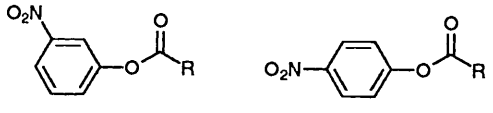
The Kinetics of Cleavage of Nitrophenyl Alkanoates by γ-Cyclodextrin and by 'Dimethyl-β-cyclodextrin' in Basic Aqueous Solution

Oswald S. Tee* and Timothy A. Gadosy
Department of Chemistry and Biochemistry, Concordia University, Montréal, Québec, Canada H3G 1M8

The cleavage of *m*- and *p*-nitrophenyl alkanoates (C_2 to C_8) in basic solution is accelerated modestly (7–17 times) by γ-cyclodextrin (γ-CD). The effects on the two isomeric series of esters are virtually the same and they hardly vary with the ester chain length. Cleavage of the same esters (C_2 to C_{10}) is retarded by 'dimethyl-β-cyclodextrin' (diMe-β-CD) by up to a factor of 8, but it is not totally inhibited, as was supposed earlier. The effects of this modified cyclodextrin on the two series of esters are very similar, but they vary significantly with the acyl chain lengths. For example, second-order rate constants (k_2) for reaction of the esters with diMe-β-CD show little change from C_2 to C_5, a steep increase from C_5 to C_7, and then a levelling off. Overall, the behaviours of γ-CD and diMe-β-CD differ significantly from those found earlier for ester cleavage by α-CD, β-CD and 'hydroxypropyl-β-CD'. These differences are discussed in terms of the relative importance of transition-stage and initial-state binding, and in relation to the structural variations among the five cyclodextrins.

The basic cleavage of aryl esters by cyclodextrins[1,†] (CDs) in aqueous solution has been the subject of many studies that are summarized in reviews.[1–5] For the most part, these investigations have involved α-CD[1] or β-CD[1] and very few studies have been carried out with γ-CD[1] or with modified CDs[1,6] due to their limited availability or high cost. These derivatives are now more accessible and further research on their chemistry is warranted, particularly since they are likely to have many practical applications in the future.[1b,7,8] For such reasons, we recently examined the binding of 'hydroxypropyl-β-cyclodextrin' (Hp-β-CD) to alkyl-bearing compounds[9] and its ability to cleave aryl esters.[10]

The present report deals with the cleavage of *m*- and *p*-nitrophenyl alkanoates (*m*NPAlk and *p*NPAlk) by γ-CD and by the modified derivative, 'dimethyl-β-cyclodextrin' (diMe-β-CD).[1b,6,7] As explained in detail in earlier work,[4,5,10,11] cleavage of the two series of isomers provide a means of probing the relative importance of aryl and alkyl binding effects in the initial state and transition state of a reaction, as well as of the sensitivity of these effects to alkyl chain length.



m NPAlk *p* NPAlk

(RCO = C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10})

The principal cyclodextrins are α-, β- and γ-CD, although others have been mooted.[12] The three main ones have six, seven and eight glucose units, respectively, joined in a toroidal fashion[1] so that the widths of their cavities increase from α-CD to γ-CD, but their depths are all the same. As is reasonable, the propensities of the three CDs to form inclusion compounds with molecules of different sizes vary accordingly.[1] It is to be expected, therefore, that γ-CD has quantitatively and qualitatively different effects on reactions because of its wider cavity.

'Dimethyl-β-cyclodextrin' (diMe-β-CD) is β-CD that has been alkylated so that, on average, all seven primary hydroxy groups and about 7 of the 14 secondary hydroxy groups are methylated.[1b,6] One effect of this modification is greatly to enhance solubility in water and to increase the ability of the CD to solubilize organics, relative to β-CD.[1b,7,8c] Additionally, the presence of seven methoxy groups around the narrower primary rim[1] of the cavity and another seven around the wider secondary rim[1] may be expected to influence the chemistry of the β-CD moiety. The primary methoxy groups may afford a floor to the bottom of the CD cavity, as proposed for other functionalities,[1b,13] thereby altering its binding characteristics. Also, the secondary methoxy groups may prevent or hinder the acyl transfer to an ionized secondary hydroxy group[1,5] during ester cleavage, as well as modifying inclusion.

Results

We have measured the kinetics of basic cleavage of *m*NPAlk and *p*NPAlk esters (C_2 to C_7) by γ-CD in a phosphate buffer of pH 11.6. The studies were more limited than planned, owing to a problem of precipitation with longer esters (see the Experimental). Pseudo-first-order rate constants (k_{obs}) were obtained over a range of CD concentrations and, as anticipated from earlier studies with other CDs, reaction of the esters with γ-CD shows saturation kinetics[1–5,10,11] (*e.g.*, Fig. 1). This behaviour conforms to reaction in the medium [eqn. (1)] and to reaction through a substrate•CD complex [eqn. (2)] or its kinetic equivalent.[4,5,11] For these two processes, with the usual condition that $[S•CD] < [S]_t \ll [CD]$, the variation of the observed rate constant with [CD] may be represented by eqn. (3).

$$S \xrightarrow{k_u} \text{products} \quad (1)$$

$$S + CD \underset{K_s}{\rightleftharpoons} S•CD \xrightarrow{k_c} \text{products} \quad (2)$$

$$k_{obs} = \frac{(k_u K_s + k_c[CD])}{(K_s + [CD])} \quad (3)$$

Data for four esters (two *meta* and two *para* isomers) are presented in Fig. 1 as examples of the observed behaviour, along with saturation curves calculated using eqn. (3). Fitted

† IUPAC-recommended names: cyclomaltohexaose (α-CD), cyclomaltoheptaose (β-CD), cyclomaltooctaose (γ-CD).

