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The Cleavage of Aryl Alkanoate Esters by Cyclodextrins

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A Thesis

in

The Department

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ABSTRACT

The Cleavage of Aryl Alkanoate Esters by Cyclodextrins

Xianxian Du

The influences of α- and β-cyclodextrin (α- or β-CD) on the rates of cleavage of several substituted phenyl alkanoates were studied in phosphate buffer solution (pH 11.6-11.7) at 25°C. The principal object was to look at the effect of alkanoate chain lengths on the ease of ester cleavage. The following esters were studied: p- and m-nitrophenyl alkanoates (C2, C3, C4, C5, C6), 4-carboxy-2-nitrophenyl alkanoates (C2, C3, C4, C5, C6, C7, C8), the corresponding 2-ethylhexanoate and 4-methylpentanoate, and 2-carboxy-4-nitrophenyl alkanoates (C2, C4, C6, C8).

The cleavage pathway is believed to involve a cyclodextrin complex of the ester from which the anion of the phenol is released and the acyl group is transferred to a secondary hydroxyl group of the cyclodextrin. The resultant monoacyl cyclodextrin undergoes subsequent cleavage very slowly.

The p- and m-nitrophenyl alkanoates (C2 - C6) were cleaved by α- or β-CD following 1:1 complexation. Apparently the phenyl portion of short chain ester is included into the CD cavity, but the hydrocarbon chain portion of some of the longer chain esters is bound into the CD cavity. The reaction of m-nitrophenyl alkanoates are faster than corresponding p-nitrophenyl alkanoates under the same conditions.

The cleavage of 4-carboxy-2-nitrophenyl acetate and 4-methylpentanoate by α- or β-CD followed the 1:1 binding pathway, also. The 4-carboxy-2-nitrophenyl butanoate, pentanoate, hexanoate, heptanoate, and octanoate in α-CD show processes involving two α-CD molecules complexed with one ester molecule to form a stronger 2:1 binding complex. It appears that the hydrocarbon chain portion is inserted into the cavity of the first α-CD molecule, then the aryl moiety is included in the second α-CD molecule.

In contrast, 4-carboxy-2-nitrophenyl alkanoates (including the 2-ethylhexanoate) are cleaved with a second-order process in the β-CD. In this pathway, the hydrocarbon chain portion is inserted in the first CD molecule and cleavage results from attack of a second CD molecule.

The cleavage of the 2-carboxy-4-nitrophenyl acetate follows the 1:1 binding pathway while the butanoate, hexanoate, and octanoate are cleaved by a second-order process with both α- and β-CD.

In general, the stability of complex which has the hydrocarbon chain moiety inserted into the CD cavity increases with the chain length of the aryl alkanoate.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Professor O.S. Tee, for an introduction into the field of physical organic chemistry and for his guidance throughout the course of this work. I will always be indebted to him for his assistance and valuable advice during my M.Sc. program.

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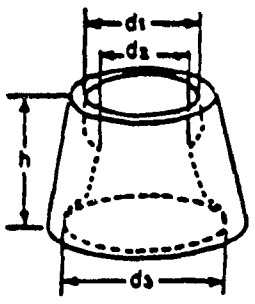
CHAPTER 1 INTRODUCTION

Cyclodextrins form inclusion complexes with many species, most notably with organic substrates.[1,2] As a result, they can have significant effects on the rates and products of organic reactions.[2] Of particular interest, they can function as catalysts in various kinds of reactions, and much attention has been paid to their ability to function as models of enzymes. In the case of ester (and amide) cleavage there is usually reaction between one of the hydroxy groups of the cyclodextrin and the ester (amide) included in its cavity.[2,3]

1.1. STRUCTURE OF α- AND β- CYCLODEXTRIN

Cyclodextrins(CD) are cyclic oligosaccharides consisting of 6, 7, or more glucose units, linked α(1-4).[1] For the case of 6 and 7 units the resulting, doughnut-shaped molecules are called α- and β-cyclodextrin, respectively (Fig. 1).



Cyclo-dextrin	d_1 (Å)	d_2 (Å)	d_3 (Å)	h(Å)
α	5.6	4.2	8.8	7.8
β	6.8	5.6	10.8	7.8

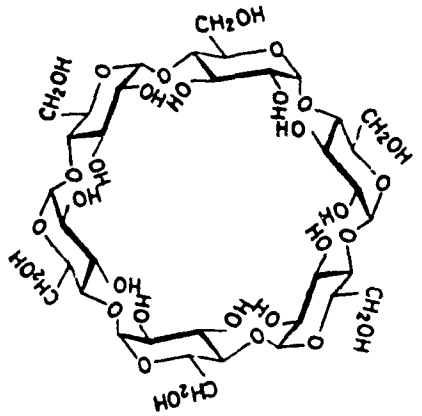


Fig. 1 Shape and structure of cyclodextrin cavity.

CDs have the form of short truncated cones about 8 A long, with the wide rim occupied by secondary 2- and 3-hydroxyl groups and the narrow one by primary 6-hydroxyls. The molecules are hydrophilic at the periphery whereas the central cavity, of diameter 5 to 8 A, is lined with ether-like oxygens and C-H hydrogens and therefore it is hydrophobic in character.[1,2] Due to their annular shape, cyclodextrins can function as hosts and they form inclusion compounds with a large variety of guest molecules.[1,2]

1.2 REVIEW OF CYCLODEXTRIN CHEMISTRY[1,2]

The initial discovery of cyclodextrins is attributed to Villiers, who isolated them as degradation products of starch in 1891. In 1904, Schardinger demonstrated that these compounds could be obtained by the action of Bacillus macerans amylase upon starch, and this is now their principal source. The structure of CDs was determined in the 1940s and it was found that they can form inclusion compounds by Freudenberg in 1948. The formation of such complexes was studied by Cramer, who also discovered that CDs have a catalytic action in some reactions. The increasing number of publications and patents dealing with cyclodextrin inclusion compounds shows the general interest in their physical and chemical properties.

Following the initial discoveries of catalytic ability by Cramer, CDs were shown to catalyze the hydrolysis of esters by Bender[3] and Chin[4] in the end of 1960's. From such studies it became apparent that specificity and selectivity are some of the important characteristics

of the cyclodextrin catalysis. Recently, Tee and Bennett have shown that α-CD catalyzes the bromination of phenols.[5] The formation of substrate.CD complexes can also control the photobehaviour of molecules included in the CD cavity.[6,7,8] The possibility of controlling the course of Diels-Alder additions by the use of cyclodextrins is currently attracting considerable interest. Such control probably depends on an externally driven compression or packing of the diene and the dienophile.[9,10,11] This ability of CDs to influence reactions and reactivity is due to the fact that they can form the host-guest complexes with a lot of organic molecules.[1,12]

1.3 HOST-GUEST COMPLEXES OF CYCLODEXTRINS

The terms "host", "guest", "complex", and their binding forces have been recently defined by Cram.[5] According to his definitions, hosts are synthetic counterparts of the receptor sites of biological chemistry, and guests the counterparts of substrates, inhibitors, or cofactors. In our case, the hosts are cyclodextrins and aryl alkanoates are the guests.

Cyclodextrins are well-known for their ability to form "inclusion complexes", also known as "host-guest complexes", with a wide range of compounds.[2] In many cases such inclusion complexes can be precipitated from aqueous solutions of the CD when excess of a guest is added. The α- and β-CD complexes thus formed can have two modes of packing of cyclodextrin molecules in the crystal lattice.[1]

The two packing modes are described as cage or channel structures,

according to the overall appearance of the cavity. In channel-type complexes, CD molecules are stacked on top of each other like coins in a roll, with the new linearly-aligned cavities producing channels in which the guest molecules are embedded. In the cage-type crystals, the cavity of one CD molecule is blocked off on both sides by adjacent CD molecules, thereby leading to isolated cavities. Two types of cage structures are often encountered, namely the brick type and the herring bone type. For β-cyclodextrin, a clear separation between channel- and cage-forming guests is not possible and so one cannot predict the nature of packing for a particular guest.[15]

Usually, the "empty" cyclodextrins are filled by water molecules. With small molecular (non-polar) guests included, α-CD units pack in a herring bone motif such that cavity of one molecule is blocked on both sides by adjacent, symmetry related α-CD molecules. If aromatic guests are considered, the situation will be changed. Apparently, the aromatic moiety induces an elliptical deformation of the α-CD molecule which then, packs no longer in a herring bone motif, but like bricks in a wall.[13,14] If ionic or extended molecular guests are included which are too long to fill only one isolated CD cage the CD macrocycles stack to form infinite columns with a central, channel-like cavity.[15] The smallest fatty acids like acetic, and propionic acids form cage-like complexes with α-CD molecules arranged in herring bone pattern whereas the longer homologs form channel-like complexs with α-CD.[15]

In solution the formation of host-guest complexes by cyclodextrins with a variety of compounds is well documented.[1,2,15] These complexes usually have a 1:1 stoichiometry[1] but, more recently, a number of 2:1

CD-substrate complexes have been characterized.[47] The formation of a 1:1 complex (CD.S) between the CD and a guest (substrate, S) may be characterized by a dissociation constant, K_d:

$$CD + S \underset{K_d}{\rightleftharpoons} CD.S \qquad (1)$$

$$K_d = \frac{[CD].[S]}{[S.CD]} \qquad (2)$$

The stability of the complex CD.S varies markedly either with the size of the CD cavity or with the structure of the substrate.

In our case, the guest molecules have two binding sites (aryl or alkyl portion) and a CD molecule has two modes of binding. However, there can be two isomeric 1:1 complexes (R-Ar.CD and Ar-R.CD) since only one end of the CDs can be entered.[29]

Although 1:1 stoichiometry is often assumed in evaluating stability constants for cyclodextrin complex formation, recent observations have revealed the presence of 1:2 complexes (S.2CD) in some systems.[40,47] If 1:2 complexes are present in the case of aryl alkanoates as guests, there can be two kinds of binding pathway:

$$R\text{-}Ar + CD \underset{K_d}{\rightleftharpoons} R\text{-}Ar.CD \overset{CD}{\underset{K_2}{\rightleftharpoons}} CD.R\text{-}Ar.CD \qquad (3)$$

$$CD + R\text{-}Ar \underset{K_d'}{\rightleftharpoons} CD.R\text{-}Ar \overset{CD}{\underset{K_2'}{\rightleftharpoons}} CD.R\text{-}Ar.CD \qquad (4)$$

The methods available for determining the constants K_d and K_2 (or

K_d' and K_s') are mainly based on the properties of solutions of the substrate and CD. The concentration of one component is gradually varied and the concentration of one of the reactants or product is followed (directly or indirectly) by a suitable analytical method. Usually the potentiometric, spectrophotometric, polarographic, conductimetric, extraction, nmr, and kinetics methods can be used to measure K_d.

The optical properties of solutions containing complexes usually differ from those of the constituent molecules. Spectrophotometric methods, such as uv/vis and ir, are based on the measurement of light absorption according to the Beer-Lambert law, assuming this is obeyed by the species in equilibrium.

With the kinetic method, the variation of a reaction rate with [CD] may be used to determine the dissociation constant K_d. As will be discussed later, this method was used in the present investigation of the cleavage of ester guest molecules.

1.4. THE CLEAVAGE OF ESTERS BY CYCLODEXTRINS

The effect of cyclodextrins on the rates of the cleavage of esters show many specificities, since the reactions involve the formation of a complex of the ester with the cyclodextrin.[2,3] Most of the CD-influenced reactions so far examined proceed through nucleophilic attack by the anion of a secondary hydroxyl group of the CD on the carbonyl group of the ester included in the cavity of CD, resulting in

the formation of a covalent intermediate, an acyl cyclodextrin.

In the CD-influenced hydrolyses of phenyl benzoate esters, for example, the formation of acyl-CD as intermediates was shown by spectroscopic analysis of reaction solutions.[16] The secondary hydroxyl groups on CDs act as weak acids and they dissociate into corresponding alkoxide ion in a strongly basic solution. The pK_a values for α- and β-CD are 12.33 and 12.20, respectively, at 25°C, as determined by means of pH potentiometry.[17] Moreover, the secondary C-2 and C-3 OH groups, rather than the primary C-6 OH groups, are involved in the ionization process.[17] A ^{1}H-NMR study has suggested that the C-3 OH hydrogens are hydrogen bonded to the C-2 OH oxygen and, therefore, the C-2 OH is more liable to dissociate than the C-3 OH.[18] Bergeron and Burton have presented ^{1}H- and ^{13}C-NMR data that cast some doubt upon the premise that the C-3 hydroxyls are inherently unreactive and therefore uninvolved in CD-induced ester cleavage.[19]

Most ester cleavages by CDs proceed by nucleophilic attack of a CD on the carbonyl group of the ester bound in the CD cavity, and an acyl CD is formed.[2,3,20] However, in a few cases it has been shown there is general base catalysis of ester hydrolysis. In these cases a CD anion assists the nucleophilic attack of a water molecule on the carbonyl of esters, by partially abstracting a proton from the water molecule.[21-23] Such behaviour has been observed for the hydrolysis of some special esters which have a good leaving groups, such as 2,2,2-trifluoroethyl 4-nitrobenzoate.[21,23,24].

Geometric selectivities are very important for the ease of ester cleavage in the presence of CDs. For example, deacylation of m-tert-

butylphenyl acetate within its β-CD complex is accelerated by a factor of 250, due to the proximity of the ester group to the lip of the CD in the complex.[3] When β-CD was modified by building in an intrusive floor, so that the cavity was well-defined and shallower, cleavage of m-tert-butylphenyl acetate was improved by another order of magnitude since the substrate binding geometry was closer to that of the transition state.[48]

More spectacular accelerations have been obtained by modifying the substrates. Breslow's group[48-50] synthesized various ferrocene-acrylate esters which exhibit accelerations up to 6 million! Also, they found enantioselectivities up to 62. In these cases the ferrocene moiety sits very precisely in the CD cavity, holding the ester function close to a 2- or 3-hydroxyl group.

1.5 OBJECTIVE

The earlier work of Bender and coworkers[3,16,20] focussed attention on the importance of the position of substituents on the phenyl ring of aryl acetates in ester cleavage by cyclodextrins in aqueous base. Because of the formation of inclusion complexes of the esters with CDs the position of the substituent, rather than its nature, greatly influences the ease of ester cleavage.[3,16] However, our interest was to study the behaviour of longer chain aryl esters to probe the importance of alkyl group inclusion in primary mode of binding of the esters and in the transiton state for ester cleavage.

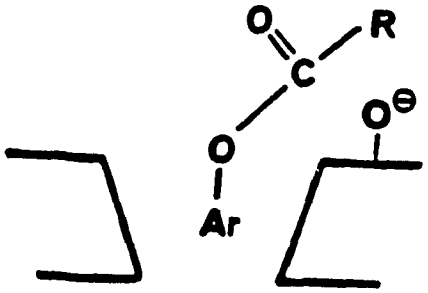
With the series of esters R-CO_2Ar, where R = Me, Et, Bu, etc., and Ar = m-and p-nitrophenyl, it was hoped that the effects of CDs on the kinetic parameters of the cleavage would reveal any changes in the mode of binding. It was imagined that the mode of insertion of the alkanoates into the CDs, which in the case of short chain alkanoates has the m- and p-nitrophenyl moiety is included in the CDs cavity, might change to have the alkyl chain included in the case of the longer chain esters.

Studies were also carried out with aryl alkanoate esters in which Ar = 4-carboxy-2-nitrophenyl and 2-carboxy-4-nitrophenyl. With these esters, where the aryl groups are more hydrophilic than with nitrophenyl esters, it was hoped that the tendency to bind the alkyl chains would be greater and so a clear dependence on the chain length would observed. The results were unexpected and gratifying; we found unusual processes which involve two molecules of α- or β-cyclodextrin.

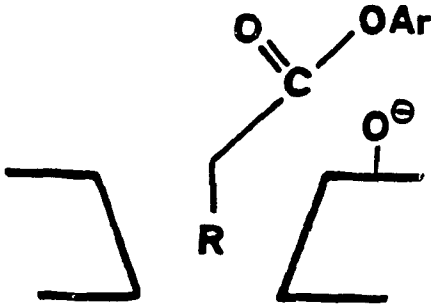
Moreover, in the case of the 4-carboxy-2-nitrophenyl esters, different behaviour was observed with α- and β-CD. Some of these results were recently published.[25]

CHAPTER 2. CLEAVAGE OF p- AND m-NITROPHENYL ALKANOATES

The cleavages of p- and m-nitrophenyl alkanoates (C2 to C6) were carried out in the presence of α- or β-CD in a phosphate buffer of pH 11.6-11.7, at 25°C. These substrates were chosen to make use of the known meta vs. para selectivity[2] as a probe of the mode of inclusion. If the ester cleavage involves a complex in which the aryl group is included in the cavity of the CD (structure A, below) then the kinetic parameters should reflect the difference between the m- and p-nitrophenyl esters. On the other hand, if cleavage takes place via alkyl group inclusion (structure B) the kinetic parameters for the m- and p-nitrophenyl esters should be similar but vary with the length of the alkyl chain.



A



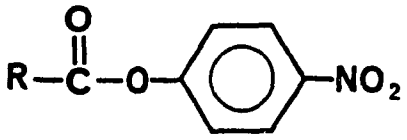
B

2.1 INTRODUCTION

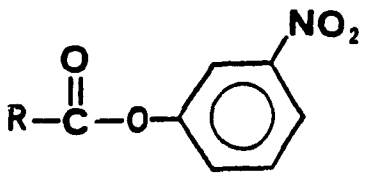
It is well-known that interaction between the hydrophobic moieties of host and guest molecules plays an important role in the formation of

complexes by cyclodextrins.[1] Like many other reactions, the cleavage of aryl esters by CDs depends on the size and shape of the substrate.[26] This reaction has been extensively investigated[3,16,20,28,29] and its mechanism is clearly established.[3] As pointed out earlier, it involves nucleophilic attack by a secondary hydroxy group of the CD on the carbonyl carbon atom of the bound substrate. The proper inclusion of the ester is necessary to allow for easy formation of the tetrahedral intermediate, rather than strong binding of the substrate.

