The Kinetics of Basic Cleavage of Nitrophenyl Alkanoate Esters by 'Hydroxypropyl-β-cyclodextrin' in Aqueous Solution

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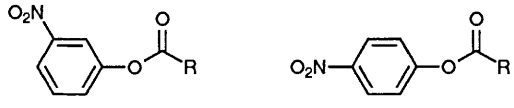
The kinetics of cleavage of *m*- and *p*-nitrophenyl alkanoates by 'hydroxypropyl-β-cyclodextrin' (Hp-β-CD) in basic aqueous solution vary significantly with the chain length of the esters (C_2 to C_{10}). For both series of esters with short chains (C_2 to C_6) simple saturation kinetics are observed, indicative of 1:1 (ester:CD) binding and reaction of one molecule of ester with one molecule of Hp-β-CD. For longer chains, there is also a cleavage process involving *two* molecules of Hp-β-CD. This type of behaviour was not found previously for the same esters reacting with α-CD and with β-CD but it has been observed for some carboxynitrophenyl alkanoates. With the longest esters there is also evidence of productive 1:2 (ester:CD) binding. For the 1:1 binding, there is a linear dependence of the strength on acyl chain length and the close similarity of the dissociation constants for the two series of esters implicate inclusion of the alkyl chains of the esters' acyl groups. Transition-state binding is more complex: for the *meta*-nitro isomers aryl-group inclusion appears dominant whereas for the *para* isomers there seems to be a switch from aryl-group inclusion to acyl-group inclusion, occurring at a chain length of C_6–C_7.

The cleavage of aryl esters by cyclodextrins (CDs)[1] in basic aqueous solution has been studied extensively over the last thirty years.[1,8] To a considerable extent, this has occurred because the reaction provides a convenient means of studying the binding properties of CD hosts and their influence on the reactivities of included guests.[7] In this way, we have found novel and interesting behaviour, using the kinetics of aryl ester cleavage as the probe.[8-14]

For example, *m*-nitrophenyl alkanoates with short and medium chain lengths (C_2 to C_6) undergo cleavage by α-CD[1] with aryl-group binding in the transition state whereas their *p*-nitrophenyl isomers react through acyl-group binding (C_3 to C_{12}), even though substrate binding in both series of esters entails inclusion of the acyl group in the CD cavity.[9] Cleavage of the esters by β-CD[1] is similar, but the picture is not so clear-cut, as will be discussed later. In contrast, the reaction of chloroaspirin derivatives with CDs entails aryl-group binding in both the initial state and the transition state.[9] For the cleavage of some carboxynitrophenyl alkanoates, we observed productive and non-productive processes involving two molecules of the CD, and these increase in importance with the chain length of the alkanoate ester.[10] We have also found that the reaction of *p*-nitrophenyl acetate with α-CD or β-CD is *not* inhibited competitively by many species which bind to the CDs and which do inhibit the corresponding reactions of the *m*-nitro isomer.[11] Furthermore, the cleavage of longer *p*-nitrophenyl alkanoates is actually catalysed by various potential inhibitors.[12] In the case of *m*-nitrophenyl hexanoate, some additives cause a switch between reaction modes: they inhibit cleavage occurring *via* aryl-group binding *but* promote reaction *via* acyl-group binding.[14]

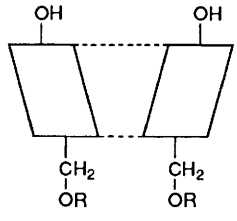
The above studies provide information about the differential binding of aryl and alkyl groups to CDs in the initial states and transition states of reactions.[7] We are now applying the same approach to probe the binding to modified CDs.[1c,d,15] The work has been undertaken because earlier studies were severely restricted owing to the limited availability of such CDs. Now that several modified CDs are being produced industrially, and they are becoming of commercial importance,[1d,16] the time is ripe to learn more about their binding properties[13] and their effects on the reactivities of included guests.