Table 1 Constants for the cleavage of *m*-nitrophenyl and *p*-nitrophenyl alkanoates by γ-cyclodextrin[a,b]

Ester	k_u/s^{-1}	k_c/s^{-1}	K_s/mmol dm^{-3}	k_2/dm^3 mol^{-1} s^{-1}	k_c/k_u	K_{TS}/mmol dm^{-3}
m-Nitrophenyl alkanoates						
C_2	0.0805	1.39 ± 0.10	22.6 ± 2.6	62	17	1.3
C_4	0.0401	0.624 ± 0.027	23.7 ± 1.7	26	16	1.5
C_6	0.0414	0.482 ± 0.077	14.4 ± 1.7	33	12	1.2
p-Nitrophenyl alkanoates						
C_2	0.104	1.61 ± 0.04	35.6 ± 1.3	45	15	2.4
C_3	0.105	1.28 ± 0.05	31.9 ± 1.8	40	12	2.7
C_4	0.0589	0.530 ± 0.032	14.7 ± 1.7	36	9.0	1.6
C_5	0.0594	0.515 ± 0.034	14.6 ± 1.8	35	8.7	1.7
C_6	0.0660	0.443 ± 0.010	10.7 ± 0.5	41	6.7	1.6
C_7	0.0558	0.434 ± 0.017	12.0 ± 1.0	36	7.8	1.5

[a] In aqueous solution, at a nominal pH of 11.6 and at 25 °C. The constants K_s and k_c were obtained by non-linear fitting of eqn. (3) to the kinetic data. The quoted uncertainties are the standard errors obtained from the fitting. [b] Four examples of the kinetic data and the fits to them are plotted in Fig. 1.

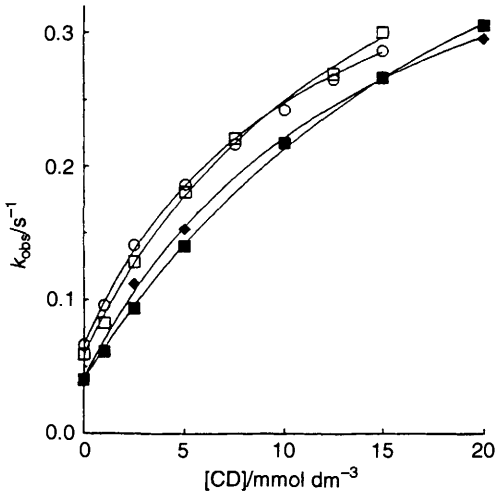


Fig. 1 Dependence of rate constants for the cleavage of four nitrophenyl alkanoates on the concentration of γ-CD. The esters are *m*NPAlk, C_4 (■), C_6 (◆); *p*NPAlk, C_4 (□), C_6 (○). The curves conform to saturation kinetics, calculated from eqn. (3), using the parameters in Table 1.

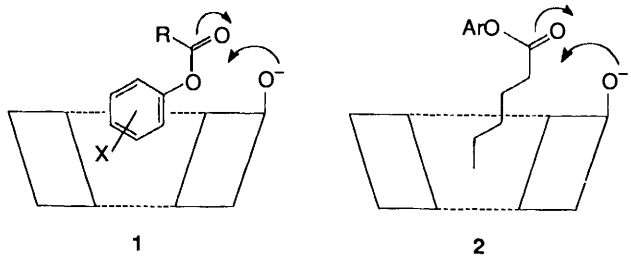
constants for all the esters studied are collected in Table 1. Note that k_{obs} values rise with increasing [γ-CD] because $k_c > k_u$, as is the case for *m*NPAlk and *p*NPAlk esters reacting with α-CD, β-CD and Hp-β-CD[10,11] but not for all types of ester.[5,14]

More extensive kinetic studies were possible for reaction of the two series of esters with diMe-β-CD because solubility was much less of a problem. Fig. 2(*a*) and (*b*) presents typical data for some of the esters, all of which show similar *downward* curvature of k_{obs}. Even though ester cleavage is slowed down by the CD in all cases, it is not totally inhibited ($k_c = 0$), as was presumed in an earlier, limited study of diMe-β-CD,[15] and the calculated curves still correspond to eqn. (3) but now with $k_c < k_u$. Fitted parameters for reaction of the esters with diMe-β-CD are presented in Table 2.