In the case of the aryl esters studied so far it has been assumed that the aromatic portion of the molecule is included in the cavity of the CD.[2,27] We have investigated the kinetic consequences of alkyl group versus aryl group insertion in the cleavage of the p- and m-nitrophenyl alkanoates (I and II): acetate (C2), propanoate (C3), butanoate (C4), pentanoate (C5), hexanoate (C6).



I



II

$R = CH_3(CH_2)_n-$: n = 0, 1, 2, 3, 4.

Molecular models show that, as the length of the hydrocarbon chain increases, the alkyl portion may fill the CD cavity better than the nitrophenyl portion.

2.2 RESULTS

The kinetics of the cleavage of the esters I and II in aqueous basic solutions containing α- or β-CD have been studied using the stopped-flow technique.[28] Before presenting these results it is necessary to outline how the kinetic data were analyzed to obtain the desired kinetic parameters.

For a substrate S reacting in a medium (eq 5) and via a substrate-CD complex S.CD (eq 6) the expected form of the observed rate constant

$$S \xrightarrow{k_u} \text{Products} \qquad (5)$$

$$S + CD \underset{K_d}{\rightleftharpoons} S.CD \xrightarrow{k_c} \text{Products} \qquad (6)$$

$$k_{obs} = (k_u K_d + k_c[CD])/(K_d + [CD]) \qquad (7)$$

is given by eq 7, assuming that [CD] >> [S]. The constant k_u is the "uncatalyzed" rate constant, k_c is that for the S.CD complex, and K_d = [S].[CD]/[S.CD] is the dissociation constant of the complex. Equation 7 gives rise to a non-linear dependence on [CD], characteristic of saturation kinetics.[2] In other words, at high [CD], where nearly all of S will be in the form of S.CD, k_{obs} tends to level out at the value of k_c.

Analysis of the data in terms of eq 7 was carried out in three ways:
1. linear regression of $1/(k_{obs}-k_u)$ vs $1/[CD]$, a Lineweaver-Burk approach;[2]

Table 1. Constants for the cleavage of nitrophenyl alkanoates in the presence of α-cyclodextrin.[a]

Acyl	K_d, mM	k_u, s^{-1}	k_c, s^{-1}	k_c/k_u	k_2 $M^{-1}s^{-1}$	K_{TS} mM
(a) p-Nitro:						
C-2	10.1	0.0956	0.267	2.79	26.4	3.62
C-3	11.6	0.0992	0.203	2.05	17.5	5.66
C-4	5.00	0.0580	0.113	1.95	22.6	2.56
C-5	3.37	0.0450	0.0928	2.06	27.5	1.64
C-6	2.88	0.0494	0.146	2.96	50.7	0.973
C-8[b]	0.98	0.00095	0.0033	3.6	3.37	0.272
C-12[b]	0.37	0.0003	0.0032	10.6	8.65	0.035
(b) m-Nitro:						
C-2	25.0	0.0858	24.6	287	984	0.0871
C-3	6.51	0.0396	4.50	114	691	0.0571
C-4	5.37	0.0293	3.16	108	588	0.0497
C-5	4.07	0.0284	1.99	70.1	489	0.0581
C-6	3.49	0.0238	1.96	82.4	562	0.0424

a At pH 11.6 - 11.7; buffer: 0.2M Na_2HPO_4 - 0.13M NaOH; 0.1%(v/v) MeOH. Errors in K_d, k_u, and k_c are 5-10%. The constant $k_2 = k_c/K_d$ (see p 22).

[b] At pH 10.4. Taken from reference 32.

2. linear regression of $(k_{obs}-k_u)$ vs $(k_{obs}-k_u)/[CD]$, an Eadie approach;[2]

3. non-linear fitting of eq 7, keeping k_u fixed at the experimental value.[28]

All three approaches gave excellent fits and very similar values for k_c and K_d. In what follows we simply use the values obtained by method 2, since the Eadie approach is statistically superior to method 1.

Table 1 contains values of k_u, k_c and K_d obtained for the esters I and II in the presence of α-CD. The examples in Figures 2 and 3 show that at fixed pH and varying CD concentration the observed first-order rate constants (k_{obs}) vary in the curvilinear manner of eq 7. The rate constants k_u decrease with increasing hydrocarbon chain length of p- and m-nitrophenyl alkanoates, as expected.[29]

The cleavage of the p-nitrophenyl alkanoates is faster than that of the corresponding m-nitrophenyl esters in the absence of cyclodextrin. Under similar conditions and in the presence of α-CD, the rate constant (k_c) of the cleavage reaction decreases first then increases with the chain length for p-nitrophenyl alkanoates. A decrease with increasing chain length of m-nitrophenyl alkanoate is also observed. The cleavage reaction of p-nitrophenyl alkanoate is slower than corresponding m-nitrophenyl alkanoate in α-CD. The dissociation constants Kd decreases with increasing chain length for both p- and m-nitrophenyl ester with α-CD.

Table 2 contains values of k_u, k_c and K_d of I and II in basic aqueous β-CD solution from this study. The dissociation constant Kd and rate constant kc of cleavage reaction decrease with chain length for

Table 2. Constants for the cleavage of nitrophenyl alkanoates in the presence of β-cyclodextrin.[a]

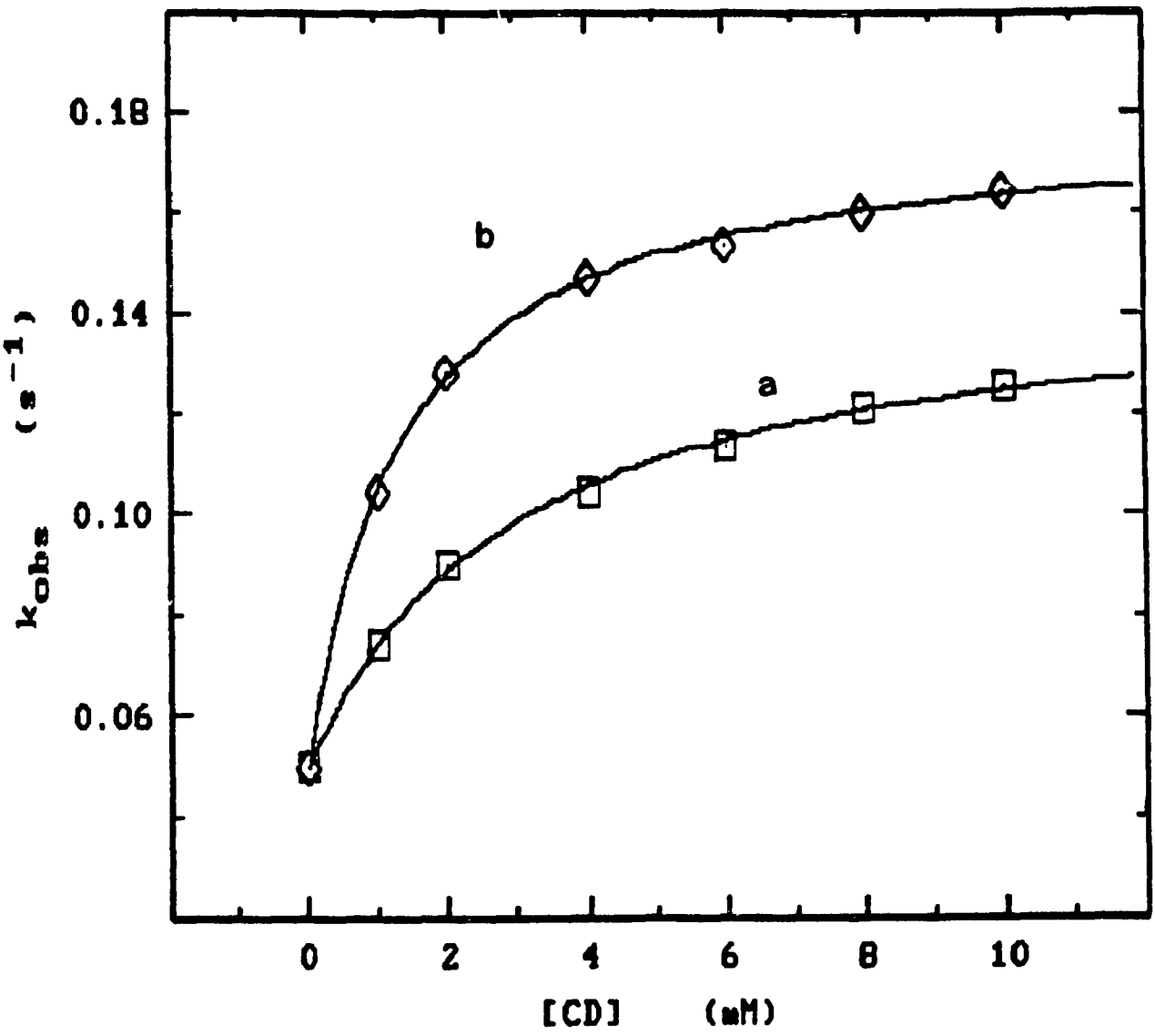
Acyl	K_d, mM	k_u, s^{-1}	k_c, s^{-1}	k_c/k_u	k_2 M^{-1} s^{-1}	K_{TS} mM
(a) p-Nitro:						
C-2	7.83	0.0956	0.775	8.11	99.0	0.966
C-3	5.18	0.0998	0.468	4.69	90.3	1.10
C-4	2.70	0.0575	0.268	4.66	99.3	0.579
C-5	1.95	0.0466	0.181	3.88	92.8	0.503
C-6	1.34	0.0494	0.179	3.62	134	0.370
C-8[b]	1.9	0.00095	0.0093	9.8	4.9	0.194
C-12[b]	0.75	0.0003	0.0202	67	26.9	0.011
(b) m-Nitro:						
C-2	12.3	0.0858	5.25	61.2	427	0.201
C-3	5.23	0.0401	1.65	41.2	316	0.127
C-4	3.68	0.0274	0.914	33.4	248	0.110
C-5	2.41	0.0284	0.662	23.3	275	0.103
C-6	1.81	0.0232	0.606	26.1	335	0.0694

[a] At pH 11.6 - 11.7; buffer: 0.2M Na_2HPO_4 - 0.13M NaOH; 0.1%(v/v) MeOH. Errors in K_d, k_u, and k_c are 5-10%. The constant $k_2 = k_c/K_d$ (see p 22).

[b] At pH 10.4. Taken from reference 32.

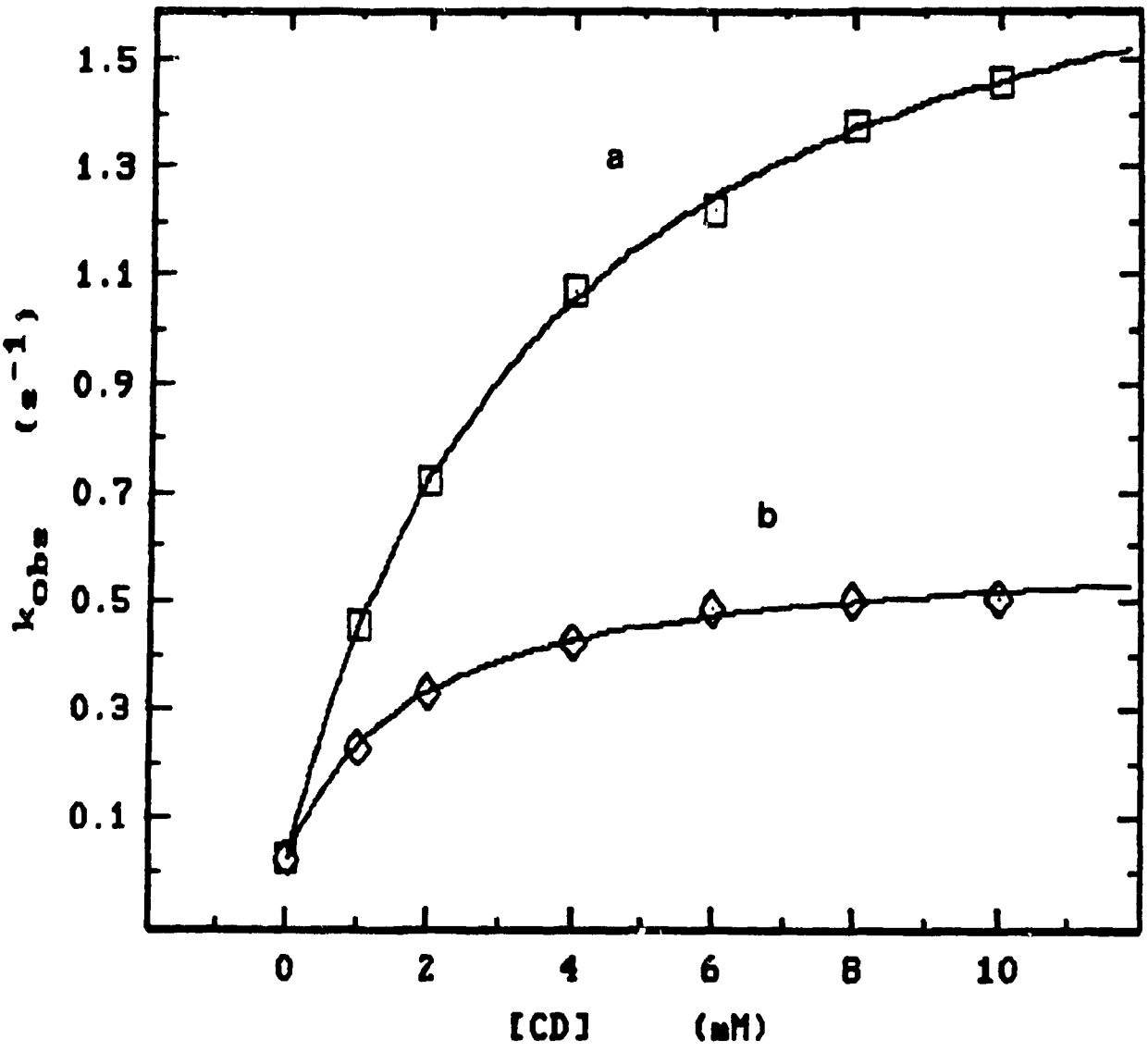
p- and m-nitrophenyl alkanoate in β-CD. Also, the constants (Kd) of p-nitrophenyl alkanoates are smaller than of the corresponding m-nitrophenyl esters in β-CD. The cleavage reaction of p-nitropheny] alkanoate is slower than corresponding m-nitrophenyl alkanoate in the presence of β-CD.

The cleavage reaction of p-nitrophenyl alkanoate is faster in β-CD than in α-CD (Fig. 2). However this same reaction is faster in α-CD than in β-CD for m-nitrophenyl alkanoate (Fig. 3).



(a). α-CD ; (b) β-CD.

Figure 2. Rates of cleavage of p-nitrophenyl hexanoate as a function of cyclodextrin concentration.



(a) α-CD ; (b) β-CD.

Figure 3. Rates of cleavage of m-nitrophenyl hexanoate as a function of cyclodextrin concentration.

2.3 DISCUSSION

The results show that cleavage of nitrophenyl alkanoates is enhanced by CD. However, the effects of the CDs show differences since ester cleavage involves complexation of the substrate with CDs prior to the chemical transformation. From Table 1 and Table 2, the values of k_c exhibit a meta-para specificity in the cleavage of phenyl esters. Here the meta-para specificity means that the cleavage of the m-nitrophenyl ester, such as m-nitrophenyl acetate, is catalyzed by α- and β-CD to a much greater extent than that of the corresponding p-compound. This specificity can be attributed to the difference in the distance between the ester carbonyl and the secondary alkoxide ion of CDs because the first step in the mechanism for cleavage of a CD-complexed aryl ester is nucleophilic attack by a secondary hydroxyl group of CD which ultimately results in a monoacyl CD (Figure 4). Under the reaction conditions, the monoacyl CD usually undergoes subsequent deacylation slowly. (N.B., In Figure 4, the dissociation constant (Kd) is described by k_1 and k_{-1}, and the cleavage rate constant k_c is shown by k_2).