The present paper reports studies of the basic cleavage of *m*- and *p*-nitrophenyl alkanoates (*m*NPAlk and *p*NPAlk) by 'hydroxypropyl-β-cyclodextrin' (Hp-β-CD).[1d] This material is produced by treating β-CD (**1**) with propylene oxide in alkaline solution so that most of the seven primary hydroxy groups of β-CD are replaced by $-OCH_2CH(OH)CH_3$ (**2**). Conceivably, this modification, which leads to much greater solubility in water, may have either of two consequences at the molecular level. Firstly, it may simply extend the depth of the cavity of β-CD. Secondly, if the 2-hydroxypropyl groups turn inwards, towards the central axis of the β-CD cavity, they may form an 'intrusive floor', closing off the bottom of the cavity, as was envisaged earlier for other functionalities.[4a,c,5b]



*m*NPAlk *p*NPAlk

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



1 β-CD, R = H
2 Hp-β-CD, R = $CH_2CH(OH)CH_3$

Results

We have measured the kinetics of cleavage of the esters *m*NPAlk and *p*-NPAlk (acetate to decanoate, C_2 to C_{10}) by Hp-β-CD in a phosphate buffer of pH 11.6. The reactions were followed by monitoring the first-order production of the nitrophenolate anions[9] and rate constants (k_{obs}) were obtained over a range of [Hp-β-CD] (Figs. 1 and 2).

For both series of esters, reaction of the shorter chains (C_2 to

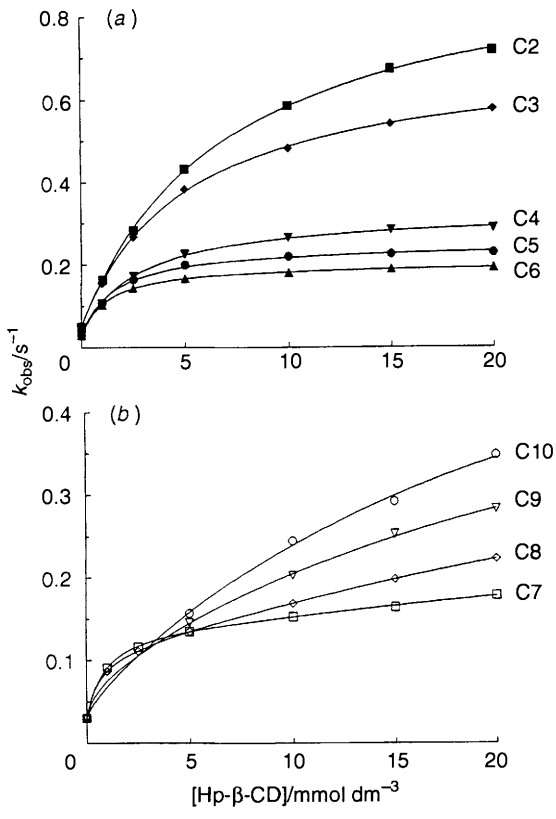


Fig. 1 Dependence of rate constants for the cleavage of *m*-nitrophenyl alkanoates on the concentration of Hp-β-CD: (*a*) for the acetate to hexanoate (C_2 to C_6) the curves conform to simple saturation kinetics, eqn. (3); (*b*) for the heptanoate (C_7) the data follow eqn. (5); for the octanoate to decanoate (C_8 to C_{10}) the curves are described by eqn. (7)—see the text