Previously, we observed significant 1:2 (ester:CD) binding for some 4(or 2)-carboxy-2(or 4)-nitrophenyl alkanoates reacting with α-CD and β-CD,[14b,c] and also more recently for the cleavage of the longer *m*NPAlk and *p*NPAlk esters by Hp-β-CD.[10] During the initial, exploratory phases of the present work we found evidence of such 1:2 binding with γ-CD at high concentration (up to 50 mmol dm^{-3}). This behaviour was not pursued as we were primarily interested in the 1:1 binding and its consequences. No evidence of 1:2 (ester:CD) binding was observed from the reaction of the esters with diMe-β-CD.

Discussion

The present study employs the same strategy as previous studies of the cleavage of *m*NPAlk and *p*NPAlk esters by CDs.[10,11] In brief, if the acyl transfer reaction occurs through a transition state having aryl group binding (**1**) we expect appropriate kinetic parameters to reflect the position of the aryl substituent and to be relatively insensitive to the alkanoate chain. On the other hand, if the transition state is stabilized by acyl group inclusion (**2**) the kinetic parameters should depend more on



1 **2**

the acyl chain length and not on the position of the aryl substituent.*

Upon inspection of the constants in Tables 1 and 2 one is immediately struck by the close similarity of the parameters for the reaction of the isomeric esters with each CD† but the behaviours of γ-CD and diMe-β-CD are quite dissimilar. By way of comparison, esterolyses by α-CD, β-CD and Hp-β-CD show significant differences between the *meta* and *para* series of esters but broad similarities for the three CDs.[10,11] Thus, both γ-CD and diMe-β-CD show different overall behaviours with the *m*NPAlk and *p*NPAlk esters than have been found hitherto. To facilitate subsequent discussion and comparisons, Table 3 contains some constants for *m*- and *p*-nitrophenyl hexanoate reacting with all five CDs.

Substrate Binding (K_s).—For the binding of the *m*NPAlk and *p*NPAlk esters to γ-CD, values of pK_s ($= -\log K_s$) show little variation with the alkanoate chain length (Fig. 3) and they are

* At the outset,[11] these expectations were based partly on the knowledge that the cleavage of *m*-substitued phenyl acetates by α-CD and by β-CD is generally more efficient than that of their *para* isomers.[2,5] It turns out that this distinction is absent for γ-CD (Table 1) and so it is not applicable as a potential probe of transition state binding. Still, the presence or absence of a firm dependence of kinetic parameters on the chain length of the alkanoate esters may be used as a criterion.[4,5,10,11,16]

† We note that Bender and co-workers[15a] found that the *m*- and *p*-*tert*-butylphenyl acetates also have quite similar kinetic parameters for reaction with γ-CD, but not for reaction with α-CD.

Table 2 Constants for the cleavage of *m*-nitrophenyl and *p*-nitrophenyl alkanoates by 'dimethyl-β-cyclodextrin'[a,b]

Ester	k_u/s^{-1}	k_c/s^{-1}	K_s/mmol dm^{-3}	k_2/dm^3 mol^{-1} s^{-1}	k_c/k_u	K_{TS}/mmol dm^{-3}
m-Nitrophenyl alkanoates						
C_2	0.080 7	0.039 2 ± 0.000 6	5.66 ± 0.24	6.9	0.49	12
C_3	0.081 2	0.021 0 ± 0.000 5	2.88 ± 0.08	7.3	0.26	11
C_4	0.050 4	0.008 87 ± 0.000 17	1.69 ± 0.03	5.3	0.18	9.6
C_5	0.049 1	0.006 50 ± 0.000 04	0.942 ± 0.024	6.9	0.13	7.1
C_6	0.049 7	0.010 2 ± 0.001 2	0.467 ± 0.041	22	0.21	2.3
C_7	0.050 3	0.009 33 ± 0.001 19	0.264 ± 0.028	35	0.19	1.4
C_8	0.048 9	0.009 83 ± 0.000 75	0.219 ± 0.017	45	0.20	1.1
p-Nitrophenyl alkanoates						
C_2	0.103	0.055 8 ± 0.000 6	4.64 ± 0.18	12	0.54	8.6
C_3	0.082 3	0.029 2 ± 0.001 1	4.15 ± 0.28	7.0	0.36	12
C_4	0.058 6	0.015 0 ± 0.000 3	2.27 ± 0.06	6.6	0.26	8.9
C_5	0.049 6	0.008 24 ± 0.000 31	1.42 ± 0.05	5.8	0.17	8.5
C_6	0.051 6	0.010 8 ± 0.001 2	0.767 ± 0.060	14	0.21	3.7
C_7	0.048 7	0.012 2 ± 0.000 7	0.378 ± 0.022	32	0.25	1.5
C_8	0.050 5	0.013 8 ± 0.001 0	0.176 ± 0.020	78	0.27	0.64
C_9	0.050 5	0.014 9 ± 0.000 4	0.160 ± 0.009	93	0.30	0.54
C_{10}	0.040 6	0.014 9 ± 0.002 0	0.197 ± 0.006	76	0.37	0.54

[a] As in Table 1. [b] Six examples of the kinetic data are presented in Fig. 2, together with the fitted curves.