During the formation of the CD-ester complex, it seems likely that if the aryl portion is larger than hydrocarbon portion, the phenyl moiety will be included in the cavity of CD. On the other hand, it is possible that the hydrocarbon chain could be inserted into the cavity of CD when the phenyl portion is much smaller than the alkyl group. One would expect the functional groups and their position to influence the rate of the cleavage reaction if the phenyl portion is included in

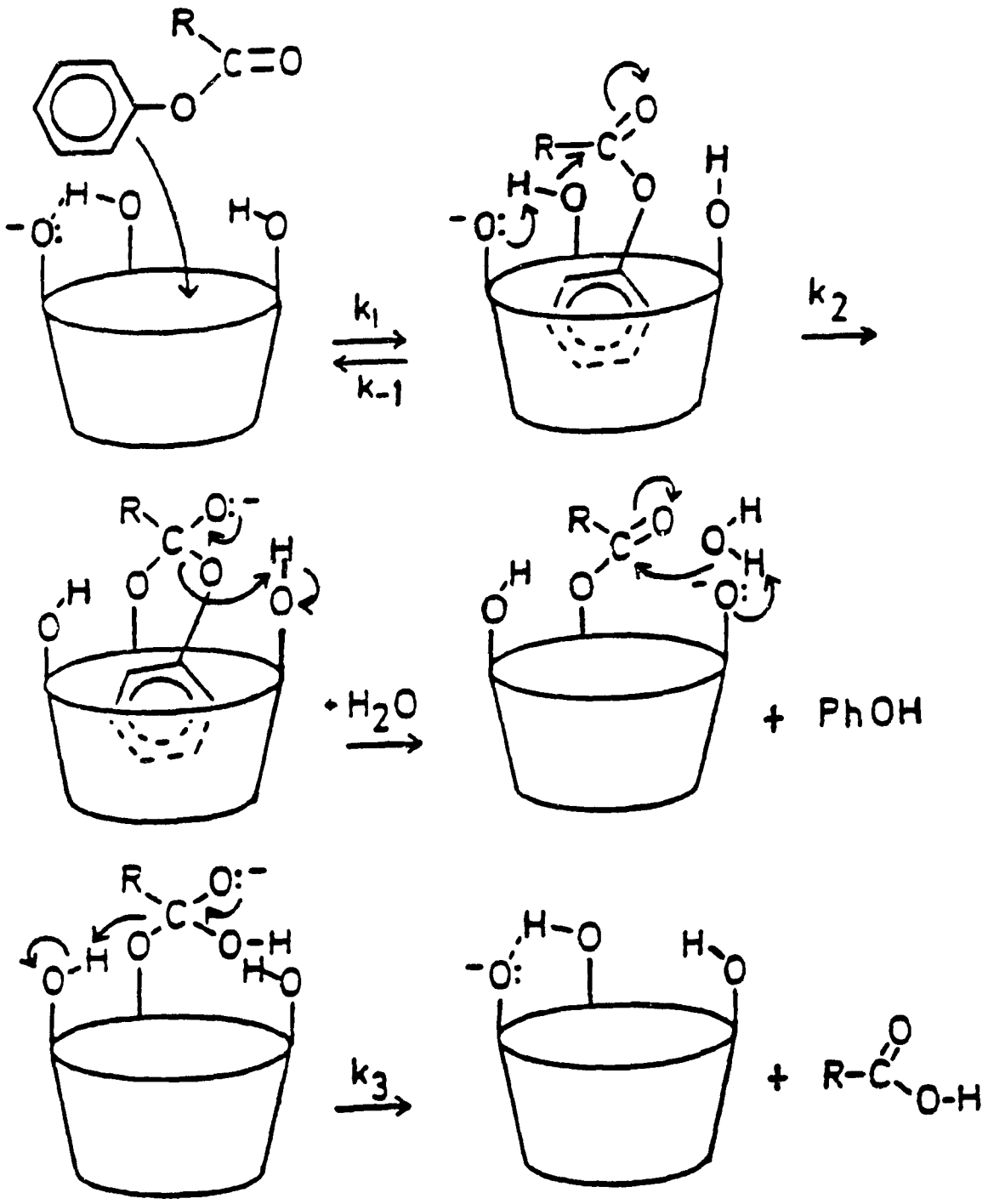


Figure 4. Schematic representation of the hydrolysis of phenyl alkanoate catalyzed by cyclodextrin.

the CD cavity. Conversely, one would expect the length of the alkyl chain to influence this reaction if it is bound in the cavity of CD. In the alkaline hydrolysis of carboxylic esters lengthening of the alkyl chain usually results in the reduction of the rates.[30]

The effect of CD on the cleavage of nitrophenyl acetate, propanoate, butanoate, pentanoate, and hexanoate was studied to investigate the conformation of the guest molecule in α-CD or β-CD. The following is a reaction scheme which describes the cleavage of esters by CDs.

$$\text{Products} \xleftarrow{k_u} S + CD \underset{K_d}{\rightleftharpoons} S.CD \xrightarrow{k_c} \text{Products} \qquad (8)$$

Overall:

$$S + CD \xrightarrow{k_2} \text{Products} \qquad (9)$$

where

$$k_2 = k_c/K_d \qquad (10)$$

Table 1 contains values of the apparent second order rate constants (k_2) for the cleavage by α-CD, calculated from equation 10. The values of k_2 first decreases (except p-nitrophenyl acetate) and then increases with increasing hydrocarbon chain length. It seems that the structure of the complex (S.CD) changes with chain length.

The catalytic ratios (k_c/k_u) are also shown in Table 1. They first decrease and then increase with increasing chain length also. It is possible that the orientation of the substrate in the α-CD cavity in the transition state changes with increasing the chain length. In the case of I this change may occur at p-nitrophenyl butanoate and in the

case of II, at m-nitrophenyl pentanoate.

From k_2 and the ratios k_c/k_u (Table 1) it seems that the binding pathway changes, and that this change starts after C3 for I and II. It strongly suggests that the alkyl portion of some of the longer esters is inserted into the α-CD cavity.

From Table 1, the rate constants k_2 for m-nitrophenyl alkanoates are much larger than for the p-nitrophenyl esters, which indicates that in the transition state for cleavage insertion of the aryl portion of m-nitrophenyl ester into the cavity is more important than that of the alkyl portion. This is an example of the "meta" effect,[3,31] first observed with phenyl acetates.[3] For these esters a meta group holds the ester in a better arrangement for reaction than does the corresponding para group (see Figure 5), and so m-X-phenyl esters are cleaved more readily.

The following observations can be made from the data for β-CD in Table 2. (a) The ratios k_c/k_u are rather modest for I, but larger for the II, as is usually observed.[3,26] They decrease with alkyl chain length for I; they first decrease then increase with chain length for II. (b) The second order rate constants k_2 for the attack of CD on the esters decrease and then increase with chain length for the m-nitrophenyl alkanoate. For the p-nitrophenyl esters there is not much change in k_2 when the chain length is shorter than C6. The k_2 values for II are much larger, due the "meta" effect, discussed above. Overall, the results indicate that it is possible that the transition state structure changes for chains longer than the pentanoate for I and the butanoate for II.

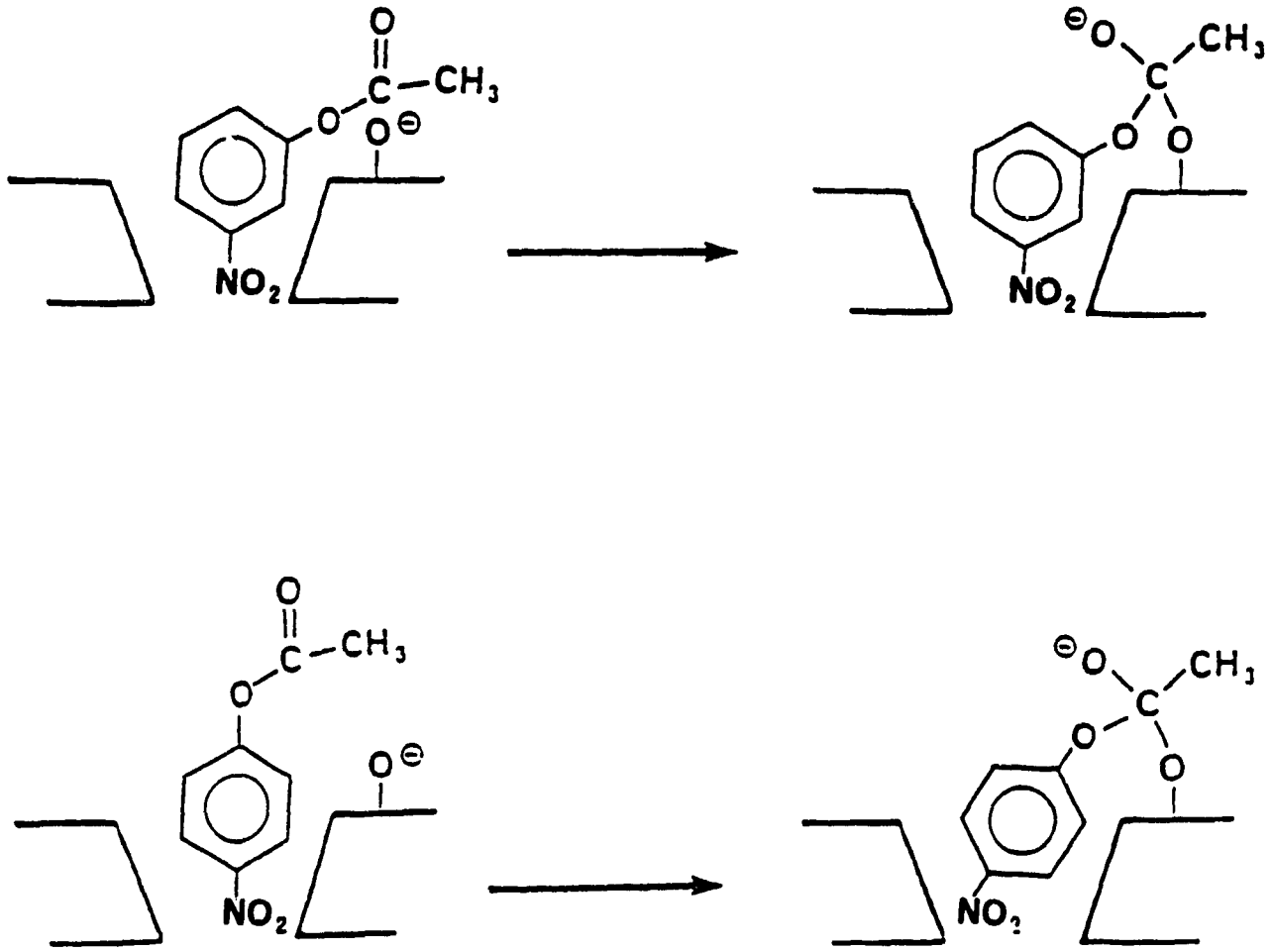


Figure 5. meta-Nitrophenyl acetate is a "better" substrate than its para isomer.

2.3.1 ANALYSIS OF DISSOCIATION CONSTANTS

The values of Kd decrease with increasing chain length for both p- and m-nitrophenyl alkanoates with both α- and β-CD (Tables 1 and 2). If only the aryl portion of the ester was inserted into the CD cavity, Kd should be fairly insensitive to the chain length. It seems, therefore, that most of the esters have their alkyl chains inserted into the CD cavity. Clearly, the stability of the ester.complex (S.CD) increases with increasing chain length.

The saturation kinetics observed in this work show that both p- and m-nitrophenyl alkanoates (I and II) form 1:1 complexes with α- and with β-CD. The guest molecules I and II have two hydrophobic sites: the aryl group or the alkyl group. Accordingly, there are two possible modes of complexation: aryl inclusion (eq 11) or alkyl chain inclusion (eq 12).

$$R\text{-}Ar + CD \underset{K_{Ar}}{\rightleftharpoons} R\text{-}Ar.CD \qquad (11)$$

$$CD + R\text{-}Ar \underset{K_{R}}{\rightleftharpoons} CD.R\text{-}Ar \qquad (12)$$

If both modes are important, the dissociation constant K_d is composite and has the form:

$$K_d = K_{Ar}.K_R/(K_{Ar} + K_R) \qquad (13)$$

When $K_{Ar} >> K_R$ only the alkyl moiety has been included in CD cavity and equation (13) will be reduced to:

$$K_d = K_R \qquad (14)$$

Alternatively, if $K_R >> K_{Ar}$ then binding of the aryl portion is more

important and equation (13) becomes:

$$K_d = K_{Ar} \qquad (15)$$

Tables 1 and 2 show that the values of K_d for the complexes of p- and m-nitrophenyl alkanoates with α- and β-CD decrease with increasing chain length of I and II. Therefore, it seems that the alkyl portion of these esters are bound in the CD cavity.

In order to investigate the binding pathway for the compounds I and II with CD it is necessary to compare them with other substrates having alkyl substituents. For this purpose we may use alcohols, alkylphenols, and acylphenols, all of which form complexes with α- and β-CD.[26,51]

At constant temperature, the Gibbs energy of binding to CD can be represented by the logarithm of the dissociation constant, K_d. For the compounds listed above the values of K_d can be related to chain length by equation 16:

$$\ln(K_d) = aN + b \qquad (16)$$

where the slope "a", similar to ρ in the Hammett equation, measures the sensitivity of binding to the chain length; N is the number of carbons in the alkyl chain of the substrate; "b" is the intercept. The results of analysis in terms of equation 16 for the binding energy of alkyl- and acylphenols, p- and m-nitrophenyl alkanoates, and linear alcohols with CD are shown in Table 3, and in Figures 6 and 7. In all cases reasonably straight lines are observed.

The values of the slopes "a" for p- and m-(alkyl or acyl)phenols are similar to those for alcohols with α- or β-CD (Table 3). The values for of m- and p-nitrophenyl alkanoates are similar to each other but they are lower than those of the alcohols. This probably results because the

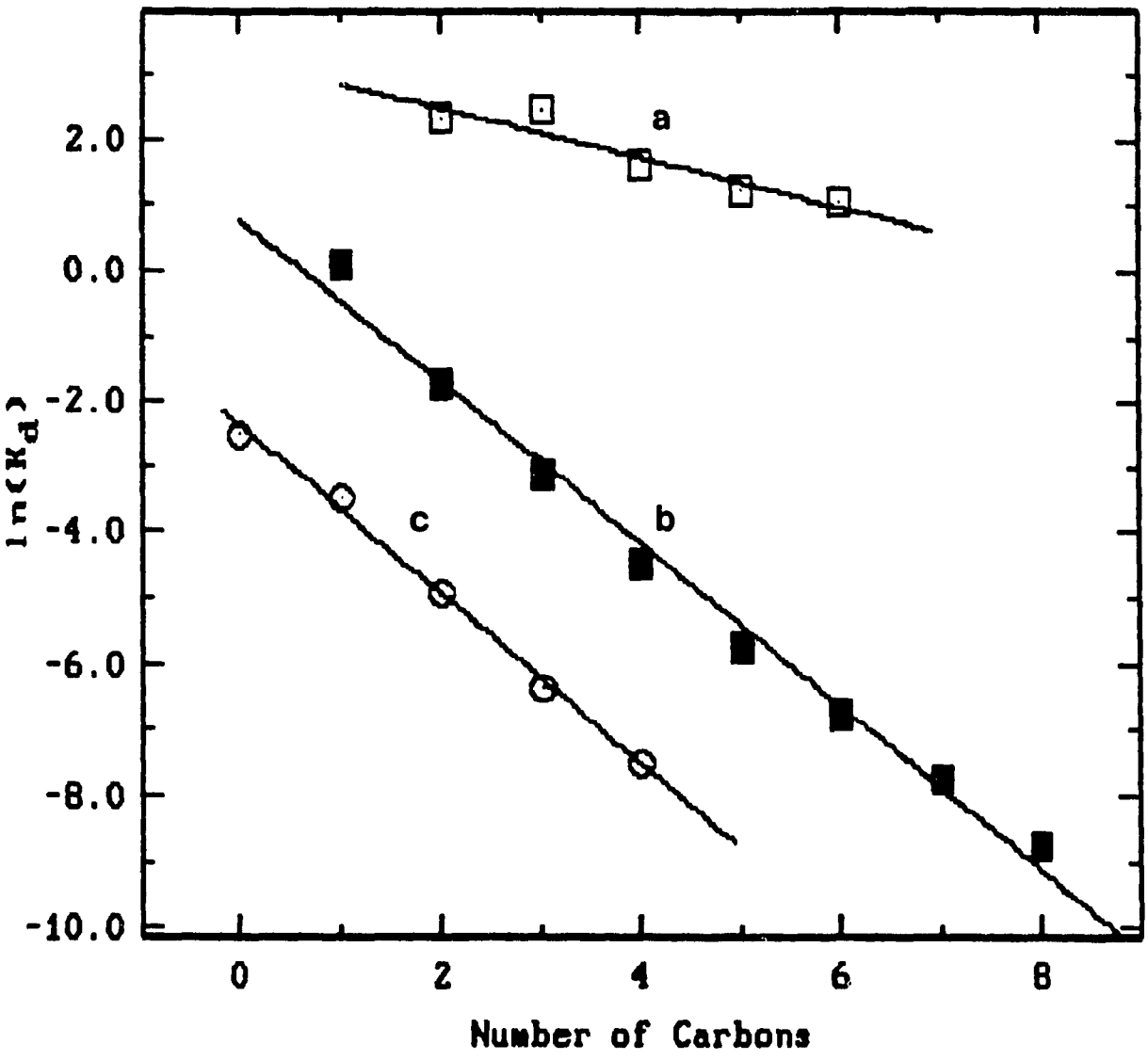
aryl portion of the esters is not particularly hydrophilic, as is the case with the hydroxyl group, and so the difference between binding the two ends is not so large. Regardless, the significant slopes for the esters indicate an appreciable sensitivity to chain length, consistent with alkyl group binding.

Table 3. Results of analysis for dissociation constants (K_d) of CD complex.[a]

Substrate	α-CD		β-CD	
	a	b	a	b
Alcohol	-1.24	0.775	-1.26	2.37
p-alkylphenol	-1.29	-2.36	-1.23	-4.27
m-alkylphenol	-1.11	-2.22	-1.27	-3.58
p-acylphenol	-	-	-1.01	-2.99
m-acylphenol	-	-	-1.46	-1.69
p-nitrophenyl alkanoates[b]	-0.375	3.23	-0.451	2.93
m-nitrophenyl alkanoates[b]	-0.215	2.52	-0.361	2.73

[a] Based on data from references 26 and 51.