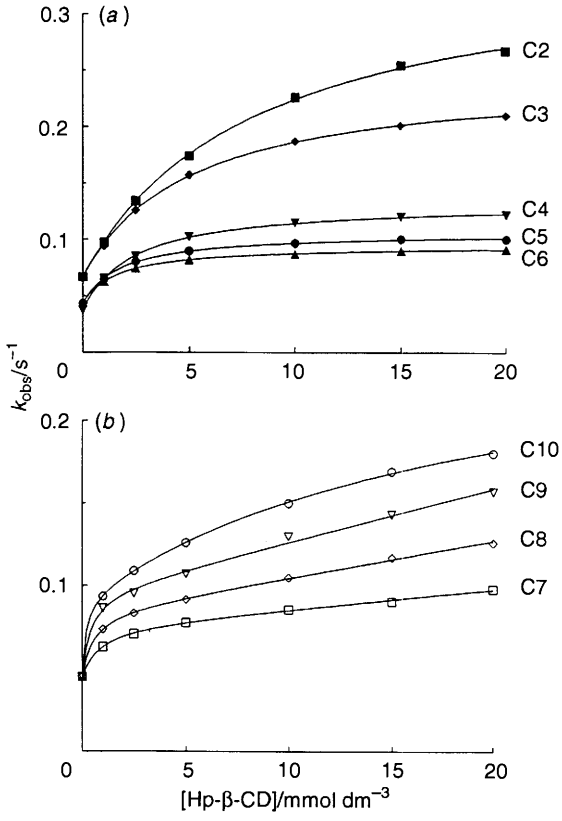


Fig. 2 Dependence of rate constants for the cleavage of *p*-nitrophenyl alkanoates on the concentration of Hp-β-CD: (*a*) for the acetate to hexanoate (C_2 to C_6) the curves conform to simple saturation kinetics, eqn. (3); (*b*) for the heptanoate to nonanoate (C_7 to C_9) the data follow eqn. (5); for the decanoate (C_{10}) the curve is described by eqn. (7)—see the text

C_6) shows simple saturation kinetics,[1] as shown in Figs. 1(*a*) and 2(*a*). This behaviour may be ascribed to reaction in the medium [eqn. (1)] and reaction with the CD [eqn. (2)], and it can be expressed algebraically by eqn. (3).[7-9] Measured values

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

$$S + CD \underset{k_1}{\rightleftharpoons} S{\cdot}CD \xrightarrow{k_c} \text{products} \quad (2)$$

$$k_{obs} = \frac{(k_u K_1 + k_c[CD])}{(K_1 + [CD])} \quad (3)$$

of k_u, and fitted values of k_c and K_1 are collected in Table 1.

With the longer esters (C_7 to C_{10}) the cleavage kinetics are not satisfactorily described by eqn. (3) because at high [Hp-β-CD] the values of k_{obs} rise beyond that expected for simple saturation [Figs. 1(*b*) and 2(*b*)]. For some of these derivatives, where the rise at high [Hp-β-*CD*] is essentially linear, we postulate the attack of a *second* molecule of Hp-β-CD on the ester·CD complex, as in eqn. (4). The incursion of this additional process, which has been observed for other long aryl alkanoate esters reacting with α- and β-CD,[10] means that the overall behaviour is now given by eqn. (5).

$$S{\cdot}CD + CD \xrightarrow{k_{c2}} P \quad (4)$$

$$k_{obs} = \frac{(k_u K_1 + k_c[CD] + k_{c2}[CD]^2)}{(K_1 + [CD])} \quad (5)$$

Eqn. (5) suffices to account for the data of several esters but with the longest esters the increase at high [Hp-β-CD] is decidedly curved downwards [Figs. 1(*b*) and 2(*b*)], suggesting the onset of a second saturation. This effect is attributable to the involvement of a discrete 1:2 (ester:CD) complex, as in eqn. (6), the presence of which requires that eqn. (5) be replaced by eqn. (7). The fitted parameters for both eqns. (5) and (7) are also

$$S{\cdot}CD + CD \underset{K_2}{\rightleftharpoons} S{\cdot}CD_2 \xrightarrow{k_{cc}} \text{products} \quad (6)$$

$$k_{obs} = \frac{(k_u K_1 K_2 + k_c K_2[CD] + k_{cc}[CD]^2)}{(K_1 K_2 + K_2[CD] + [CD]^2)} \quad (7)$$

collected in Table 1. For the purposes of comparing the two third-order processes, eqns. (4) and (6), we have entered $k_{c2} = k_{cc}/K_2$ in Table 1, also.

The low solubility of the longer esters in water presents a practical problem, particularly with *m*NPAlk esters because of the relatively low extinction coefficient of the *m*-nitrophenolate ion used to monitor their cleavage. To circumvent this problem, we made use of the knowledge that k_u is essentially constant for the C_4 and longer esters, as long as steps are taken to prevent aggregation.[17] So, k_u values for the C_7 to C_{10} esters were taken to be the same as those for the two C_6 esters, as indicated in Table 1. With increasing [Hp-β-CD] solubility of the esters is enhanced and the problem is alleviated.