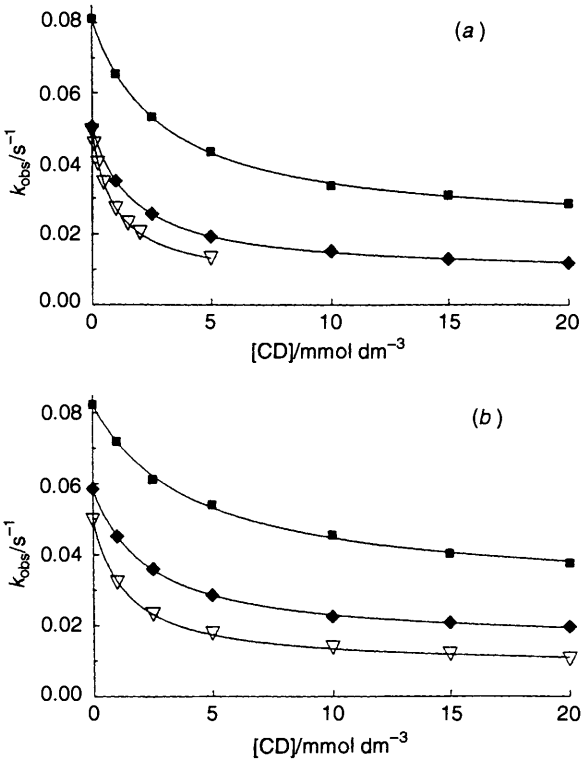


Fig 2. Dependence of rate constants for the cleavage of six nitrophenyl alkanoates on the concentration of diMe-β-CD: (*a*) *m*NPAlk esters; (*b*) *p*NPAlk esters. The acyl chain lengths are C_3 (■), C_4 (◆) and C_5 (▽). The curves conform to saturation kinetics, calculated from eqn. (3), using the parameters in Table 2.

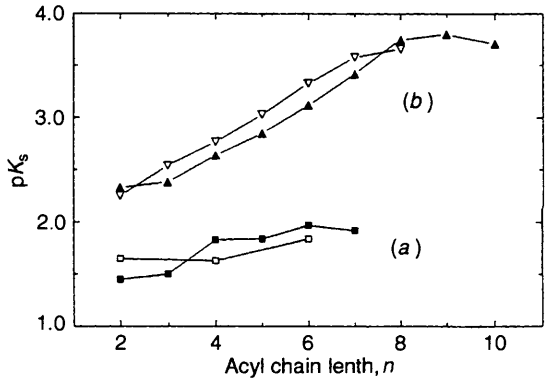


Fig. 3 Dependence of substrate binding (pK_s) of *m*- and *p*-nitrophenyl alkanoates on acyl chain length (*n*) for (*a*) γ-CD and (*b*) diMe-β-CD. The open symbols are for the *m*NPAlk esters and the solid symbols are for the *p*NPAlk esters.

hardly different for the two series of esters (Table 1). Moreover, γ-CD binds the esters more weakly than does either α-CD or β-CD[11,16] (*e.g.*, Table 3). We conclude that γ-CD, with its wider cavity,[1] probably binds to the *m*NPAlk and *p*NPAlk esters by aryl group inclusion,‡ unlike α-CD, β-CD or Hp-β-CD which form 1:1 complexes with most alkanoate esters by acyl group inclusion.[9-11,16]

In contrast with those found for γ-CD, the pK_s values for diMe-β-CD vary significantly with the ester chain length (Fig. 3) but they are quite similar for each pair of isomeric esters (Table 2); comparable behaviour was found earlier for α-CD, β-CD and Hp-β-CD.[9-11,16] For those CDs it was argued that they bind to *m*NPAlk and *p*NPAlk esters longer than the acetate by acyl group inclusion and the same conclusion is made here for diMe-β-CD.

Another feature of the binding to diMe-β-CD, not previously found for the *m*NPAlk and *p*NPAlk esters,[10,11,15] is the levelling of pK_s beyond the C_7 esters. Such levelling, which is evident for transition state binding also (*vide infra*), has been observed with alkanes and aliphatic surfactants complexing with α-CD and β-CD, but generally at longer alkyl chain lengths ($> C_8$).[17] It is attributable to the finite depths of CD cavities which are such that only six or seven methylene groups of an extended *n*-alkyl chain can be included and thereby shielded from the aqueous medium.[17d]

Rate Acceleration (k_c/k_u).—This ratio measures the limiting acceleration or retardation of the reaction at saturating levels of the CD. Esterolysis of the *m*NPAlk and *p*NPAlk esters is accelerated by γ-CD ($k_c > k_u$), as it is by α-CD and β-CD.[11,15]

‡ It is possible that γ-CD binds to the esters by acyl group inclusion but that the fit of alkyl chains in the wide cavity is so loose and inexact that a strong dependence on chain length, such as is found with α- and β-CD,[11] is not observed.