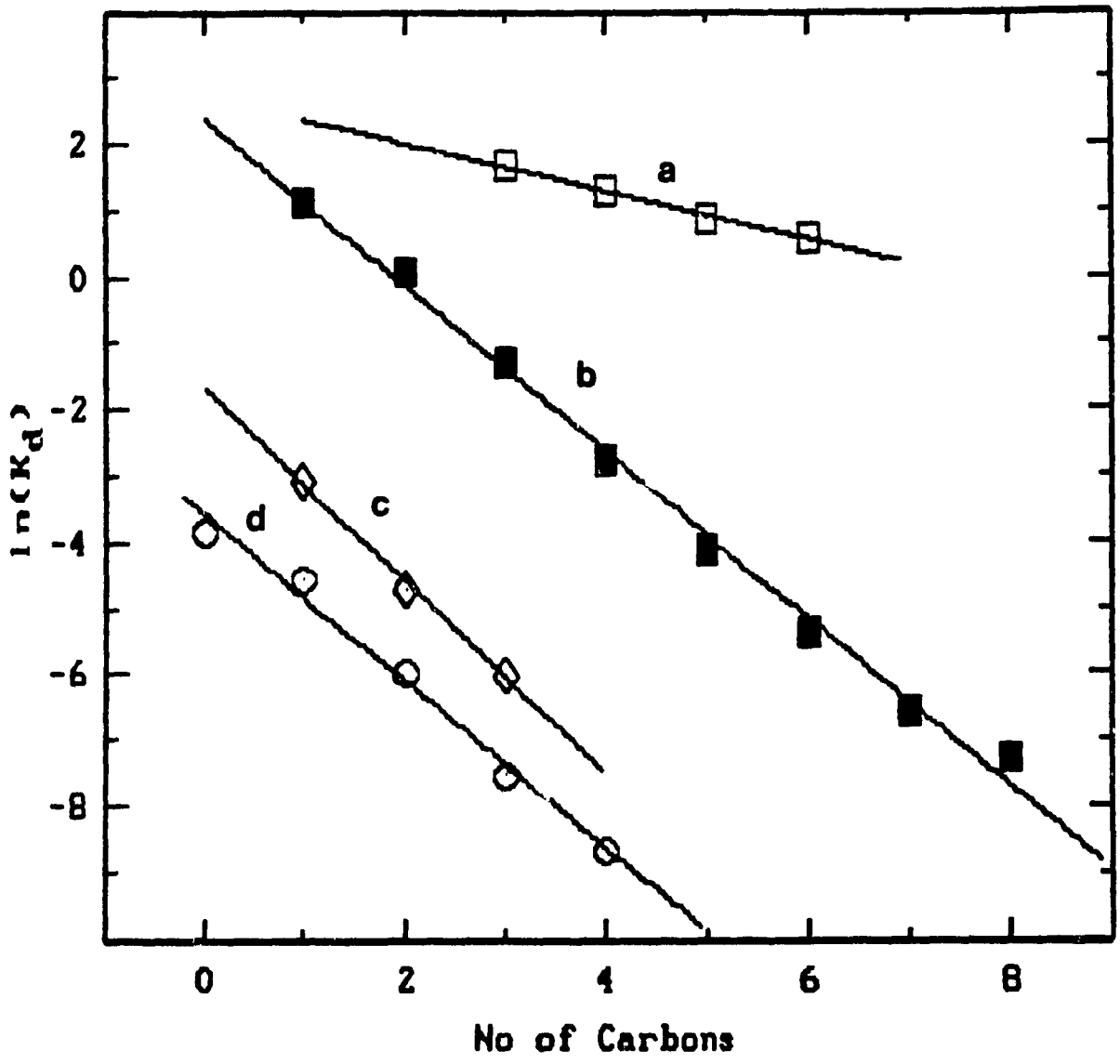
[b] This work.



(a) p-nitrophenyl alkanoates; (b) linear alcohols;

(c) p-alkylphenols.

Figure 6. Plot of $\ln(K_d)$ vs Number of carbons in the hydrocarbon chain of substrates which complex with the α-CD.



(a) m-nitrophenyl alkanoates; (b) linear alcohols;

(c) m-acylphenols; (d) m-alkylphenols.

Figure 7. Plot of $\ln(K_d)$ vs Number of carbons in the hydrocarbon chain of substrates which complex with the β-CD.

2.3.2. TRANSITION STATE OF CLEAVAGE

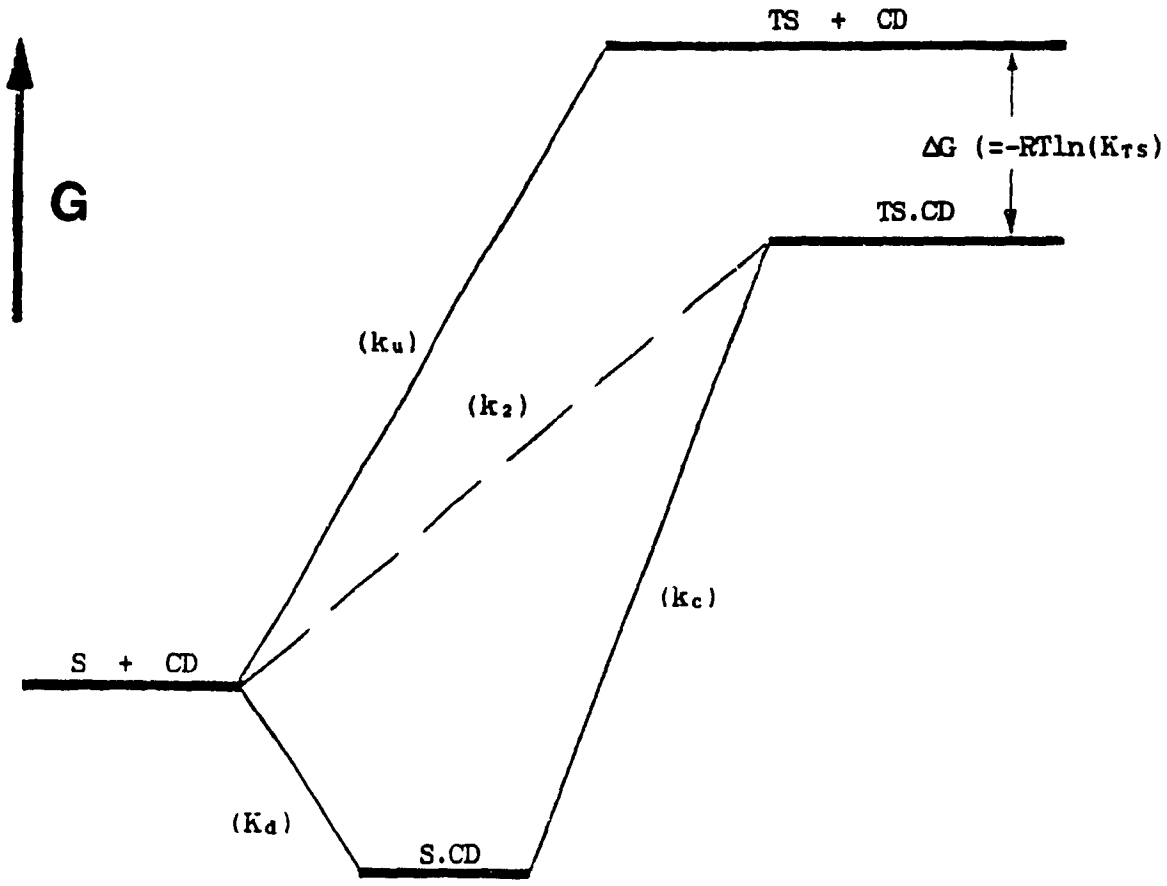
The transition state of a catalyzed reaction may be considered as being composed of the normal transition state (TS) and the catalyst, here a CD.[52] An apparent dissociation constant of the transition state can be calculated according to:[52,55,56]

$$K_{TS} = [TS][CD]/[TS.CD] = k_u.K_d/k_c \qquad (17)$$

K_{TS} is obviously not a real equilibrium constant, but it does give an indication of the energy difference between the transition states of the "catalyzed" and "uncatalyzed" pathways, and thus of the effect of CD on the stability of the transition state. The significance of K_{TS} may be appreciated by considering the Gibbs energy diagram in Figure 8. From that, it is obvious that the transition state energy of the bound substrate is lower than that of the unbound substrate, since the CD-mediated reaction is the more rapid of the two.

Table 1 contains values of K_{TS} for the p-nitrophenyl esters (I) with α-CD. From the propanoate onwards they decrease with increasing chain length, suggesting that the transition state for cleavage has the alkyl group bound in the cavity of the CD. In contrast, the values of K_{TS} for the m-nitrophenyl esters with α-CD (Table 1) show little change with chain length. It seems that in these cases the aryl group is included in the CD cavity.

Generally similar results were found with β-CD (Table 2). The value of K_{TS} for p-nitrophenyl propanoate is close to that for the acetate which suggests that the structure of their transition states are



Where:

S: Substrate; CD: Cyclodextrin;

TS: Transition state;

TS.CD: Transition state-Cyclodextrin complex;

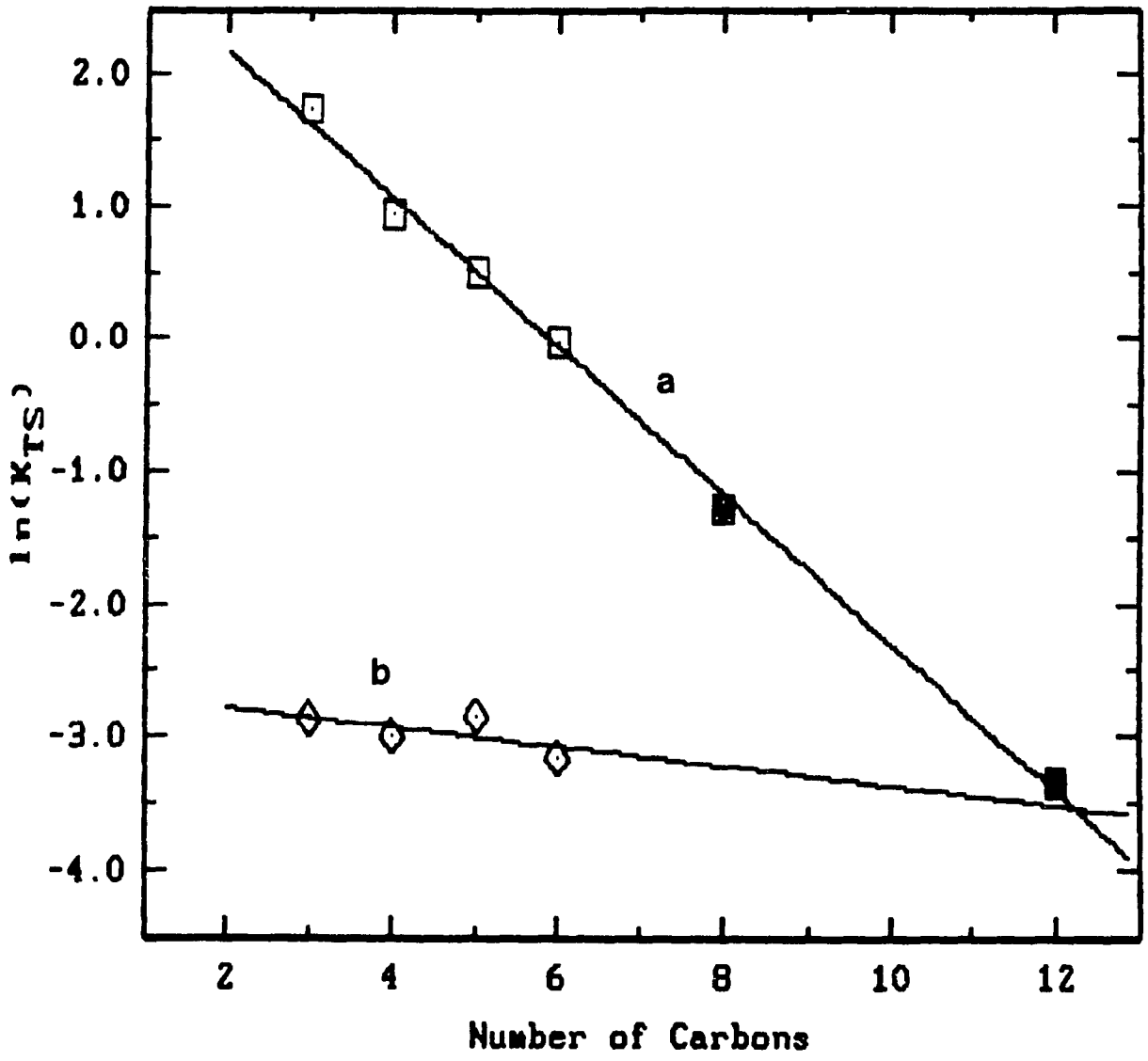
S.CD: Substrate-Cyclodextrin complex.

Figure 8. Gibbs Energy Diagram

similar. Beyond the propanoate the values of K_{TS} decrease substantially for the p-nitrophenyl esters, again suggesting that they bind with their alkyl groups in the CD cavity.

For the m-nitrophenyl substrates (II) with β-CD (Table 2) the values of K_{TS} of propanoate, butanoate, and pentanoate are very similar, and seemingly different from those for the acetate and hexanoate. However, the overall changes in K_{TS} are smaller than those in K_d and so it seems probable that the structure of transition state in the cleavage of the m-nitrophenyl alkanoate does not change much for acyl chains of C2 to C6.

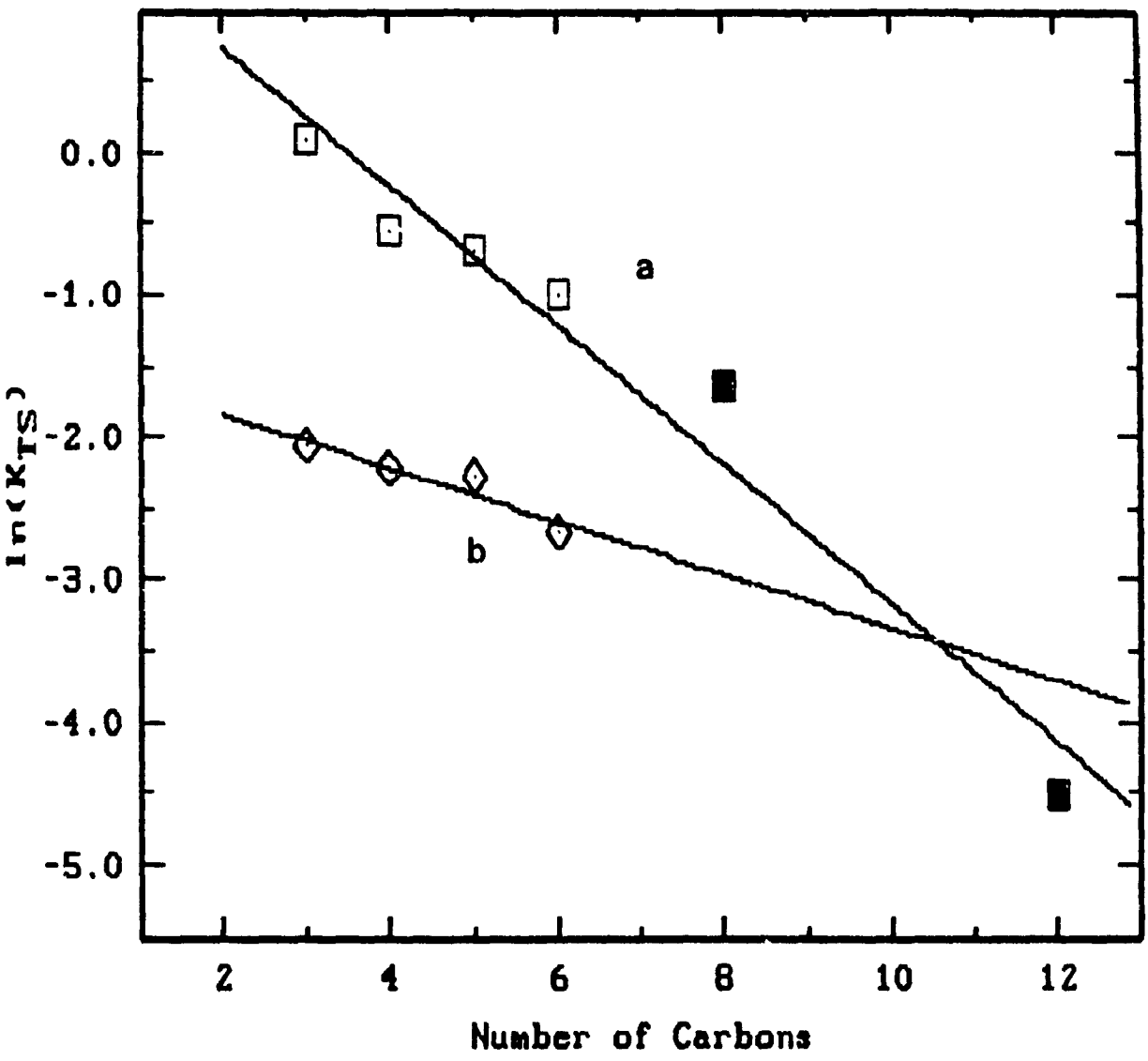
At fixed temperature, here 25°C, the logarithm of K_{TS} is directly proportional to the free energy difference between the transition states of the "normal" and "catalyzed" reactions (see Figure 8). Thus, we consider the effect of structural variation on $\ln(K_{TS})$. Figures 9 and 10 show plots of $\ln(K_{TS})$ vs the number of carbons on the acyl chain for the two series of esters, I and II. Particularly in Figure 9 (for α-CD) there seem to be linear relationships. More importantly, the slopes of the plots are quite different for the m- and p-nitrophenyl alkanoates. The much steeper slopes for the p-nitrophenyl esters show a much greater sensitivity to the length of the acyl chain. This strong variation in transition state energy with the alkyl chain length of p-nitrophenyl alkanoates supports the view that during cleavage the alkyl moiety of these esters is included in the cavity of CDs (for acyl chain lengths longer than C2).



(a) p-nitrophenyl alkanoates;

(b) m-nitrophenyl alkanoates.

Figure 9. Plot of $\ln(K_{TS})$ vs acyl chain length for the cleavage of nitrophenyl alkanoates by α-CD:



(a) p-nitrophenyl alkanoates;

(b) m-nitrophenyl alkanoates.

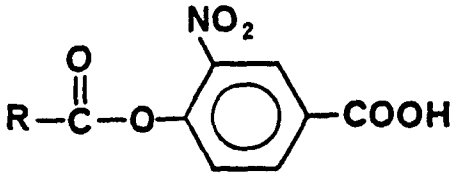
Figure 10. Plot of $\ln(K_{TS})$ vs acyl chain length for the cleavage of nitrophenyl alkanoate in the β-CD.

CHAPTER 3. CLEAVAGE OF III AND IV

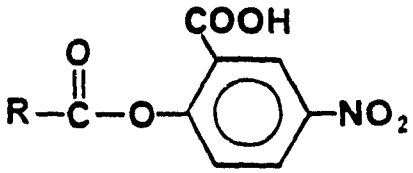
The rates of cleavage of 4-carboxy-2-nitrophenyl and 2-carboxy-4-nitrophenyl alkanoates (III and IV) were also measured in a phosphate buffer solution of pH 11.60 containing α- or β-CD at 25°C. Analysis of the variation of k_{obs} with [CD] shows that with the longer chain esters there are processes involving two CD molecules. With α-CD a 2:1 binding leads to inhibition of cleavage for III, whereas with β-CD a process which is second-order in CD provides rate enhancement for III and IV. Similarly, with α-CD there is also a second-order process for IV. In the case of the 2:1 binding it seems likely that with the shorter chain esters the phenyl moiety is first inserted into the CD cavity whereas for longer chain esters the alkyl chain is first included.

