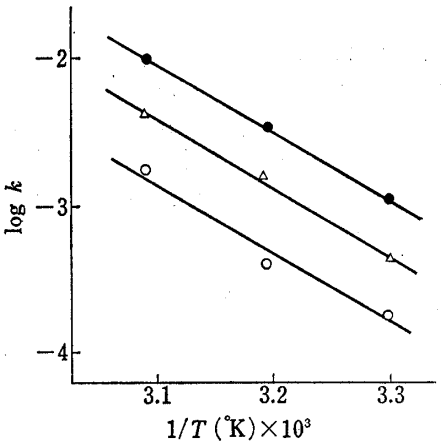

Fig. 5. Arrhenius Plots for k_o and k_c

●: k_o, ($E_a^{\ddagger}$=20.8 kcal/mole)
○: k_c for α-CyD complex, ($E_a^{\ddagger}$=22.6 kcal/mole)
△: k_c for β-CyD complex, ($E_a^{\ddagger}$=21.7 kcal/mole)

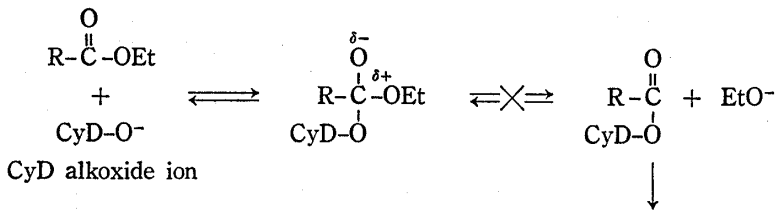

Figure 5 shows Arrhenius plots for k_o and k_c, where the activation energy change by the inclusion is not significant, which may also support the absence of the specific mechanism in the complexed ester.

Table I summarizes the results on k_o, k_c, the ratio k_c/k_o, and K_c. The inclusion by α-CyD retarded the hydrolysis in all cases but that by β-CyD accerelated for NO_2 and CN substituted

8) T. Higuchi and K.A. Connors, *Advan. Anal. Chem. Instr.*, **4**, 117 (1965).
9) R.L. VanEtten, J.F. Sebastian, G.A. Clowes, and M.L. Bender, *J. Am. Chem. Soc.*, **89**, 3242 (1967).

Table I. Hydrolysis Rates and Stability Constants of Ethyl Substituted Cinnamate-CyD System[a)]

Substituent		$k_o \times 10^3$ (min^{-1})	α-CyD system			β-CyD system		
			$k_c \times 10^3$ (min^{-1})	k_c/k_o	K_c (M^{-1})	$k_c \times 10^3$ (min^{-1})	k_c/k_o	K_c (M^{-1})
trans	H	3.51	0.33	0.09	189	1.59	0.54	118
	o-CH_3	2.77	—	—	—	—	—	—
	m-CH_3	2.88	0	0	182	1.23	0.43	236
	p-CH_3	2.28	0	0	273	1.03	0.45	104
	o-NO_2	11.7	—	—	—	—	—	—
	m-NO_2	7.55	3.59	0.48	150	11.1	1.47	74.4
	p-NO_2	8.86	1.97	0.22	84.4	17.8	2.01	168
	m-OCH_3	3.14	0	0	148	1.94	0.62	222
	p-OCH_3	1.61	0	0	200	1.05	0.65	173
	m-Cl	4.58	1.38	0.30	125	—	—	—
	p-Cl	3.87	0	0	100	5.20	1.34	143
	p-CN	7.94	0	0	128	11.7	1.47	249
cis	H	1.59	—	—	—	—	—	—

a) obtained at pH 10.50 and 40°

derivatives. For bulky substances such as *cis*-cinnamate and *ortho* substituted derivatives k_c and K_c could not be determined because of the insignificant change of reaction rate. This may be ascribed to the steric hindrance by the bulkiness of the guest molecules. Figure 6 is Hammett plots for log k_o and log k_c where ρ value for k_c (=1.23) is significantly larger than that for k_o (=0.642). These results indicate that in the included ethyl cinnamate molecule