Table 3 Constants for the reaction of *m*- and *p*-nitrophenyl hexanoate with various CDs[a]

CD	k_c/k_u	K_s/mmol dm^{-3}	k_2/dm^3 mol^{-1} s^{-1}	K_{TS}/mmol dm^{-3}
m-Nitrophenyl hexanoate				
α	83	3.5	570	0.042
β	27	1.8	340	0.067
γ	12	14	33	1.2
Hp-β-CD	6.6	1.3	152	0.20
diMe-β-CD	0.21	0.47	22	2.3
p-Nitrophenyl hexanoate				
α	3.1	2.9	52	0.94
β	3.7	1.3	140	0.35
γ	6.7	11	41	1.6
Hp-β-CD	2.1	1.6	59	0.76
diMe-β-CD	0.21	0.77	14	3.7

[a] The values for α-CD and β-CD are taken from ref. 11 and those for Hp-β-CD are from ref. 10. The remaining values are from the present study (Tables 1 and 2).

However, with γ-CD the acceleration is modest (7–17 times) and virtually the same for both series of esters (Table 1) whereas with α-CD and β-CD it is generally greater for the *meta* isomers (e.g., Table 3) for chain lengths below C_{10}.[10,11]

Basic cleavage of the two series of esters is retarded by diMe-β-CD ($k_c < k_u$), and to a similar extent in each series (Fig. 2, Table 2). Presumably, the methyl groups on some of the secondary OH groups of the CD, where the acyl transfer takes place,[1-5] hinder reaction compared to that with β-CD or Hp-β-CD. Furthermore, the hindrance is more severe for the *meta* isomers because they are generally more reactive towards β-CD.[11] For example, as seen in Table 3, the *m*NPAlk C_6 ester reacts with β-CD such that k_c/k_u is 27 but for diMe-β-CD the ratio is 0.21, so that the overall reduction in k_c is 130-fold. For the isomeric *p*NPAlk C_6 ester, which is less reactive with β-CD, the rate reduction is only 18-fold. Clearly, some of the methyl groups around the secondary rim of diMe-β-CD prevent the *meta* isomer from reacting in the normally preferred geometry,[2-5] thereby wiping out the advantage that the cleavage of *meta* isomers usually enjoys.

Substrate Selectivity (k_2).—At fixed pH, the second-order rate constant for the reaction S + CD ⟶ products, k_2 [$=k_c/K_s$, eqn. (2)] measures the reactivity of the CD towards the ester (here, S). Thus, for a particular CD, values of k_2 indicate its ability to select between different esters under non-saturating conditions, and variations with ester structure may provide clues to the mode of binding of the esters to the CD in the transition state of the cleavage reaction.[4,5,10,11]

From this point of view, γ-CD is not very discriminating in its reaction with the two series of nitrophenyl esters. Values of k_2 show little variation with ester chain length and little difference between each pair of *meta* and *para* isomers. This behaviour is in sharp contrast with that shown by the three CDs studied previously.[10,11] For cleavage of the same two series of esters by α-CD, the *meta* isomers are much more reactive and k_2 values vary little with the chain length of the ester whereas for the *para* isomers k_2 increases significantly with the chain length. For cleavage by β-CD and Hp-β-CD the picture is similar but more complex for *p*NPAlk, as discussed later. The lack of sensitivity to chain length shown by the reaction with γ-CD presumably results from its wider cavity in which the binding of linear *n*-alkyl chains is loose and less energetically favourable than for the other, smaller CDs.[1] Likewise, the absence of the usual distinct preference for reaction with *m*-nitrophenyl esters[3-5,15] must mean that the γ-CD cavity is too wide to hold the *m*-nitrophenyl group in a geometry that is particularly favourable for acyl transfer.

The values of k_2 for the reaction of *m*NPAlk and *p*NPAlk with diMe-β-CD, and their structural dependence, are different in several ways from those found for other CDs.[10,11] Firstly, they are relatively low (Table 3) and they are similar for the two series of esters (Table 2). Secondly, they show modest variation at short chain lengths (C_2 to C_5), then they increase substantially, but they level off at around C_8 (Table 2). These features are seen in Fig. 4 which shows log k_2 values for diMe-β-CD, plotted along with those for Hp-β-CD reacting with same esters,[10] for comparison. For the reaction of Hp-β-CD with the *meta* isomers, log k_2 barely rises from C_2 to C_{10} suggesting that cleavage involves aryl group inclusion (**1**) throughout the series. For the *para* isomers reacting with Hp-β-CD, there is a very shallow rise in log k_2 from C_2 to C_6, followed by a steep rise to C_{10}. This abrupt change in chain-length dependence, which is seen in other kinetic parameters,[10] is attributed to a switch from a transition state featuring aryl group inclusion (**1**), or even no inclusion (*vide infra*), to one having acyl group inclusion (**2**). With diMe-β-CD, there seems to be a similar switch at about C_5 for *both* series of esters (Fig. 4). The levelling off that occurs after C_7 (Fig. 4) is ascribed to methyl groups on the primary side of the diMe-β-CD making its cavity shallower than normal.