Table 1 Constants for the cleavage of *m*-nitrophenyl and *p*-nitrophenyl alkanoates by 'hydroxypropyl-β-cyclodextrin'[a]

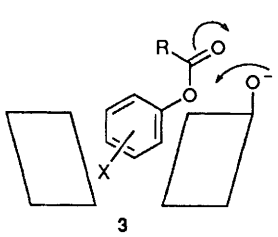
Ester	k_u/s^{-1}	k_c/s^{-1}	K_1/mmol dm^{-3}	k_{c2}/dm^3 mol^{-1} s^{-1}	K_2/mmol dm^{-3}	k_{cc}/s^{-1}
m-Nitrophenyl alkanoates						
C_2	0.0502	0.956 ± 0.010	6.98 ± 0.19			
C_3	0.0515	0.707 ± 0.007	5.08 ± 0.16			
C_4	0.0293	0.325 ± 0.004	2.68 ± 0.12			
C_5	0.0306	0.247 ± 0.004	1.63 ± 0.13			
C_6	0.0306	0.202 ± 0.001	1.33 ± 0.03			
C_7	0.0306[b]	0.143 ± 0.003	0.899 ± 0.060	2.06 ± 0.13		
C_8	0.0306[b]	0.112 ± 0.010	0.587 ± 0.211	8.77[c]	75.9 ± 8.0	0.666 ± 0.043
C_9	0.0306[b]	0.0721 ± 0.0156	0.372[d]	22.2[c]	25.1 ± 9.1	0.557 ± 0.084
C_{10}	0.0306[b]	0.0495 ± 0.0369	0.245[d]	29.8[c]	23.2 ± 14.0	0.692 ± 0.178
p-Nitrophenyl alkanoates						
C_2	0.0653	0.343 ± 0.007	8.18 ± 0.48			
C_3	0.0660	0.247 ± 0.002	5.05 ± 0.15			
C_4	0.0402	0.135 ± 0.001	2.67 ± 0.15			
C_5	0.0431	0.105 ± 0.001	1.94 ± 0.09			
C_6	0.0444	0.0932 ± 0.0005	1.59 ± 0.08			
C_7	0.044[b]	0.0769 ± 0.0018	0.792 ± 0.146	1.13 ± 0.09		
C_8	0.044[b]	0.0858 ± 0.0014	0.497 ± 0.068	2.17 ± 0.07		
C_9	0.044[b]	0.0980 ± 0.0048	0.391 ± 0.170	3.14 ± 0.027		
C_{10}	0.044[b]	0.0925 ± 0.0055	0.159 ± 0.100	13.7[c]	19.5 ± 3.9	0.268 ± 0.014

[a] In aqueous solution, at pH 11.6 and at 25 °C. Apart from k_u values, which were measured, constants were obtained by non-linear fitting of eqns. (3), (5) or (7) to the data in Figs. 1 and 2. The quoted uncertainties are the standard errors obtained from the fitting. [b] Assumed to be the same as for the C_6 ester (see the text). [c] Calculated from k_{cc}/K_2. [d] Estimated by extrapolation of eqn. (9a).

A related difficulty with the C_9 and C_{10} *m*NPAlk esters, also due to their low solubility, was that we were unable to measure k_{obs} at low enough [Hp-β-CD] to determine K_1 reasonably. Accordingly, for these two esters we estimated K_1 values by extrapolation of the linear relationship between pK_1 and acyl chain length, as discussed below.

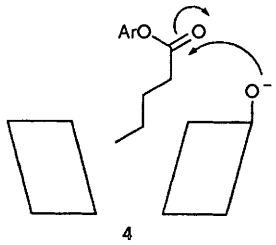
Discussion

The basic strategy behind the present work was the same as that used in our earlier study of the cleavage of *m*NPAlk and *p*NPAlk esters by α-CD and β-CD.[9] In brief, we hypothesized that if the reaction proceeds through a transition state involving aryl-group binding (**3**) then appropriate kinetic parameters should be sensitive to the position of the aryl substituent (*meta* or *para*) and insensitive to the acyl chain length. Alternatively, for a transition state with acyl-group inclusion (**4**) the parameters should reflect the chain length and be insensitive to the position of the aryl substituent.