3.1. INTRODUCTION

To further explore the interaction of longer chain alkanoates with CDs, and to probe the binding of their alkyl chains during esterolysis, we have also studied the cleavage by α- and β-CD of various 4-carboxy-2-nitrophenyl esters (III): acetate, propanoate, butanoate, pentanoate, hexanoate, heptanoate, octanoate, 2-ethylhexanoate, and 4-methylpentanoate, and the 2-carboxy-4-nitrophenyl esters (IV): acetate, butanoate, hexanoate, and octanoate. These two series of compounds have the same functional groups, but in different positions, and so more subtle geometrical effects may be probed.



III



IV

	R		R
C2	CH_3- ;	C7	$CH_3(CH_2)_4CH_2-$;
C3	CH_3CH_2- ;	C8	$CH_3(CH_2)_5CH_2-$;
C4	$CH_3CH_2CH_2-$;	2-Et-C6	$CH_3(CH_2)_3\underset{\underset{Et}{\vert}}{CH}-$;
C5	$CH_3(CH_2)_2CH_2-$;		
C6	$CH_3(CH_2)_3CH_2-$;	4-Me-C5	$(CH_3)_2CHCH_2CH_2-$.

The depths of the cavities of α- and β-CD are the same, but their widths are different.[2] Therefore, the effects on ester cleavage may be different for α- and β-CD. The phenomena which are caused by the guest or the CD in the esterolysis might be distinguishable by changing the positions of functional group on the phenyl ring.

3.2 RESULTS

We have measured the kinetics of cleavage of 4-carboxy-2-nitrophenyl (III) and 2-carboxy-4-nitrophenyl (IV) esters by α- or β-CD using the stopped-flow technique. The reactions were followed by monitoring the increase in absorbance at around 407nm for III and 370nm for IV.

It was observed that the shorter chain esters behave like many of the other esters previously studied.[2,30,32] They exhibit saturation

kinetics, consistent with equation 7. The results for III are listed in Tables 4 and 5, those for IV in Table 6.

With α-CD, the C4, C5, C6, C7, and C8 esters of III show saturation kinetics and an inhibitory process at high [CD] (Figure 11). These results may be explained by a non-productive 2:1 (CD:ester) binding:

$$S.CD + CD \underset{K_2}{\rightleftharpoons} S.2CD \qquad (18)$$

The presence of this additional equilibrium requires that eq 7 be changed to:

$$k_{obs} = \frac{(k_u.K_d + k_c[CD])K_2}{(K_d.K_2 + K_2[CD] + [CD]^2)} \qquad (19)$$

Equation 19 gives excellent fits to the data for the C4, C5, C6, C7, and C8 esters of III (Figure 11); the appropriate constants are given in Table 4. The results show that the K_d values of III decrease with increasing hydrocarbon chain length in α-CD, but the K_2 values do not. The uncatalyzed rate constants, ku, change very little from C3 to C8 whereas the rate constants for the reaction of the ester.CD complexes increase with chain length, with the exception of the acetate and propanoate esters.

In contrast to the esters just discussed, with α-CD the 4-carboxy-2-nitrophenyl acetate, 2-ethylhexanoate, and 4-methylpentanoate show only simple 1:1 binding and their behavior may be explained by eq 7.

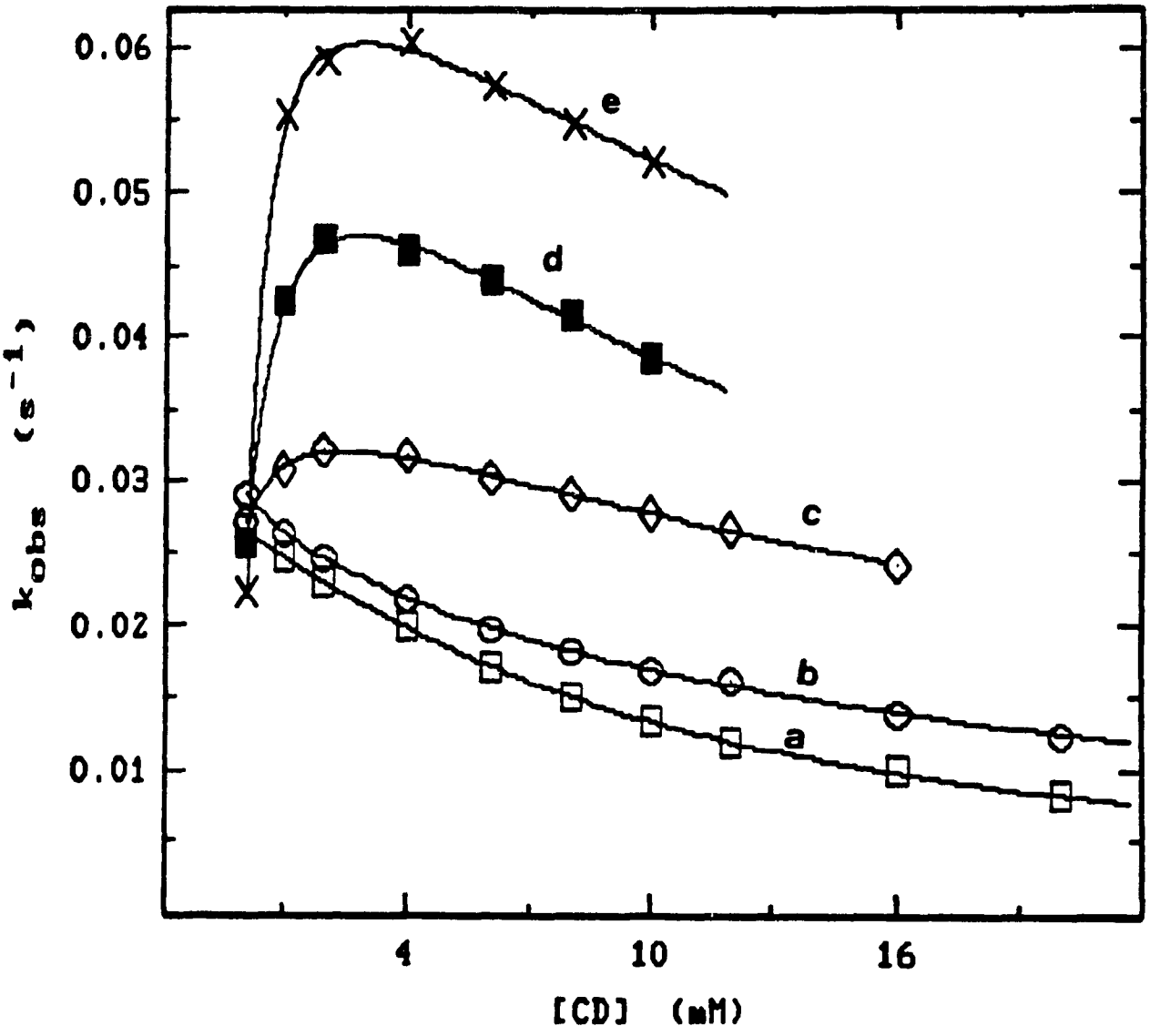
With β-CD the longer chain esters: C4, C5, C6, C7, and C8 of III, and C4, C6, and C8 of IV, have another catalytic process which becomes important at high [CD] (see Figures 12 and 13). Similar behavior was

Table 4. Constants for the cleavage of 4-carboxy-2-nitrophenyl alkanoate esters in the presence of α-cyclodextrin.[a]

Acyl	K_d, mM	K_2 mM	k_u, s^{-1}	k_c, s^{-1}
C2	8.88	-	0.0964	0.171
C3	50.4	10.8	0.0529	0[b]
C4	7.66	14.3	0.0265	0.0126
C5	2.07	21.8	0.0290	0.0222
C6	1.43	28.7	0.0271	0.0375
C7	1.05	16.8	0.0256	0.0629
C8	0.503	26.2	0.0222	0.0737
2-Et-C6	2.28	-	0.00104	0.00188
4-Me-C5	1.08	-	0.0254	0.0174

[a] At 25°C, in a 0.4M phosphate buffer of pH 11.6 containing 0.1%(v/v) MeOH. Errors in K_d, K_2, k_u, and k_c are 5-10%. The constant $k_2 = k_c/K_d$ (see p 22).

[b] Assumed value. Fitting this parameter gives a small negative value.



(a) C4; (b) C5; (c) C6;

(d) C7; (e) C8.

Figure 11. Rates of cleavage of 4-carboxy-2-nitrophenyl alkanoates as a function of α-cyclodextrin concentration

Table 5. Constants for the cleavage of 4-carboxy-2-nitrophenyl alkanoate esters in the presence of β-cyclodextrin.[a]

Acyl	K_d, mM	k_u, s^{-1}	k_c, s^{-1}	k_{c2} $M^{-1}\ s^{-1}$
C2	5.89	0.0964	0.276	-
C3	5.51	0.0501	0.0330	-
C4	1.54	0.0291	0.00777	-
C5	0.920	0.0309	0.00208	0.218
C6	0.380	0.0235	0.00669	0.149
C7	0.272	0.0256	0.0142	0.236
C8	0.789	0.0222	0.0263	0.535
2-Et-C6	0.454	0.00104	9.35×10^{-5}	8.53×10^{-3}
4-Me-C5	0.255	0.0255	0.00479	0.104

[a] At 25°C, in a 0.4M phosphate buffer of pH 11.6 containing 0.1%(v/v) MeOH. Errors in K_d, k_u, k_c and k_{c2} are 5-10%. The constant $k_2 = k_c/K_d$ (see p 22).

also observed with α-CD and the longer esters (C4, C6, and C8) of IV (Figure 14). It may be ascribed to the attack of a second molecule of CD on the ester-CD complex:

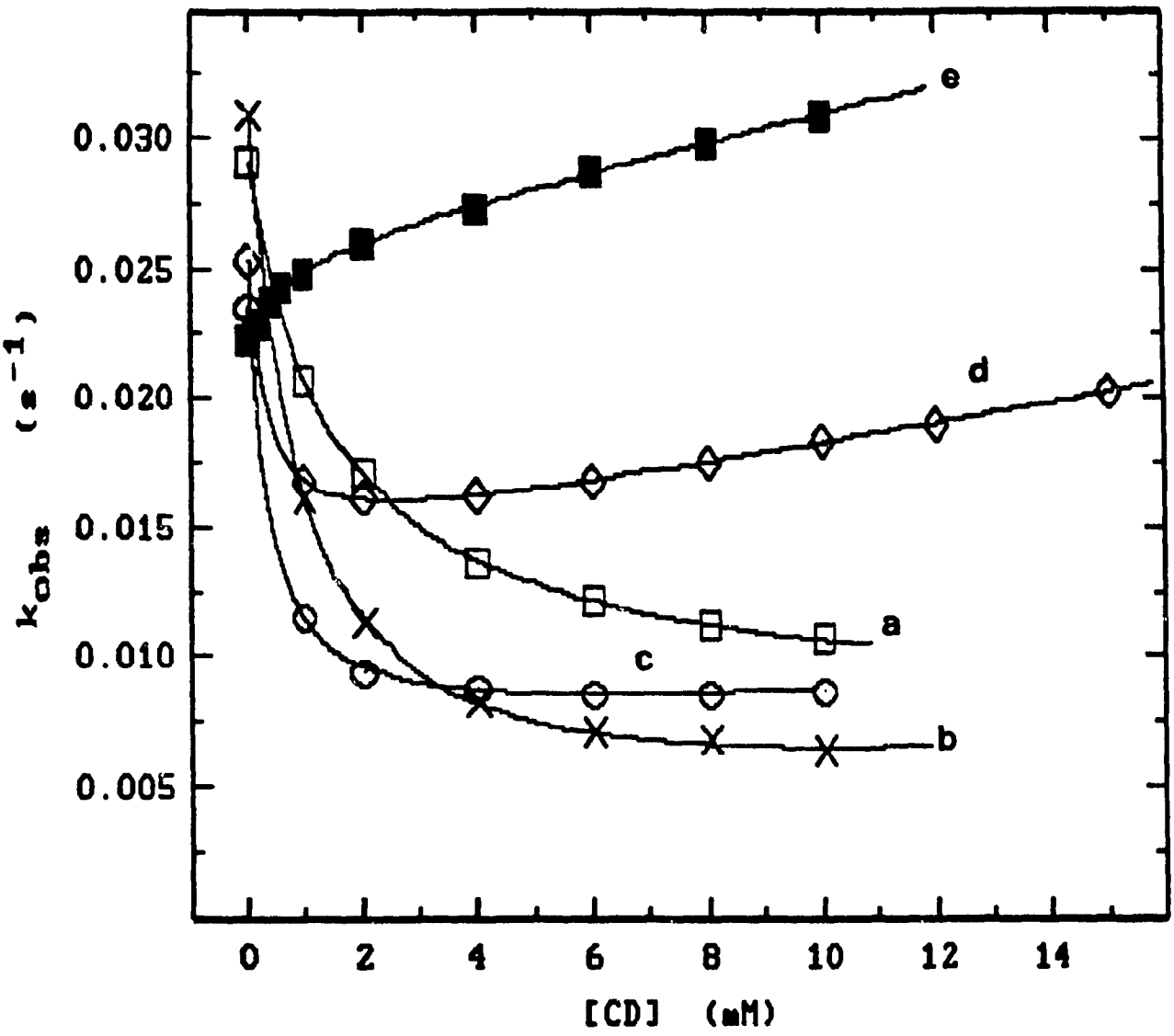
$$S.CD + CD \xrightarrow{k_{c2}} \text{Products} \qquad (20)$$

In this case, eq 7 must be expanded to equation 21 which fits our data for the longer chain esters (Fig. 12, 13, 14) with the constants given in Tables 5 and 6.

$$k_{obsd} = \frac{(k_u K_d + k_c[CD] + k_{c2}[CD]^2)}{(K_d + [CD])} \qquad (21)$$

The constants ku, kc, and Kd are the same as previously, and k_{c2} is the rate constant for the reaction of the complexed ester reacting with a second CD molecule.

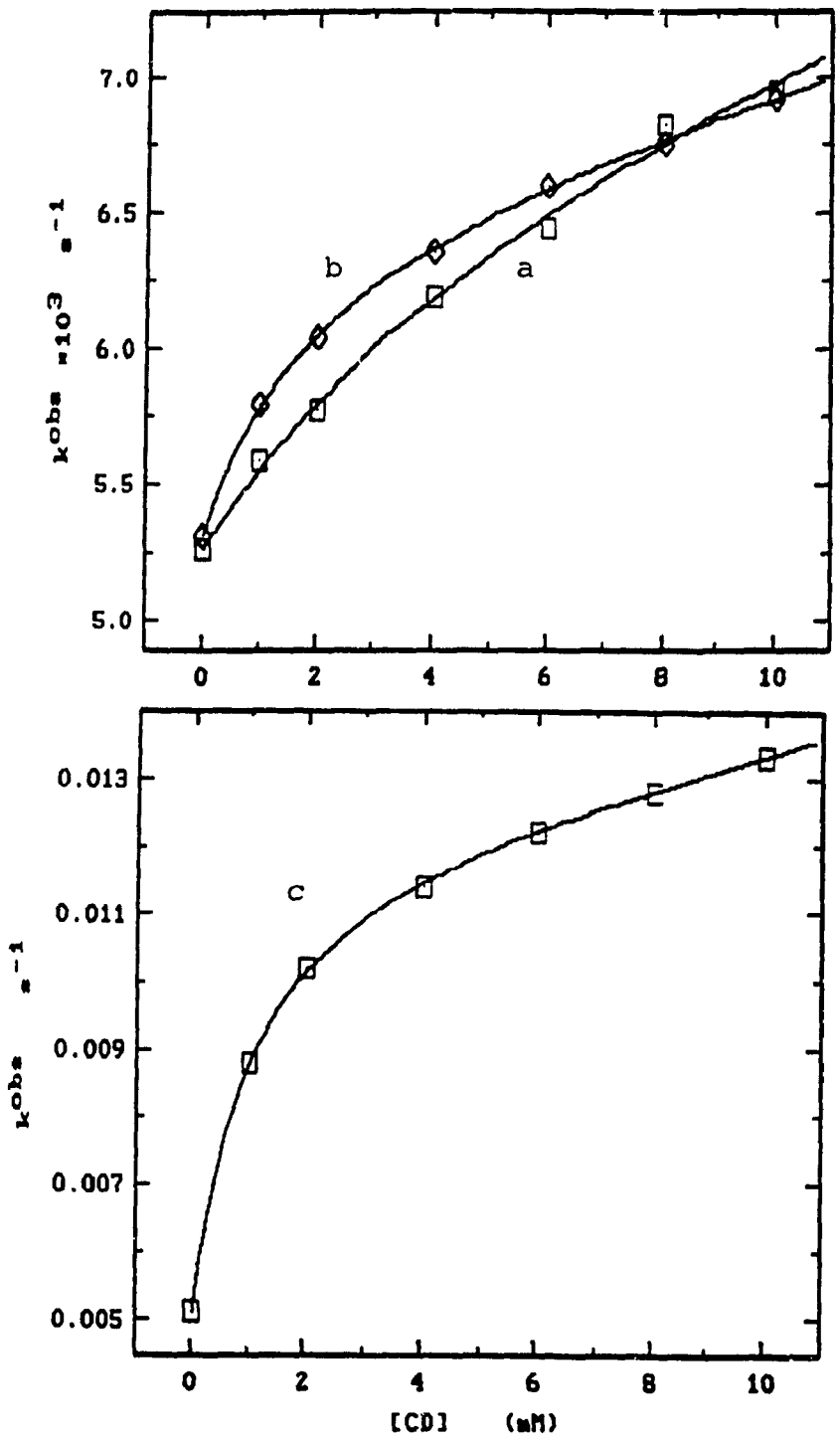
Generally speaking, the constants Kd for the dissociation of the complexes of III with β-CD (Table 5) and of α- and β-CD with IV (Table 6) decrease with increasing chain length. The rate constants k_c for the cleavage of the complexes of the esters of III and IV with β-CD first decrease and then increase with increasing chain length. In the case of IV with α-CD the three values of k_{c2} which were accessible remain almost constant.