Transition State Binding (K_{TS}).—In this section, we use an approach developed by Kurz[18] for quantifying the stabilization of a transition state by a catalyst. We have found it useful for reactions mediated by CDs (and other species),[5] especially where different modes of transition state binding are possible.[4,5,10,11,14b,c,18-20] Following Kurz,[18] we define an apparent dissociation constant (K_{TS}) of the transition state of the CD-mediated reaction (TS•CD), into the transition state of the normal reaction (TS) and the CD [eqn. (4)]. This constant affords a measure of the stabilization of the reaction transition state by the CD.[4,5]

$$K_{TS} = \frac{[TS][CD]}{[TS{\cdot}CD]} = \frac{k_u K_s}{k_c} = \frac{k_u}{k_2} \quad (4)$$

Variations of K_{TS} with structure, sometimes in the form of linear free energy relationships (LFERs), can serve as probes of the mode of transition state binding of the CD.[4,5] In particular, a significant variation of pK_{TS} ($= -\log K_{TS}$) on acyl chain length (n) can be used to provide evidence of acyl group binding during the course of the reaction of alkanoate esters with CDs.[4,5,10,11] For the reaction with α-CD, the relative invariance

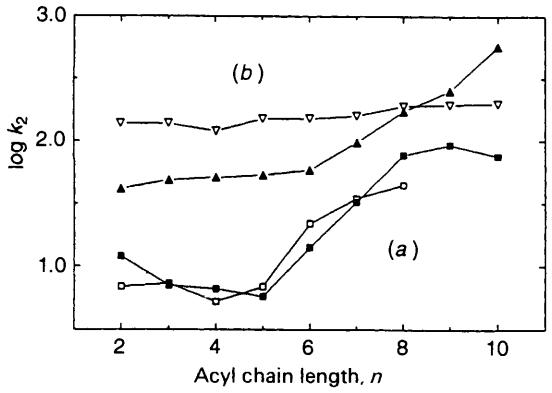


Fig. 4 Chain-length dependence of the substrate selectivity (k_2) for the cleavage by *m*- and *p*-nitrophenyl alkanoates by (*a*) Hp-β-CD [10] and (*b*) diMe-β-CD. The open symbols are for the *m*NPAlk esters and the solid symbols are for the *p*NPAlk esters.

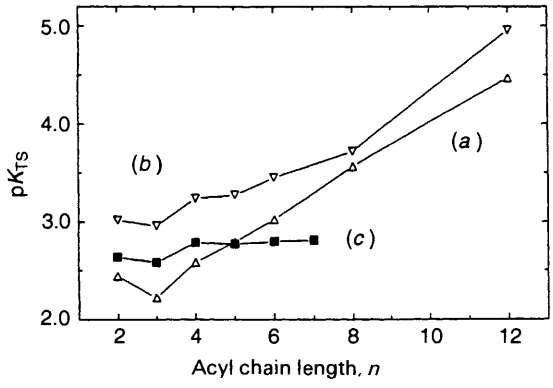


Fig. 5 Chain-length dependence of the transition state binding (pK_{TS}) for the basic cleavage of *p*-nitrophenyl alkanoates by the three main CDs: (*a*) α-CD[11]; (*b*) β-CD[11]; (*c*) γ-CD. The chain-length dependence for the *m*-nitro isomers reacting with these CDs is minimal.

of pK_{TS} with *n* for the more efficient cleavage of *m*NPAlk esters is evidence of aryl group binding (**1**), while the systematic linear increase of pK_{TS} with *n* for the *para* isomers (beyond the acetate) (Fig. 5) is indicative of acyl group binding in the transition state (**2**).[11] With β-CD and Hp-β-CD, the behaviour is more complex but still the two series of isomeric esters behave differently.[10,11] For the faster reaction of the *m*NPAlk esters with β-CD or Hp-β-CD, pK_{TS} varies little with the chain length, consistent with aryl group binding (**1**) and the variations of pK_{TS} for *p*NPAlk are also modest for short acyl chains (C_2 to C_6), consistent with aryl group binding (**1**) (or no inclusion), but for longer *p*NPAlk esters (C_6 to C_{10} or C_{12}) pK_{TS} increases steeply (*e.g.*, Fig. 5), implying acyl group inclusion (**2**). Thus, with the *para* isomers there seems to be a switch in the mode of transition state binding, occurring at the hexanoate.[10,§]

The present results for esterolyses by γ-CD differ markedly from those by α-CD, β-CD and Hp-β-CD, just outlined. For γ-CD, the values of pK_{TS} do not vary significantly with *n* as they are constant for the C_4 to C_7 esters and only slightly lower for the C_2 and C_3 derivatives (Fig. 5). Moreover, the *m*NPAlk and *p*NPAlk series of esters have very similar values of K_{TS} (Table 1). The insensitivity of pK_{TS} to *n*, which mirrors that of pK_s (Fig. 3), could mean that aryl group binding is dominant in the transition state *or* it may mean that inclusion of the ester in the cavity of γ-CD is unimportant with respect to cleavage, as concluded below.