3

Sensitive to position of X; insensitive to length of RCO



4

Insensitive to position of X; sensitive to length of RCO

In applying this strategy, we make use of an approach developed by Kurz[18] for estimating the stabilization of a transition state by a catalyst. This approach, which has been of considerable influence in enzymology,[19] is useful for reactions mediated by CDs (and other 'catalysts'),[7] particularly ones where different modes of transition-state binding are possible.[7,9-12] Following Kurz,[18] we define K_{TS} as given in eqn. (8). This quantity is the apparent dissociation constant of the

$$K_{TS} = \frac{[TS][CD]}{[TS{\cdot}CD]} = \frac{k_u K_1}{k_c} \quad (8)$$

transition state of the CD-mediated reaction, symbolized by TS·CD, into the transition state of the normal reaction (TS) and the CD. It provides a measure of the stabilization of the cleavage transition state by the CD, and variations of K_{TS} with structure, often in the form of linear free-energy relationships (LFERs), can serve as probes of the transition-state binding of the CD.[7,9-12]

Before discussing transition-state binding in detail, we consider substrate binding, rate accelerations and substrate selectivity, as a function of structure—the acyl chain length and the position of the substituent on the aryl group.

Substrate Binding (K_1 *and* K_2).—There are two main features to note about the binding of *m*NPAlk and *p*NPAlk to Hp-β-CD. First, the values of K_1 for the two series of esters are almost the same (Table 1), and within the accuracy of the measurements they may be the same. Secondly, as shown in Fig. 3, the values of pK_1 (= −log K_1) for the two series of esters vary systematically and linearly with the acyl chain length (n). As discussed previously,[7,9] in the context of binding to α- and β-CD, such relationships are effectively LFERs since both the size of alkyl groups and various measures of their hydrophobicities increase monotonically with n. The solid lines in Fig. 3 are given by eqns. (9a) and (9b).* The slopes of these two equations are

*m*NPAlk: $pK_1 = 0.180\ (\pm 0.009)n + 1.81\ (\pm 0.05)$; ($r = 0.993$, 7 points) (9a)

*p*NPAlk: $pK_1 = 0.201\ (\pm 0.009)n + 1.70\ (\pm 0.06)$; ($r = 0.993$, 9 points) (9b)

* On the basis of the good linearity of the two relationships, eqns. (9a) and (9b), we estimated K_1 values for the C_9 and C_{10} *m*NPAlk esters (Table 1) by simple extrapolation of eqn. (9a).

Table 2 Derived constants for the cleavage of *m*-nitrophenyl and *p*-nitrophenyl alkanoates by 'hydroxypropyl-β-cyclodextrin'[a]

Ester	k_c/k_u	k_2/dm^3 mol^{-1} s^{-1}	K_{TS}/mmol dm^{-3}	$K_{TS'}$/mmol dm^{-3}
m-Nitrophenyl esters				
C_2	19.0	137	0.367 ± 0.006	
C_3	13.7	139	0.370 ± 0.008	
C_4	11.1	121	0.242 ± 0.008	
C_5	8.07	152	0.202 ± 0.013	
C_6	6.60	152	0.201 ± 0.009	
C_7	4.67	159	0.192 ± 0.009	69.4 ± 2.9
C_8	3.66	191	0.160 ± 0.043	12.8 ± 0.6
C_9	2.36	193	0.158 ± 0.034	3.25 ± 0.02
C_{10}	1.62	202	0.151 ± 0.113	1.66 ± 0.66
p-Nitrophenyl esters				
C_2	5.25	41.9	1.56 ± 0.06	
C_3	3.74	48.9	1.35 ± 0.03	
C_4	3.36	50.6	0.795 ± 0.039	
C_5	2.44	54.1	0.796 ± 0.029	
C_6	2.10	58.6	0.757 ± 0.034	
C_7	1.75	97.1	0.453 ± 0.073	68.1 ± 3.8
C_8	1.95	173	0.255 ± 0.031	39.5 ± 0.6
C_9	2.23	251	0.176 ± 0.068	31.2 ± 1.3
C_{10}	2.10	582	0.0756 ± 0.0431	6.73 ± 0.59

[a] In aqueous solution at pH 11.6, and at 25 °C. Calculated from the constants in Table 1, as follows: $k_2 = k_c/K_1$, $K_{TS} = k_uK_1/k_c$, $K_{TS'} = k_c/k_{c2}$. The errors in K_{TS} and $K_{TS'}$ are calculated from the uncertainties in Table 1, using error analysis.