(a) C4; (b) C5; (c) C6;

(d) C7; (e) C8.

Figure 12. Rates of cleavage of 4-carboxy-2-nitrophenyl alkanoates as a function of β-cyclodextrin concentration.



(a) C4; (b) C6; (c) C8.

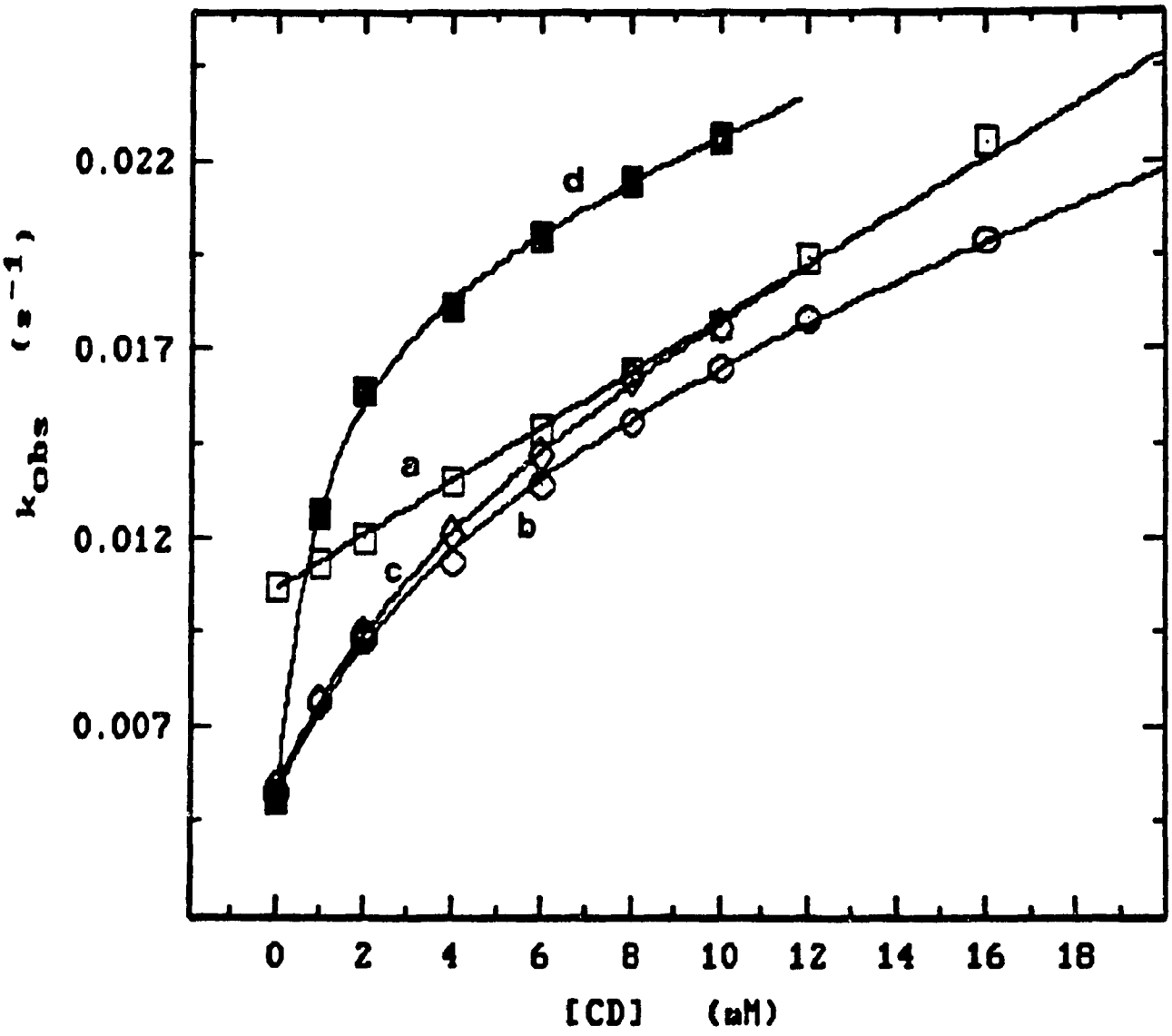
Figure 13. Rates of cleavage of 2-carboxy-4-nitrophenyl alkanoates as a function of β-cyclodextrin concentration

Table 6. Constants for the cleavage of 2-carboxy-4-nitrophenyl alkanoate esters in the presence of α- and β-cyclodextrin.[a]

Acyl	K_d, mM	k_u, s^{-1}	k_c, s^{-1}	k_c/k_u	k_{c2} $M^{-1}\ s^{-1}$	k_c/k_d $M^{-1}\ s^{-1}$
(a) α-cyclodextrin						
C2	-[b]	0.0107	-[b]	-[b]	-	0.712[b]
C4	4.98	0.00519	0.0182	3.51	0.382	3.66
C6	4.72	0.00538	0.0179	3.33	0.556	3.60
C8	0.944	0.00508	0.0196	3.86	0.466	20.8
(b) β-cyclodextrin						
C2	24.2	0.0100	0.0859	8.59	-	3.55
C4	8.81	0.00526	0.00832	1.58	-	0.941
C6	1.93	0.00531	0.00664	1.25	0.0588	3.44
C8	0.976	0.00510	0.0122	2.39	0.186	12.5

[a] At pH 11.60.

[b] Value not available since saturation kinetics were not observed (for [CD] = 0 up to 20 mM). The plot of k_{obs} vs [CD] was linear, from which the slope = k_2 provides the value of k_c/K_d.



(a) C2; (b) C4; (c) C6; (d) C8.

Figure 14. Rates of cleavage of 2-carboxy-4-nitrophenyl alkanoates as a function of α-cyclodextrin concentration.

3.3 DISCUSSION

The longer chain esters of III and IV have two potential binding sites: the aryl group and the alkyl group. Therefore, they can form two kinds of 1:1 complexes and one 1:2 complex with CDs. The 1:2 complexation may be described by equation (22):

$$S + CD \underset{K_d}{\rightleftharpoons} S.CD \overset{CD}{\underset{K_2}{\rightleftharpoons}} S.2CD \qquad (22)$$

for which the "total" (overall) dissociation constant (K_{dt}) will be:

$$K_{dt} = [S][CD]^2/[S.2CD] = K_d.K_2 \qquad (23)$$

Of the present results, equation 23 is only valid for the case of the cleavage of III by the α-CD. In contrast, the results for III with β-CD, and for IV with α- or β-CD show no clear evidence of 2:1 binding, even though cleavage at high [CD] involves 2 molecules of CD.

From the results, the dissociation constants for 1:1 complexation, K_d (Tables 4, 5, and 6) decrease with increasing chain length of the alkyl moiety of the ester (except for 4-carboxy-2-nitrophenyl octanoate with β-CD). Overall, this decrease indicates that the stability of the CD-ester complex is enhanced by acyl chain length, suggesting that binding takes place through the alkyl group.

The second dissociation constants K_2 for the esters III in the presence of α-CD shows little change with increasing chain length. This means that the second complexation of S.CD with another α-CD molecule is not influenced significantly by changes in the length of alkyl chain. The most probable explanation is that the second binding corresponds to the aryl moiety being inserted into the cavity of the

second α-CD molecule. Since the same binding site is involved in all six cases, it is reasonable that K_2 shows little variation.

The rate constants (k_c) for the cleavage of the 1:1 complexes of the esters III and IV are larger for α-CD than for β-CD. This difference in behaviour for the longer esters must relate to the cavity widths of α- and β-CD, since their depths are the same.[2] In the case of the smaller sized cavity of the α-CD the alkyl chain of the acyl group must be held more rigidly. Presumably the attacking group, an ionized secondary hydroxyl on CD, is much closer to the acyl group of guest ester in α-CD than in β-CD.

For both α- and β-CD, the variations of k_c/k_u with chain length (Table 7, 8, and 9) are similar to those for the corresponding p-nitrophenyl esters: substrate binding becomes stronger whereas the catalytic ratio k_c/k_u decreases and then increases. Apparently, with a shorter, bound alkyl chain, such as with the C4 ester, the ester function sits too deeply in the CD cavity to be easily attacked by an ionized hydroxyl group. With the longer esters, the ester function is held progressively higher, and is more accessible to nucleophilic attack. Thus, the data for α-CD (Table 7) shows that the ratio k_c/k_u increases from 0.48 to 3.3, as the acyl chain length increases from C4 to C8. For β-CD (Table 8) the increase is from 0.067 to 1.2 for C5 to C8.

This behaviour is quite reasonable since, as discussed earlier, the kinetic parameters for the cleavage of p-nitrophenyl alkanoates depend on their chain length. Also, as mentioned in Chapter 2, the binding to CDs of alcohols, alkylphenols, acylphenols,[26] and of alkane sulfonate

ions[35,36] is strongly dependent on the length of their alkyl groups.

The results for the esters III with α-CD (Table 4) show that two molecules of CD can bind one molecule of the longer chain III esters. This formation of a 2:1 complex differs from the more normal 1:1 complexation[1-3,37-39] and 2:2 complexation.[40,41] With the present guests, 4 carboxy-2-nitrophenyl (III) esters, which possess a carboxy group, there may be the formation of a hydrogen bond to the second molecule of CD. As already disccused, the values of K_d and K_2 are consistent with insertion of the ester moiety into the cavity of the first CD molecule to form a 1:1 complex (K_d), and then inclusion of the aryl portion in a second molecule of CD to form the 2:1 complex. Presumably, the carboxy group undergoes hydrogen bonding with a primary hydroxyl group on the narrow end of the torus.

From the data in Table 4, the following constants can be calculated: (a) the rate constants k_2 (= k_c/K_d) for the reaction of the esters with α-CD; (b) the apparent dissociation constants K_{TS}, calculated from eq 17. Both sets of values are listed in Table 7.

As seen in Figure 15, the values of k_2 increase with the acyl chain length of ester, showing that the reactivity of α-CD becomes greater for longer alkanoates. Similarly, the values of K_{TS} decrease with chain length. Both sets of values are consistent with alkyl binding in the transition state for esterolysis.

For an increase of one methylene group, the chain length will be increase by approximately 1.25Å. When the length of the alkyl portion is longer than the aryl moiety, it is probable that the alkyl moiety inserts into the α-CD cavity in preference to the aryl portion.

Table 7. Constants calculated from Table 4 for the cleavage of 4-carboxy-2-nitrophenyl alkanoate esters in the presence of α-cyclodextrin.[a]

Acyl	K_d, mM	K_2 mM	k_c/k_u	k_2 M^{-1} s^{-1}	K_{TS} mM
C2	8.88	-	1.77	19.3	5.02
C3	50.4	10.8	0[b]	0[c]	-[b]
C4	7.66	14.3	0.476	1.65	16.1
C5	2.07	21.0	0.766	10.7	2.70
C6	1.43	28.7	1.38	26.2	1.04
C7	1.05	16.8	2.46	59.9	0.427
C8	0.503	26.2	3.32	147	0.152
2-Et-C6	2.28	-	1.81	0.825	1.26
4-Me-C5	1.08	-	0.685	16.1	1.58

[a] At 25°C, in a 0.4M phosphate buffer of pH 11.6 containing 0.1%(v/v) MeOH; [ester] = 0.05mM.

[b] Since $k_c = 0$ (see Table 4) this quantity is not calculable.

[c] Assuming $k_c = 0$ (see Table 4).

Table 8. Constants calculated from Table 5 for the cleavage of 4-carboxy-2-nitrophenyl alkanoate esters in the presence of β-cyclodextrin.[a]

Acyl	k_c, s^{-1}	k_c/k_u	k_{c2} $M^{-1}\ s^{-1}$	k_2 $M^{-1}\ s^{-1}$	K_{TS} mM	K_{TS}' mM
C2	0.276	2.86	-	46.9	2.06	
C3	0.0330	0.659	-	5.99	8.37	
C4	0.00777	0.267	-	5.05	5.77	
C5	0.00208	0.0673	0.218	2.26	13.7	9.54
C6	0.00669	0.285	0.149	17.6	1.35	44.9
C7	0.0141	0.555	0.236	52.2	0.490	60.2
C8	0.0263	1.19	0.535	33.3	0.666	49.2
2-Et-C6	9.35×10^{-5}	0.0899	8.53×10^{-3}	0.206	5.05	11.0
4-Me-C5	0.00479	0.188	0.104	18.8	1.36	46.1

[a] At 25°C, in a 0.4M phosphate buffer of pH 11.6 containing 0.1%(v/v) MeOH; [ester] = 0.05mM.

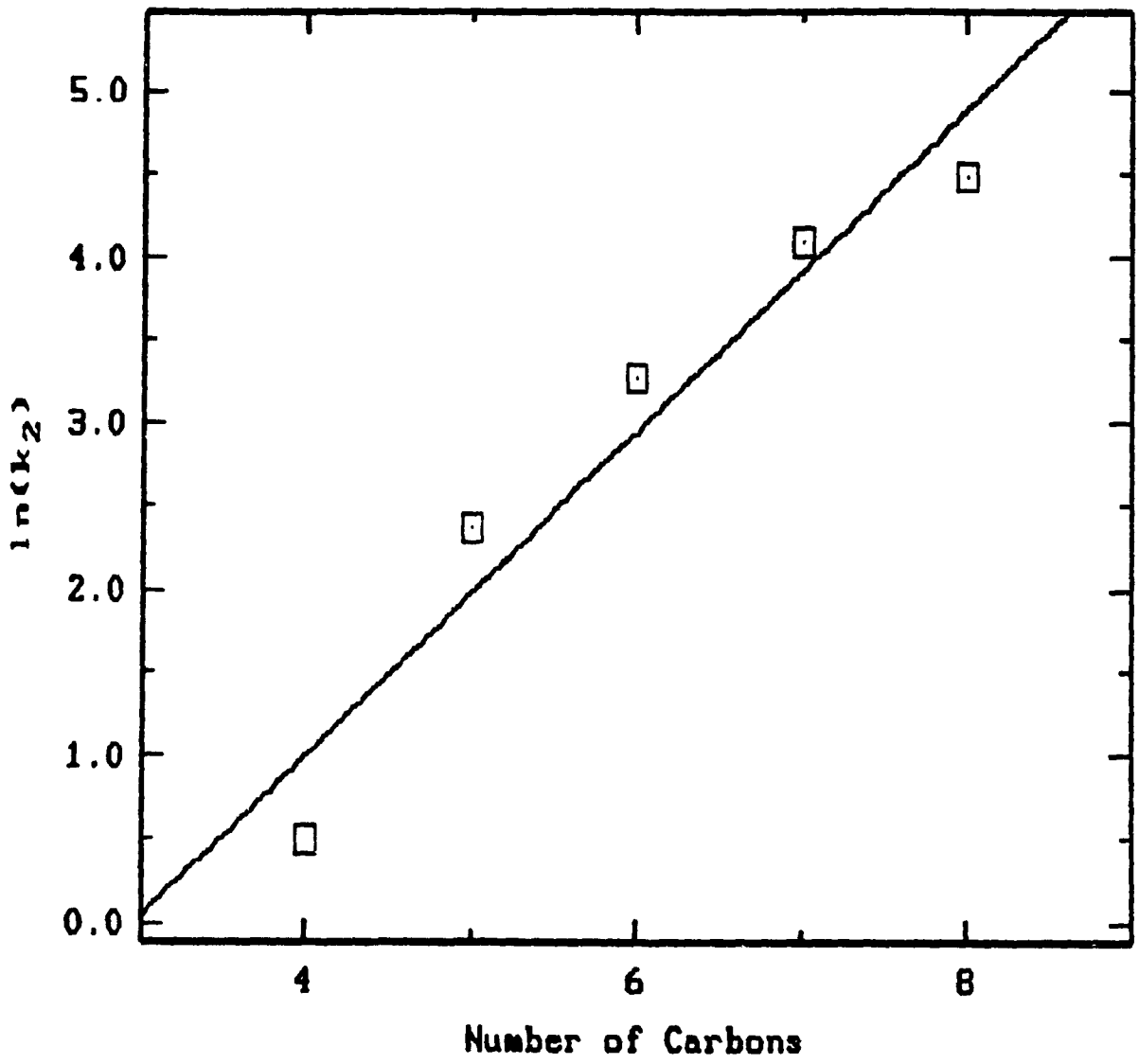


Figure 15. Plot of $\ln(k_2)$ vs acyl chain length for the cleavage of 4-Carboxy-2-nitrophenyl Alkanoates by α-CD.