§ Consistent with a switch in the mode of inclusion in the transition state, there are also distinct changes in dependences of log (k_c/k_u) and log k_2 (Fig. 4) on acyl chain length that occur at about C_6, for the reaction of *p*NPAlk esters with β-CD and Hp-β-CD.[10]

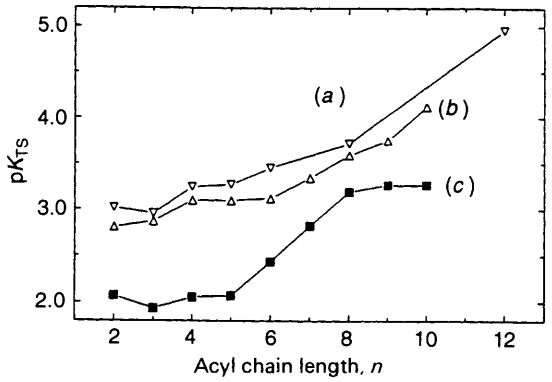


Fig. 6 Chain-length dependence of the transition state binding (pK_{TS}) for the basic cleavage of *p*-nitrophenyl alkanoates by β-CDs: (*a*) unmodified β-CD;[11] (*b*) Hp-β-CD;[10] (*c*) diMe-β-CD

We have shown that the cleavages of *p*NPAlk esters by α-CD, β-CD and Hp-β-CD are *not* totally inhibited by various potential inhibitors and may even be catalysed by some of them.[19,20] Thus, in the transition states of these reactions the CD cavity is not completely occupied by a portion of the substrate ester because the reaction basically takes place outside of it. The same situation may well be true for the cleavage of *m*NPAlk and *p*NPAlk by γ-CD since k_c/k_u and k_2 for these reactions are close to those for the cleavage of *p*-nitrophenyl acetate by the other three CDs. Also, the reactivities of all four CDs towards *p*-nitrophenyl acetate are close to that of trifluoroethanol (pK_a = 12.4)[19b,20b] and so to that expected for an 'alcohol' having the same pK_a as a CD (*ca.* 12.3).[1,21] Thus, although the *m*NPAlk and *p*NPAlk esters bind to γ-CD in the initial state, as evidenced by the saturation kinetics (Fig. 1 and Table 1), they probably do not bind significantly in the cavity of γ-CD in the transition state for cleavage.

The dependence of pK_{TS} on chain length ¶ for the cleavage of the *p*-nitrophenyl esters by diMe-β-CD is shown in Fig. 6, along with data for Hp-β-CD for comparison. As remarked above, β-CD and Hp-β-CD seem to show a change in dependence at C_6, attributed to a switch in the mode of transition state binding.[10] For ester cleavage by diMe-β-CD, pK_{TS} values are significantly lower but the chain-length dependence is similar. From the C_2 to C_5 ester, pK_{TS} values remain almost constant but rise steeply from C_5 to C_8,‖ after which they show no further increase (Fig. 6). This 'saturation' or levelling off, which is also observed in pK_s (Fig. 3 and Table 2), may result from the methoxy groups on the primary side of the CD blocking off the bottom of the cavity and providing an 'intrusive floor'.[1b,13] Suggestions of such behaviour have been made for esters reacting with other β-CDs having modifications of the primary hydroxy groups.[13] What is surprising is that Hp-β-CD does not show any levelling in pK_{TS} or pK_s, at least to the C_{10} esters.[10] We take this to mean that the 2-hydroxypropyl groups of Hp-β-CD do not intrude into the β-CD cavity, but simply extend it. Consistent with this view, the strengths of binding of various alkyl-bearing

¶ Note that since $K_{TS} = k_u/k_2$ [eqn. (4)], and k_u values are nearly constant for each series of esters (Table 2), the chain length dependences of pK_{TS} and log k_2 are very similar.

‖ It should be noted that there is a distinct change in the chain length dependence of k_c/k_u values, also. For both series of esters, the ratios decrease markedly from C_2 to C_5 and then rise slowly from C_5 to C_{10} (Table 2). Similar biphasic behaviour was observed for the cleavage of *p*NPAlk esters by Hp-β-CD, except that the break point was at the C_7 ester (see Fig. 4 of ref. 10).

derivatives (including the *m*NPAlk and *p*NPAlk esters) to Hp-β-CD are virtually the same as to β-CD.[9] By contrast, K_s and K_{TS} values for these esters reacting with diMe-β-CD (Table 2) are not the same as those for β-CD: the former are slightly smaller (stronger initial state binding) while the latter are larger (weaker transition state binding).