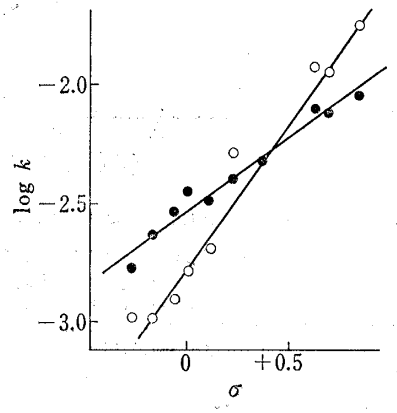

Fig. 6. Hammett Plots for log k_o and log k_c of Ethyl Cinnamates

●: k_o
○: k_c for β-CyD complex

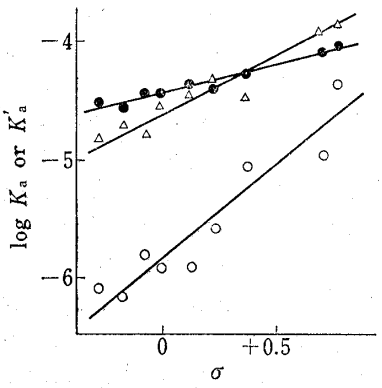

Fig. 7. Hammett σ-ρ Correlations for log K_a of Cinnamic Acids and log K_a' of their CyD Complexes

●: K_a
○: K_a' for α-CyD complex
△: K_a' for β-CyD complex

the electron mobility is facilitated comparing to that of the ester in simple aqueous solution, *i.e.* the microsolvent effect[5)] is predominant over the other effects. That benzene ring moiety which transmits the substituent polar effect is principally located in the CyD cavity is pressumed from the result of the previous study.[6)] And the ester linkage moiety is seemed to be located out of the cavity and the stereo-specific contribution of CyD may participate in minor degree on the hydrolysis mechanism. These argument may be also supported from the study on ethyl esters of serial phenylalkyl carboxylic acids which will be shown later.

That the electronic effect of the substituent is magnified in the CyD cavity is elucidated also by the re-examination on the inclusions of substituted cinnamic acid (CA) and conjugated

cinnamate ion (CA^-) which was reported in the previous study.[6] Following scheme may be appropriate for the inclusions, where K_a and K_a' are dissociation constants of free CA and included CA, CA–CyD, respectively. K_m and K_i are stability constants, (CA–CyD)/(CA)(CyD) and (CA^-–CyD)/(CA^-)(CyD), respectively.

$$\begin{array}{ccc} CA + CyD & \overset{K_m}{\rightleftharpoons} & CA\text{-}CyD \\ K_a \updownarrow & & \updownarrow K_a' \\ H^+ + CA^- + CyD & \underset{K_i}{\rightleftharpoons} & CA^-\text{-}CyD + H^+ \end{array}$$

And Eq. 2 is stoichiometrically obtained.

$$K_aK_i = K_a'K_m \qquad (2)$$

From Eq. 2 K_a' can be calculated as K_a is known[10] and K_m and K_i were determined.[6] Figure 7 shows Hammett plots for log K_a and log K_a'. Although the plots are scattered probably owing to various factors other than substituent polar effect, it is clearly observed that the included cinnamic acids have larger slope than that of free acids. The stability constants of the inclusion for CA and CA^- themselves have no Hammett type relationship, but the dissociation of the included CA is assumed to be predominated by the facilitated polar effect.

Table II. ρ-Values for Hydrolysis Rates of Ethyl Cinnamate in Various Solvents at 40°

Solvent	Dielectric constant	ρ	$r^{a)}$	$n^{b)}$
4% $CH_3CN^{c)}$		0.642	0.983	10
20% $EtOH^{d)}$	62	0.879	0.997	5
40% $EtOH^{d)}$	51	1.034	0.998	5
60% $EtOH^{d)}$	40	1.208	0.997	5
80% $EtOH^{d)}$	30	1.190	0.994	5
88% $EtOH^{e)}$	29	1.342	0.996	15

a) correlation coefficient
b) the number of compounds involved in the calculation of ρ
c) in pH 10.50 phosphate buffer
d) in 0.043N KOH
e) literary values (ref. 11)

Table III. Hydrolysis Rates and Stability Constants of Ethyl Esters of Various Acid[a)]

Compound		$k_o \times 10^3$ (min^{-1})	β-CyD system		
			$k_c \times 10^3$ (min^{-1})	k_c/k_o	K_c (M^{-1})
Ethyl benzoate	H	2.08	0.13	0.06	246
	p-NO_2	28.0	7.32	0.26	59.3
	p-Cl	3.30	0.39	0.12	223
	p-CH_3	0.843	0.086	0.10	509
ph-CH_2COOEt		12.7	1.27	0.10	124
ph-CH=CHCOOEt		3.51	1.59	0.54	118
ph-CH_2CH_2COOEt		5.98	3.17	0.53	154
ph-$CH_2CH_2CH_2$COOEt		3.48	1.87	0.46	512

a) obtained at pH 10.50 and 40°

10) G. Kortüm, W. Vogel, and K. Andrussow, "Dissociation Constant of Organic Acid in Aqueous Solution," Butterworths, London, 1961.