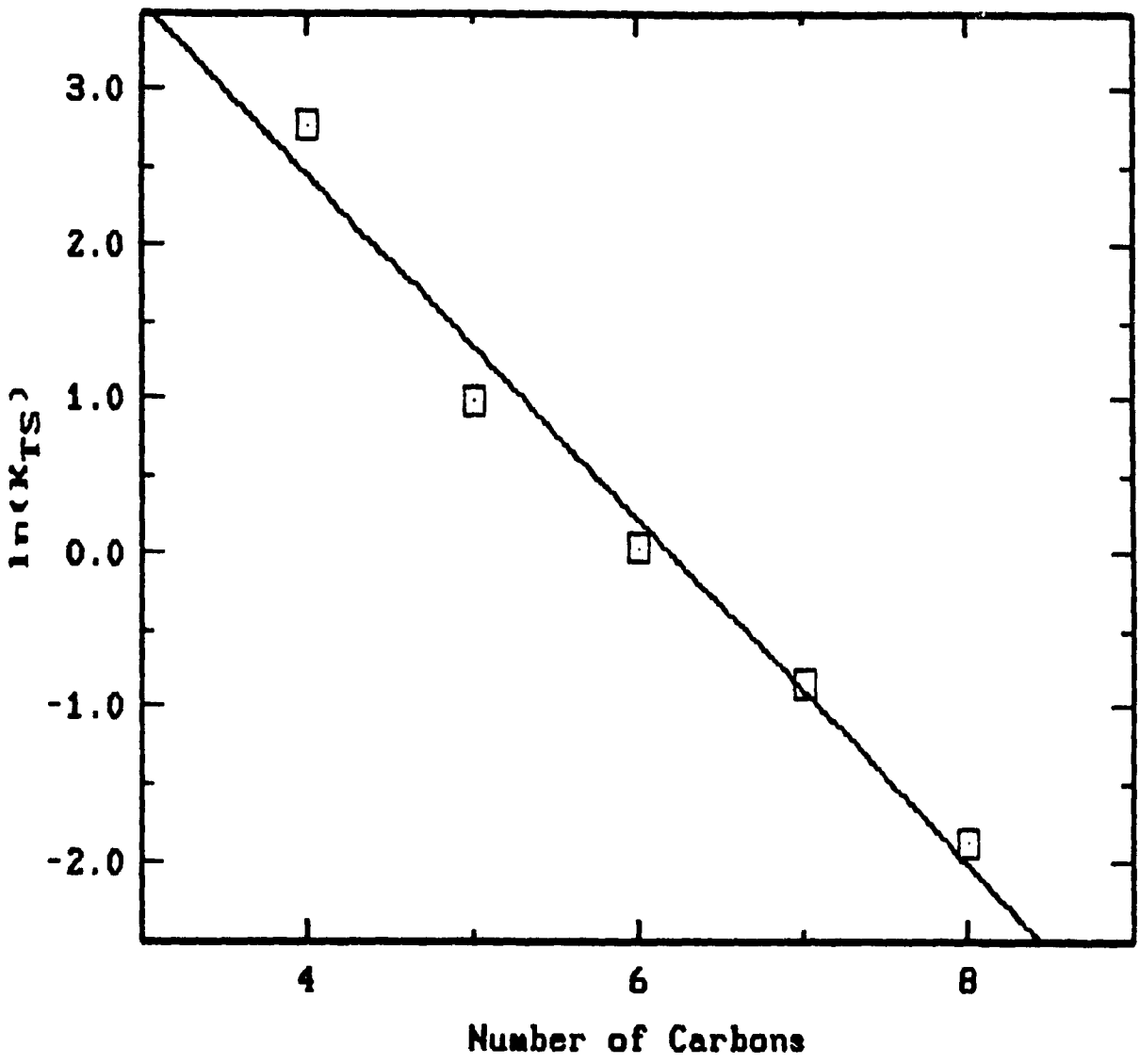


Figure 16. Plot of $\ln(K_{TS})$ vs chain length for the cleavage of 4-Carboxy-2-nitrophenyl Alkanoates by α-CD.

The basic cleavage of 4-carboxy-2-nitrophenyl 2-ethylhexanoate and 4-methylpentanoate by α-CD follows the normal pattern,[3,16] with only 1:1 complexation. No evidence was found of 2:1 binding.

As described in Chapter 2, the transition state stabilization energy can be expressed in terms of $\ln(K_{TS})$. The plot of $\ln(K_{TS})$ vs the acyl chain length for the cleavage of III (Figure 16) by α-CD shows that the transition state stabilization increases regularly with the length of the alkyl chain of esters. It clearly indicates that the alkyl moiety is included in the cavity of α-CD for the esters III whose acyl chain length is longer than C3.

The kinetics of cleavage of the esters of III and IV with β-CD, and of IV with α-CD, did not show any indication of 2:1 binding. However, they do show the involvement of a process which requires two molecules of CD, which becomes important at high [CD], after the 1:1 binding has been largely saturated (see Figures 12, 13, 14 and Tables 5, 6). This new process could arise from a 2:1 complex, but if it does the complex must be a very weak one since it does not show up in the kinetics.

The rate constants (k_{c2}) associated with the second-order process are defined for the reaction of a second CD with the 1:1 ester.CD complex (eq 20). For the limited data available, the values of these constants seem to increase with alkyl chain length (Tables 5, 6).

The rate constants k_2, for the cleavage of III by β-CD (Table 8) and of IV by α- or β-CD (Table 9), first decreases then increase with the acyl chain length. This change probably arises because the esters with longer chains have their alkyl groups inserted into the cavity of the CD in such a way that the distance between the ionized hydroxyl group

Table 9. Constants calculated from Table 6 for the cleavage of 2-carboxy-4-nitrophenyl alkanoate esters in the presence of α- and β-cyclodextrin.[a]

Acyl	k_c/k_u	k_2 $M^{-1}\ s^{-1}$	K_{TS} mM	K_{TS}' mM
(a) α-cyclodextrin				
C2	-[b]	0.712	15.0	-
C4	3.51	3.66	1.42	47.6
C6	3.33	3.79	1.42	32.2
C8	3.86	20.8	0.244	42.1
(b) β-cyclodextrin				
C2	8.59	3.55	2.82	-
C4	1.58	0.944	5.57	-
C6	1.25	3.44	1.54	113
C8	2.39	12.5	0.408	65.6

[a] At pH 11.60.

[b] not available (see foot note b in Table 6).

of the CD and acyl group of the ester decreases with increasing chain length and so the ease of reaction increases. However, this effect cannot go on indefinitely; at some point the ester must become too long. Apparently, this occurs when the chain is longer than C7, since then the rate constant k_2 of the C8 ester of III is lower (Table 8). This may indicate that the distance between acyl group and ionized secondary hydroxyl group is then increased beyond the optimal.

Table 8 also shows the apparent dissociation constants K_{TS} for the cleavage of the esters III by β-CD. The changes in K_{TS} are not very regular and there is no clear relationship between the transition state energy and the acyl chain length.

Table 9 shows the values of K_{TS} for IV decrease with increasing acyl chain length of the ester for both α- and β-CD. This trend indicates that the binding of the transition state is influenced by the length of the alkyl chain, and it demonstrates the acyl moiety of the ester is included in the CD cavity for the cleavage of IV with α- or β-CD.

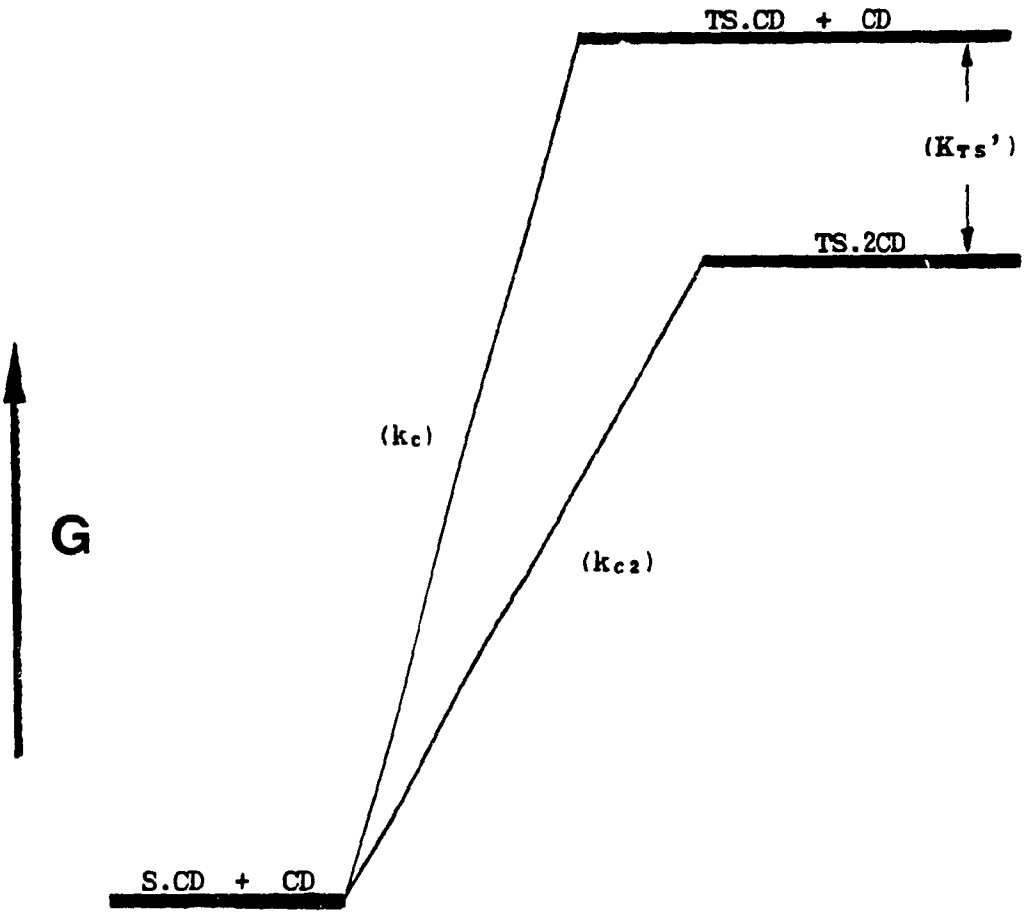
By analogy with K_{TS} (see eq 17), we may define an apparent constant K_{TS}'as follows:

$$K_{TS}' = kc/kc_2 \qquad (24)$$

Figure 17 shows the significance of this equation in a free energy diagram. Obviously, the value of $\ln(K_{TS}')$ is directly related to the energy difference between the transition state for the cleavage of the 1:1 ester.CD complex (S.CD) and that for cleavage by the second-order process (S.CD + CD $\longrightarrow$ P, eq 20).

The values of K_{TS}' in Tables 8 (for III with β-CD) and 9 (for IV with α- and β-CD) were calculated, using eq 24. Unlike the values of

K_{TS}, which vary significantly with acyl chain length, the values of K_{TS}' are quite close to each other, within a given series. This may be taken to mean that the aryl portions of the esters insert into the cavity of the second CD, during the second-order cleavage process.



Where:

CD: Cyclodextrin; S.CD: Substrate-Cyclodextrin complex;

TS.CD: Transition state-Cyclodextrin complex;

TS.2CD: Transition state-Two molecule of cyclodextrin complex.

Figure 17. The energy difference between the transition state of cleavage of 1:1 complex and the second-order complex process.

CHAPTER 4. SUMMARY

The kinetic analysis supports the cleavage of substituted phenyl alkanoates by cyclodextrins through a complexation pathway. The kinetic evidence shows that the formation of the complex has the phenyl portion inserted into the CD cavity for the short chain esters, whereas the acyl moiety is included in the CD cavity for the longer chain esters. The behavior of substituted phenyl ester cleavage is influenced by substituents on the phenyl portion, as outlined below.

The kinetics of ester cleavage of 4-carboxy-2-nitro and 2-carboxy-4-nitrophenyl acetate, p- and m-nitrophenyl acetates (C2), propanoates (C3), butanoates (C4), pentanoates (C5), and hexanoates (C6) in aqueous base containing α- or β-CD indicate that there are processes involving a normal 1:1 complex with cyclodextrins. In the cases of 4-carboxy-2-nitrophenyl propanoate to octanoate (C3 to C8) reacting with α-CD there is the formation of an unreactive 2:1 (CD.ester) binding complex. With β-CD some of these esters (C5 to C8) there is a second order process, second-order in CD, which provides an additional reaction pathway. Similar behavior was found for longer 2-carboxy-4-nitrophenyl esters, which undergo second order cleavage processes with α- and β-CD.

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CHAPTER 5. EXPERIMENTAL

5.1 MATERIALS

The cyclodextrins, acid anhydrides, acid chlorides, 3-nitrophenol and 4-hydroxy-3-nitrobenzoic acid were all obtained from Aldrich. The 4-nitrophenyl alkanoates were from Sigma. 3-Nitrophenyl alkanoates were prepared by the reaction of 3-nitrophenol with the appropriate acid anhydride, in the presence of conc. H_2SO_4.[53] Below, the melting points are uncorrected.

2-Nitro-4-carboxyphenyl esters (C2 to C5) were synthesized[42] by the following procedure: 3-Nitro-4-hydroxybenzoic acid (1.831 g, 1 mmol) was dissolved in 15.5 mL of 3 N NaOH, and a two-fold excess of the acid anhydride was added quickly to the solution, with vigorous stirring. After 4 min, 1.0 N HCl was added until a precipitate appeared. The product was filtered off and washed with water. The melting points were: acetate, 152-4°C (lit[43] 152°C); propanoate, 142-3°C; butanoate, 130-130.5°C; pentanoate: 122-3°C.

2-Nitro-4-carboxyphenyl hexanoate (and heptanoate) were prepared by combining 3-nitro-4-hydroxybenzoic acid (3.662 g, 2 mmol) with hexanoic (or heptanoic) anhydride (4 mmol) in the presence of 5 drops of conc. H_2SO_4 at 85-95°C. After 4h the product was washed with a saturated solution of sodium bicarbonate. The melting points of the products were: 88.5-90°C and 76-7°C (lit.[43] 75°C), respectively.

2-Carboxy-4-nitrophenyl acetate, butanoate, hexanoate and octanoate were prepared, using both of the above procedures, by reacting 5-nitro-2-hydroxybenzoic acid (5-nitrosalicylic acid, Lancaster Synthesis) with

acid anhydrides. The melting points of the esters were: 159-162°C for acetate, 97-9°C for butanoate, and 90-2°C for hexanoate.

2-Nitro-4-carboxyphenyl octanoate and 2-ethylhexanoate were prepared by combining 3-nitro-4-hydroxybenzoic acid with the appropriate acid chloride in benzene and refluxing the reaction mixture for 8h and 14h, respectively. The melting point of the octanoate was 68-9°C and that of the 2-ethylhexanoate 59-62°C.

2-Nitro-4-carboxyphenyl 4-methylpentanoate was synthesized as follows. A mixture of 4-methylpentanoic acid (1.74g, 15 mmoles), dicyclohexylcarbodiimide (3.095 g, 15 mmol), and p-toluene sulfonic acid (150 mg) were dissolved in a mixture of pyridine (3 mL) and benzene (50 mL). To this solution was added 3-hydroxy-4-nitrobenzoic acid (2.80 g, 15 mmol) and the mixture was allowed to react for 4 days at the room temperature.[44-46] The solvents were removed by distillation and the product was washed with 1 M hydrochloric acid and then with a saturated aqueous solution of sodium bicarbonate. The product was not crystalline.

The structure of all of the esters which were synthesized were confirmed by H1 n.m.r. spectroscopy, and by the uv-vis absorption that they gave on hydrolysis in aqueous base.

All kinetics measurements were done in a concentrated phosphate buffer of pH 11.6. The buffer was prepared by dissolving 214.5 g (0.8 moles) $Na_2HPO_4.7H_2O$ in 518.4 mL 1M NaOH and then diluting to 1 L with water. The high buffer capacity was needed to overcome changes in pH caused by the ionization of the CD hydroxyl groups (pKa 12.2).[2]

Stock solutions of the substituted phenyl esters were made by

dissolving 1 mmole of substrate in 10.00 mL methanol. Small amounts of these solutions were diluted with water to the required concentration for kinetic experiments, just before use.

5.2. KINETIC METHODS

The kinetics of cleavage of substituted phenyl esters were measured by monitoring the appearance of the phenoxide anion at the following wavelengths: p-nitrophenoxide, 405 nm; m-nitrophenoxide, 390 nm; 2-nitro-4-carboxyphenoxide, 407nm; 2-carboxy-4-nitrophenoxide, 370nm. A solution containing aqueous buffer and CD (0 - 40 mM) was mixed (1:1) with an aqueous solution of the ester (0.1 or 0.2 mM) in a stopped-flow apparatus attached to an Aminco DW-2 UV-Vis spectrophotometer. The observation cell was kept at 25°C ± 0.1°C.

The absorbance data were analyzed by an Apple II microcomputer which was interfaced to the spectrophotometer via a Cyborg Isaac 91a. Under the conditions studied, phenoxide ion appearance showed good pseudo first-order behavior for more than 90% reaction. The rate constants (k_{obs}) were determined from the slope of $\ln(A_{inf} - A_t)$ vs. time. The final absorbance value (A_{inf}) was measured after ten half lives (>99.9% reaction).

The computer program used in this study to analyze the dependence of k_{obs} on [CD] is called CDFIT, written in TURBOPASCAL by Mr. Bryan Takasaki and Dr. O. S. Tee. This program has five options for data analysis:

1. Linear least-squares fitting to both the Linewaever-Burk and Eadie-

Hofstee transforms of the normal equation (eq 7) for reaction via a 1:1 complex. Non-linear least-squares fitting to eq 7, keeping k_u fixed at the observed value, and treating k_c and K_d as parameters.

2. Non-linear least-squares fitting to eq 7, allowing k_u to vary, along with k_c and K_d.

3. Non-linear least-squares fitting to eq 19, which allows for non-productive 2:1 binding, keeping k_u fixed at the observed value, and treating k_c, K_d and K_2 as parameters.

4. Non-linear least-squares fitting to eq 21, which allows for a process that is second-order in [CD], keeping k_u fixed at the observed value, and treating k_c, K_d and k_{c2} as parameters.

5. Non-linear least-squares fitting to eq 19, which allows for non-productive 2:1 binding, keeping k_u fixed at the observed value, k_c fixed at zero (ie. totally absent from the fitting), and treating K_d and K_2 as the parameters to be fitted.

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APPENDIX

Table 10. Rate Constants for the Cleavage of m- and p-Nitrophenyl Alkanoates in the Presence of α-Cyclodextrin.[a]

[CD]	k_{obsd}, s^{-1}				
mM	C2	C3	C4	C5	C6
(a) m-nitro:					
0	0.0858	0.0396	0.0293	0.0284	0.0238
1.0	1.04	0.619	0.515	0.418	0.456
2.0	1.77	1.11	0.901	0.659	0.720
4.0	3.55	1.76	1.35	1.01	1.07
6.0	4.87	2.18	1.63	1.22	1.22
8.0	6.14	2.40	1.93	1.32	1.38
10.0	7.08	2.81	2.09	1.41	1.46
k_c (s^{-1})[b]=	24.6	4.50	3.16	1.99	1.96
sd =	4.1	0.21	0.10	0.04	0.03
K_d (mM)[b] =	25.0	6.51	5.37	4.07	3.49
sd =	4.9	0.47	0.30	0.16	0.11
r =	0.931	0.990	0.994	0.997	0.998

(b) p-nitro:

0	0.0956	0.0992	0.0580	0.0450	0.0494
1	0.111	0.107	0.0670	0.0559	0.0740
2	0.124	0.114	0.0739	0.0627	0.0897
4	0.144	0.122	0.0830	0.0713	0.104
6	0.160	0.128	0.0885	0.0759	0.113
8	0.171	0.130	0.0914	0.0788	0.121
10	0.181	0.131	0.0944	0.0801	0.125
k_c (s^{-1})[b]=	0.267	0.203	0.113	0.0928	0.146
sd =	0.002	0.023	0.001	0.0007	0.002
K_d (mM)[b] =	10.1	11.6	5.00	3.37	2.88
sd =	0.2	3.1	0.22	0.10	0.14
r =	0.999	0.9997	0.996	0.998	0.995

[a] At 25°C, in a 0.2M phosphate buffer of pH 11.6-7 containing 0.1%(v/v) MeOH.