The above considerations also relate to the retardations observed for ester cleavage by diMe-β-CD, as opposed to the accelerations found for reaction with β-CD or Hp-β-CD. According to the Kurz approach to transition state stabilization,[4,5,18] the acceleration or retardation of a CD-mediated reaction is governed by the strength of binding of the transition state to the CD relative to that of the substrate since, from eqn. (4), $k_c/k_u = K_s/K_{TS}$. From this viewpoint, the retarded cleavage observed with diMe-β-CD arises because the initial state binding is stronger than transition state binding and it is geometrically unsuitable for facile reaction. Compared with β-CD or Hp-β-CD, the much slower reaction of the esters with diMe-β-CD is due to two effects: slightly stronger substrate binding and substantially weaker transition state binding. Most likely, the latter has its origins in steric hindrance by one or more of the methylated hydroxy groups adjacent to the reacting hydroxy group.

Conclusions

Compared with other CDs, both γ-CD and diMe-β-CD show unusual behaviour in their reactions with *m*- and *p*-nitrophenyl alkanoate esters, from which several conclusions emerge. (*a*) In the initial state, both series of esters bind to γ-CD in a 1:1 manner by inclusion of their aryl groups. (*b*) Cleavage of the esters is accelerated only about tenfold by γ-CD. In contrast with the initial state, there is probably no inclusion of the ester in the CD cavity in the transition state of the acyl transfer reaction. (*c*) Binding of most of the alkanoate esters to diMe-β-CD in the initial state and in the transition state is by acyl group inclusion, the strength of which levels off around C_8. (*d*) Cleavage of the esters is slowed by diMe-β-CD and to similar extents for the two isomeric series. This retardation mainly arises from poorer transition state binding due to steric hindrance by methoxy groups on the secondary rim of diMe-β-CD, in the vicinity of the hydroxy group reacting with the ester. This hindrance also suppresses the superior cleavage of *m*-nitrophenyl alkanoates that is evident for their reaction with α-CD, β-CD and Hp-β-CD.

Experimental

Most of the *p*-nitrophenyl esters were obtained from Sigma. The *p*-nitrophenyl C_7 and C_9 esters and all of the *m*-nitrophenyl esters, were prepared by classical methods or using a DCC method, as previously described.[10,11] Appropriate precursors were purchased from Aldrich.

The cyclodextrins were obtained from Wacker-Chemie GmbH, Munich, Germany: γ-CD was purchased but the 'dimethyl-β-cyclodextrin' was a gift. The former contained particulate matter and gave cloudy solutions, even though γ-CD itself is very soluble in water.[1] Therefore, the material as received was dissolved in hot water and filtered from any particles. The filtrate was frozen overnight and the recrystallized γ-CD was filtered off. From the filtrate more γ-CD was precipitated with ethanol. The combined materials were dried overnight at 110 °C and the cake was pulverized before further use. The 'dimethyl-β-cyclodextrin' had a specified 'degree of substitution' of 1.8, meaning that an average of 1.8 hydroxy groups per glucose unit of β-CD are methylated, *i.e.*, close to dimethylation. Earlier work, including purification and structure studies,[1b,22] indicate that this dimethylation is 2,6 with respect to each of the glucose moieties, so that all the primary hydroxy groups and half the secondary hydroxy groups of β-CD are methylated. No doubt, the material is not a pure substance,[22] but a mixture of partially alkylated derivatives. However, as long as it has a consistent composition and yields reproducible results (which was the case), we believe that it can yield useful and informative results. Again, it is important that such materials be studied since they have practical applications.[1b,7,8]

Cleavage reactions were carried out by 1:1 mixing in a stopped-flow spectrophotometer, the observation cell of which was kept at 25.0 ± 0.1 °C. One syringe contained the phosphate buffer (0.4 mol dm^{-3}; pH 11.6) and the other contained ester and CD at twice the concentrations desired in the reaction. Problems were encountered with the C_8 and longer esters with γ-CD, due to the precipitation of 'complexes', even though the CD itself is quite soluble in water. Substrate solutions were made by dilution of 0.1 mol dm^{-3} stock solutions in spectral grade acetonitrile. After 1:1 mixing in the stopped-flow apparatus the ester concentrations were 20–100 μmol dm^{-3} (*m*NPAlk) or 4–50 μmol dm^{-3} (*p*NPAlk), depending on the solubility of the ester in the medium. Seven concentrations in the range 0–20 mmol dm^{-3} were used, as in Figs. 1 and 2.

The kinetics of ester cleavage were followed by monitoring the first-order production of nitrophenolate ion at 395 nm (*m*NPAlk) or 405 nm (*p*NPAlk), using an SX17MV stopped-flow apparatus obtained from Applied Photophysics (Leatherhead, Surrey, UK). Absorbance traces consisting of 400 points covering seven to twelve half-lives were collected and first-order rate constants were estimated from non-linear least squares fitting of a first-order exponential to the traces, using the software supplied with the apparatus. The recorded rate constants (k_{obs}) were the averages of 5–10 determinations.

The constants k_c and K_s in Tables 1 and 2 were obtained by non-linear least-squares fitting[23] of eqn. (3) to k_{obs} at seven concentrations, keeping k_u fixed at the experimental value.

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