It has been generally known that as solvent becomes less polar ρ value becomes larger.[11] Table II is the summary of the experimentally determined and literature ρ values, where the less polar the solvent the larger the ρ value.

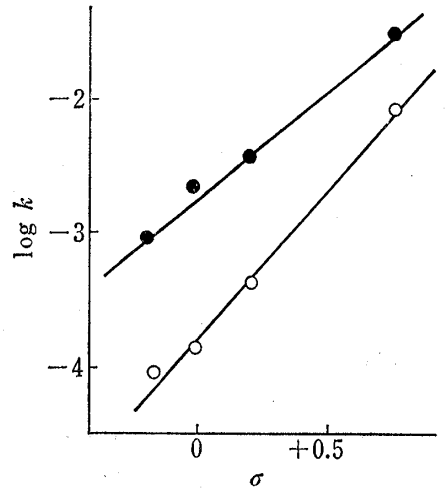

Fig. 8. Hammett σ-ρ Correlations for log k_o and log k_c of *para*-Substituted Ethyl Benzoate

●: k_o
○: k_c for β-CyD complex

With the aim of further elucidation on reaction mechanism the hydrolysis rates of *para* substituted ethyl benzoates and the ethyl esters of various phenylalkyl carboxylic acids were investigated. Table III shows k_o, k_c, k_c/k_o, and K_c values for *para* substituted ethyl benzoates, ethyl phenylacetate, ethyl 3-phenylpropionate, and ethyl 4-phenylbutyrate. Complexation by β-CyD retarded the hydrolysis for all cases. The effect of α-CyD was not quantitatively estimated because of the insignificant change of reaction rate. From k_c/k_o value shown it is noted that the longer the alkyl chain the smaller the retardation by β-CyD. By the introduction of longer alkyl group, the ester moiety may be less shielded from the hydroxide ion attack because phenyl moiety is predominantly included in CyD.[6] Value of k_c/k_o for ethyl 3-phenylpropionate is almost equivalent to that for ethyl cinnamate (see Table III). That no correlation was observed between K_c and k_c/k_o values may indicate that the distance between phenyl and ester groups is more important for the decelerating effects comparing to the complex stability. The relatively larger retardation effects observed on *para* substituted ethyl benzoates may be attributed to the proximity of ester group to the CyD cavity.

Figure 8 shows Hammett plots for log k_o and log k_c of *para* substituted ethyl benzoates. Here also ρ value for k_c (=2.09) is larger than that for k_o (=1.55). This further indicates that microsolvent effect by β-CyD is primarily responsible for the retardation of ester hydrolyses.

Appendix

The presence of acetonitrile has effect on the reaction rate as inhibitant. The competitive inclusion may be represented as;

$$\text{CyD} + \text{acetonitrile} \overset{K_{acet}}{\rightleftarrows} \text{acetonitrile-CyD}$$

$$\text{CyD} + \text{ester} \overset{K_o}{\rightleftarrows} \text{ester-CyD}$$

$$K_{acet} = \frac{(\text{acetonitrile-CyD})}{(\text{CyD})(\text{acetonitrile})} \tag{A1}$$

$$K_o = \frac{(\text{ester-CyD})}{(\text{CyD})(\text{ester})} \tag{A2}$$

$$(\text{CyD})_{total} = (\text{CyD})_f + (\text{acetonitrile-CyD}) + (\text{ester-CyD}) \tag{A3}$$

where $(\text{CyD})_{total}$ is the total concentration of CyD added and K_o is the real (not apparent as K_c) stability constant of ester inclusion. From Eq. A1, A2, and A3, (CyD) can be represented as;

$$(\text{CyD}) = \frac{(\text{CyD})_{total}}{1+K_{acet}(\text{acetonitrile})+K_o(\text{ester})}$$

$$\fallingdotseq \frac{(\text{CyD})_{total}}{1+K_{acet}(\text{acetonitrile})} \tag{A4}$$

11) H.H. Jaffé, *Chem. Rev.*, 53, 191 (1953).

where the term of K_o (ester) may be negligible because (ester) is in the order of 10^{-4} and K_o estimated is 240 at most. The term of K_{acet} (acetonitrile) is practically constant as acetonitrile added was in excess. Then the real stability constant of ester inclusion K_o is proportional to K_c which was determined as stated in experimental.

$$K_o = \frac{K_c}{1 + K_{acet}(\text{acetonitrile})} \qquad \text{(A5)}$$