[b] These results were obtained from Eadie-Hofstee analysis.

Table 11. Rate Constants for the Cleavage of m- and p-Nitrophenyl Alkanoates in the Presence of β-Cyclodextrin.[a]

[CD],	k^{obsd}, s^{-1}				
mM	C2	C3	C4	C5	C6
(a) m-nitro:					
0	0.0858	0.0401	0.0274	0.0284	0.0232
1.0	0.486	0.291	0.213	0.214	0.229
2.0	0.801	0.495	0.343	0.312	0.330
4.0	1.29	0.731	0.503	0.432	0.424
6.0	1.75	0.910	0.576	0.479	0.483
8.0	2.21	1.03	0.639	0.518	0.499
10.0	2.44	1.08	0.669	0.532	0.505
k_c (s^{-1})[b]=	5.25	1.65	0.914	0.662	0.606
sd =	0.45	0.04	0.023	0.009	0.010
K_d (mM)[b] =	12.3	5.23	3.68	2.41	1.81
sd =	1.5	0.23	0.18	0.08	0.08
r =	0.999	0.996	0.995	0.997	0.996

(b) p-nitro:

0	0.0956	0.0998	0.0575	0.0466	0.0494
1.0	0.173	0.159	0.113	0.0917	0.104
2.0	0.232	0.202	0.149	0.114	0.128
4.0	0.321	0.263	0.186	0.140	0.147
6.0	0.400	0.295	0.202	0.149	0.153
8.0	0.438	0.324	0.213	0.154	0.160
10.0	0.473	0.341	0.222	0.156	0.164
k_c (s^{-1})[b]=	0.775	0.468	0.268	0.181	0.179
sd =	0.024	0.006	0.005	0.003	0.002
K_d (mM)[b] =	7.83	5.18	2.70	1.95	1.34
sd =	0.42	0.14	0.13	0.11	0.05
r =	0.994	0.999	0.996	0.994	0.997

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[a] At 25 °C, in a 0.2M phosphate buffer of pH 11.6-7 containing 0.1%(v/v) MeOH.

[b] These results were obtained from Eadie-Hofstee analysis.

Table 12. Rate Constants for the Cleavage of 4-Carboxy-2-nitrophenyl Alkanoates in the Presence of α-Cyclodextrin.[a]

[CD], mM	k_{obsd}, s^{-1}	k_{calcd}, s^{-1}	
acetate:			
0	0.0964	0.09640	
1.0	0.104	0.1037	k_c (s^{-1})[b]= 0.171
2.0	0.110	0.1098	sd = 0.002
4.0	0.119	0.1193	K_d (mM)[b] = 8.8
6.0	0.126	0.1263	sd = 0.34
8.0	0.132	0.1317	r = 0.9970
10.0	0.136	0.1360	
propionate:			
0	0.0529	0.0[illegible]0	
2.0	0.0503	0.05052	K_d (mM)[c]= 50.4
4.0	0.0477	0.04771	sd = 1.2
6.0	0.0448	0.04464	K_2 (mM)[c]= 10.8
8.0	0.0414	0.04146	sd = 0.5
10.0	0.0384	0.03829	
12.0	0.0352	0.03523	k_c (s^{-1}) = 0.0[d]
[illegible].0	0.0295	0.02962	
20.0	0.0249	0.02485	r = 0.9999

butanoate:

0	0 0265	0.02650	
1.0	0.0246	0.02470	
2.0	0.0230	0.02296	
4.0	0.0199	0.01984	k_c (s^{-1})^c= 0.0126
6.0	0.0172	0.01723	sd = 0.0024
8.0	0.0151	0.01510	K_d (mM)^c = 7.66
10.0	0.0134	0.01336	sd = 1.05
12.0	0.0118	0.01193	K_2 (mM)^c = 14.3
16.0	0.00985	0.009749	sd = 1.70
20.0	0.00817	0.008192	r = 0.9999

pentanoate:

0	0.0290	0.02900	
1.0	0.0264	0.02640	
2.0	0.0246	0.02457	
4.0	0.0219	0.02189	k_c (s^{-1})^c= 0.0222
6.0	0.0198	0.01989	sd = 0.0021
8.0	0.0182	0.01829	K_d (mM)^c = 2.07
10.0	0.0169	0.01695	sd = 0.87
12.0	0.0161	0.01580	K_2 (mM)^c = 21.8
16.0	0.0140	0.01394	sd = 2.9
20.0	0.0123	0.01248	r = 0.9997

hexanoate:

0	0.0271	0.02710	
1.0	0.0308	0.03092	
2.0	0.0320	0.03185	k_c (s^{-1}), c = 0.0375
4.0	0.0316	0.03150	sd = 0.0005
6.0	0.0301	0.03034	K_d (mM) c = 1.43
8.0	0.0290	0.02902	sd = 0.16
10.0	0.0278	0.02771	K_2 (mM) c = 28.7
12.0	0.0265	0.02646	sd = 1.30
16.0	0.0242	0.02421	r = 0.9989

heptanoate:

0	0.0256	0.02560	k_c (s^{-1}) c = 0.0629
1.0	0.0424	0.04257	sd = 0.0016
2.0	0.0468	0.04644	K_d (mM) c = 1.05
4.0	0.0461	0.04640	sd = 0.11
6.0	0.0439	0.04399	K_2 (mM) c = 16.8
8.0	0.0415	0.04123	sd = 1.28
10.0	0.0385	0.03859	r = 0.9995

octanoate:

0	0.0222	0.02220	k_c (s^{-1})[c] = 0.0737
1.0	0.0553	0.05507	sd = 0.0016
2.0	0.0591	0.05971	K_d (mM)[c] = 0.503
4.0	0.0604	0.05983	sd = 0.056
6.0	0.0575	0.05756	K_2 (mM)[c] = 26.2
8.0	0.0548	0.05489	sd = 2.37
10.0	0.0522	0.05225	r = 0.9996

2-ethylhexanoate:

0	0.00104	0.001040	
1.0	0.00130	0.001296	k_c (s^{-1})[b] = 0.00188
2.0	0.00142	0.001433	sd = 0.00002
4.0	0.00157	0.001576	
6.0	0.00166	0.001649	K_d (mM)[b] = 2.28
8.0	0.00172	0.001694	sd = 0.12
12.0	0.00173	0.001747	
16.0	0.00177	0.001776	r = 0.9923

4-methylpentanoate:

0	0.0254	0.02540	k_c (s^{-1})[b]=	0.0174
1.0	0.0215	0.02167	sd =	0.0004
4.0	0.0195	0.01910	K_d (mM)[b] =	1.08
6.0	0.0185	0.01857	sd =	0.18
10.0	0.0179	0.01809	r =	0.9739

[a] At 25°C, in a 0.4 phosphate buffer of pH 11.6 containing 0.1%(v/v) MeOH.

[b] These results were obtained from Eadie-Hofstee analysis.

[c] These values were obtained from fitting the equation:

$$k_{obs} = \frac{(k_u.K_d + k_c[CD])K_2}{(K_d.K_2 + K_2[CD] + [CD]^2)}$$

[d] Fixed at zero. Fitting this parameter gives a small negative value.

Table 13. Rate Constants for the Cleavage of 4-Carboxy-2-nitrophenyl Alkanoates in the Presence of β-Cyclodextrin.[a]

[CD], mM	k^{obsd}, s^{-1}	k^{calcd}, s^{-1}	
acetate:			
0	0.0964	0.0964	k_c (s^{-1})[b]= 0.276
1.0	0.123	0.1216	sd = 0.005
2.0	0.141	0.1408	K_d (mM)[b] = 5.89
4.0	0.167	0.1682	sd = 0.293
6.0	0.187	0.1868	r = 0.9950
8.0	0.200	0.2003	
10.0	0.211	0.2105	
propionate:			
0	0.0501	0.05010	
1.0	0.0475	0.04747	
2.0	0.0455	0.04554	k_c (s^{-1})[b]= 0.0330
4.0	0.0428	0.04290	sd = 0.0002
6.0	0.0414	0.04118	
8.0	0.0399	0.03997	K_d (mM)[b] = 5.51
10.0	0.0390	0.03907	sd = 0.14
12.0	0.0384	0.03838	
15.0	0.0376	0.03759	r = 0.9980

butanoate:

0	0.0291	0.02910	k_c (s^{-1})[b] = 0.00777
1.0	0.0207	0.02069	sd = 0.00008
2.0	0.0171	0.01704	
4.0	0.0136	0.01370	K_d (mM)[b] = 1.54
6.0	0.0121	0.01213	sd = 0.02
8.0	0.0112	0.01122	
10.0	0.0107	0.01062	r = 0.9998

pentanoate:

0	0.0309	0.03090	k_c (s^{-1})[c] = 0.00208
1.0	0.0161	0.01600	sd = 0.00048
2.0	0.0113	0.01146	k_{c2} (M^{-1} s^{-1})[c] = 0.218
4.0	0.00820	0.008176	sd = 0.046
6.0	0.00706	0.007042	K_d (mM)[c] = 0.920
8.0	0.00674	0.006613	sd = 0.039
10.0	0.00640	0.006500	r = 0.9999

hexanoate:

0	0.0235	0.02350	k_c (s^{-1})[c] = 0.00669
1.0	0.0115	0.01143	sd = 0.00030
2.0	0.00945	0.009624	k_{c2}(M^{-1} s^{-1})[c] = 0.149
4.0	0.00878	0.008693	sd = 0.032
6.0	0.00860	0.008532	K_d (mM)[c] = 0.380
8.0	0.00857	0.008591	sd = 0.030
10.0	0.00871	0.008741	r = 0.9999

heptanoate:

0	0.0256	0.02560	
0.20	0.0206	0.02077	
0.40	0.0189	0.01884	
0.60	0.0180	0.01782	k_c (s^{-1})[c] = 0.0142
1.0	0.0168	0.01679	sd = 0.0002
2.0	0.0158	0.01594	k_{c2} (M^{-1} s^{-1})[c]= 0.236
4.0	0.0158	0.01576	sd = 0.021
6.0	0.0160	0.01600	K_d (mM)[c] = 0.272
8.0	0.0163	0.01635	sd = 0.013
10.0	0.0168	0.01675	r = 0.9995

octanoate:

0	0.0222	0.02220	
0.20	0.0229	0.02304	
0.40	0.0237	0.02364	
0.60	0.0242	0.02409	k_c (s^{-1})[c] = 0.0263
1.0	0.0247	0.02477	sd = 0.0003
2.0	0.0259	0.02588	k_{c2} (M^{-1} s^{-1})[c]= 0.235
4.0	0.0273	0.02738	sd = 0.024
6.0	0.0287	0.02863	K_d (mM)[c] = 0.709
8.0	0.0298	0.02979	sd = 0.103
10.0	0.0309	0.03092	r = 0.9997

2-ethylhexanoate:

0	0.00104	0.001040	k_c (s^{-1})[c]= 9.35 x 10^{-5}
1.0	0.000394	0.0003950	sd = 8.05 x 10^{-6}
2.0	0.000284	0.0002826	k_{c2} (M^{-1} s^{-1})[c]= 0.00853
4.0	0.000223	0.0002207	sd = 0.00085
6.0	0.000205	0.0002077	K_d (mM)[c]= 0.454
8.0	0.000206	0.0002089	sd = 0.015
10.0	0.000219	0.0002162	r = 0.9999

4-methylpentanoate:

0	0.0255	0.02550	k_c (s^{-1})[c] = 0.00479
1.0	0.00914	0.009082	sd = 0.00026
2.0	0.00719	0.007317	k_{c2} (M^{-1} s^{-1})[c]= 0.104
4.0	0.00641	0.006422	sd = 0.029
6.0	0.00635	0.006233	K_d (mM)[c] = 0.255
8.0	0.00629	0.006236	sd = 0.019
10.0	0.00623	0.006319	r = 0.9999

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[a] At 25°C, in a 0.4 phosphate buffer of pH 11.6 containing 0.1%(v/v) MeOH.

[b] These results were obtained from Eadie-Hofstee analysis.

[c] These values were obtained from fitting the equation:

$$k_{obs} = (k_u.K_d + k_c[CD] + k_{c2}[CD])/(K_d + [CD])$$

Table 14. Rate Constants for the Cleavage of 2-Carboxy-4-nitrophenyl Alkanoates in the Presence of α-Cyclodextrin.[a]

[CD], mM	k^{obsd}, s^{-1}	k^{calcd}, s^{-1}	
acetate:			
0	0.0107	0.01070	
1.0	0.0113	0.01142	
2.0	0.0120	0.01214	
4.0	0.0135	0.01357	k_2 (M^{-1} s^{-1}) = 0.712[b]
6.0	0.0149	0.01500	
8.0	0.0164	0.01642	sd = 0.0119
10.0	0.0176	0.01783	
12.0	0.0194	0.01924	
16.0	0.0225	0.02203	
20.0	0.0245	0.02479	r = 0.9989
butanoate:			
0	0.00519	0.005190	
1.0	0.00764	0.007431	
2.0	0.00930	0.009140	
4.0	0.0114	0.001167	k_c (s^{-1})[c]= 0.0182
6.0	0.0134	0.001356	sd = 0.0016
8.0	0.0151	0.001510	k_{c2} (M^{-1} s^{-1})[c]= 0.382
10.0	0.0165	0.001644	sd = 0.048
12.0	0.0178	0.001764	K_d (mM)[c] = 4.98
16.0	0.0199	0.001979	sd = 0.92

20.0	0.0216	0.002174	r = 0.9996
hexanoate:			
0	0.00538	0.005380	k_c (s^{-1})[c]= 0.0179
1.0	0.00769	0.007668	sd = 0.0023
2.0	0.00946	0.00[illegible]440	k_{c2} (M^{-1} s^{-1})[c]= 0.556
4.0	0.0121	0.01215	sd = 0.103
6.0	0.0142	0.01426	K_d (mM)[c] = 4.72
8.0	0.0162	0.01606	sd = 1.08
10.0	0.0176	0.01767	r = 0.9999
octanoate:			
0	0.00508	0.005080	k_c (s^{-1})[c]= 0.0196
1.0	0.0126	0.01278	sd = 0.0009
2.0	0.0159	0.01556	k_{c2} (M^{-1} s^{-1})[c]= 0.466
4.0	0.0181	0.01831	sd = 0.083
6.0	0.0200	0.02002	K_d (mM)[c]= 0.944
8.0	0.0214	0.02137	sd = 0.148
10.0	0.0226	0.02258	r = 0.9996

[a] At 25°C, in a 0.4 phosphate buffer of pH 11.6 containing 0.1%(v/v) MeOH.

[b] Values of k_c and K_d are not available since saturation kinetics were not observed (for [CD] = 0 up to 20 mM). The plot of k_{obs} vs [CD] was linear, from which the slope = k_2 provides the value of k_c/K_d.

[c] These results were obtained from fitting the equation:

$$k_{obs} = (k_u.K_d + k_c[CD] + k_{c2}[CD])/(K_d + [CD])$$

Table 15. Rate Constants for the Cleavage of 2-Carboxy-4-nitrophenyl Alkanoates in the Presence of β-Cyclodextrin.[a]

[CD], mM	k_{obsd}, s^{-1}	k_{calcd}, s^{-1}	
acetate:			
0	0.0100	0.01000	k_c (s^{-1})[b]= 0.0859
1.0	0.0131	0.01297	sd = 0.0096
2.0	0.0155	0.01574	
4.0	0.0206	0.02073	K_d (mM)[b] = 24.2
6.0	0.0253	0.02512	sd = 3.7
8.0	0.0292	0.02901	
10.0	0.0323	0.03247	r = 0.9998
butanoate:			
0	0.00526	0.005260	k_c (s^{-1})[b]= 0.00832
1.0	0.00559	0.005541	sd = 0.00042
2.0	0.00577	0.005784	
4.0	0.00619	0.006184	K_d (mM)[b] = 8.81
6.0	0.00644	0.006501	sd = 1.79
8.0	0.00682	0.006757	
10.0	0.00695	0.006968	r = 0.9980

hexanoate:

0	0.00531	0.005310	k_c (s^{-1})[c]= 0.00664
1.0	0.00579	0.005784	sd = 0.00009
2.0	0.00604	0.006047	k_{c2} (M^{-1} s^{-1})[c]=0.0588
4.0	0.00636	0.006366	sd = 0.0062
6.0	0.00660	0.006584	K_d (mM)[c] = 1.93
8.0	0.00675	0.006761	sd = 0.22
10.0	0.00692	0.006918	r = 0.9999

octanoate:

0	0.00510	0.005100	k_c (s-1)[c]= 0.0122
1.0	0.00878	0.008806	sd = 0.0002
2.0	0.0102	0.01015	k_{c2} (M^{-1} s^{-1})[c]= 0.186
4.0	0.0114	0.01144	sd = 0.013
6.0	0.0122	0.01220	K_d (mM)[c] = 0.976
8.0	0.0128	0.01279	sd = 0.049
10.0	0.0133	0.01330	r = 0.9999

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[a] At 25°C, in a 0.4 phosphate buffer of pH 11.6 containing 0.1%(v/v) MeOH.

[b] These results were obtained from Eadie-Hofstee analysis.

[c] These values were obtained from fitting the equation:

$$k_{obs} = (k_u.K_d + k_c[CD] + k_{c2}[CD])/(K_d + [CD])$$