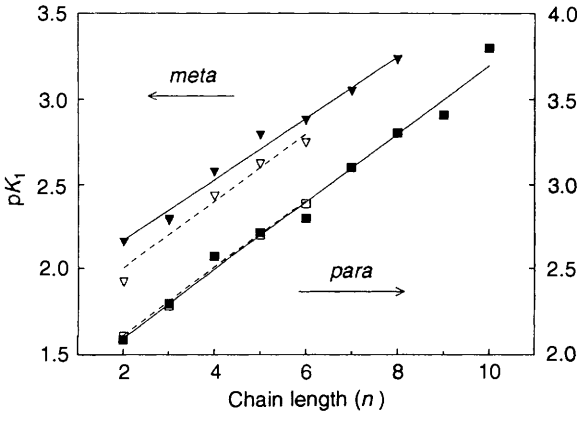


Fig. 3 Dependence of the binding (pK_1) of *m*- and *p*-nitrophenyl alkanoates to Hp-β-CD and β-CD on the acyl chain length, *n*. Solid symbols and lines are for Hp-β-CD [Table 1, eqns. (9a) and (9b)]; open symbols and dashed lines are for β-CD.[9] The scales for the *meta* and *para* isomers are offset for clarity; otherwise all four sets of data would be nearly coincident.

virtually the same as those (0.198 and 0.197) for the binding of β-CD to the same esters (see Fig. 3). We conclude that both series of esters bind to Hp-β-CD (and to α- and β-CD)[9] through their acyl chains.

Another feature of the K_1 values for the binding of the *m*NPAlk and *p*NPAlk esters to Hp-β-CD merits comment: they are almost the same as those for binding to β-CD, and the same is true of other alkyl-bearing guests, as we have noted recently.[13] We concluded that such guests bind to *both* β-CD and Hp-β-CD in essentially the same manner, and to the wider secondary side of the CD cavity. Moreover, the depth of penetration of the guests into the cavity must be such that they barely encounter the hydroxypropyl groups on the distal, primary side of Hp-β-CD.[13]

In four cases (*m*NPAlk, C_8, C_9, C_{10}; *p*NPAlk, C_{10}) there is good evidence of 1:2 (ester:CD) binding with Hp-β-CD. Such binding was not observed previously for these esters with α- and β-CD,[2g,9] probably because of the narrow ranges of [CD] used.* However, it has been found for some carboxynitrophenyl alkanoates, with $K_2 \sim 20$ mmol dm^{-3} for α-CD and ~ 50 mmol dm^{-3} for β-CD.[10] As in those cases, the present values of K_2 (20–70 mmol dm^{-3})† probably correspond to aryl-group binding, occurring after initial binding of the acyl group.

Rate Acceleration (k_c/k_u).—The ratio k_c/k_u (Table 2) is the maximal acceleration attainable at saturation of the 1:1 binding equilibrium [eqn. (2)]. For the reaction of the *m*NPAlk esters with Hp-β-CD, $\log(k_c/k_u)$ decreases linearly with the acyl chain length for all nine esters (Fig. 4). By contrast, for the *p*NPAlk isomers, $\log(k_c/k_u)$ decreases linearly for C_2 to C_7, but then it rises modestly from C_7 to C_{10} (Fig. 4), suggesting a change in behaviour at C_7.‡

In terms of the Kurz approach, the acceleration is governed by the strength of binding of the transition state relative to that of the substrate since, from eqn. (8),

$$k_c/k_u = K_1/K_{TS} \quad (10)$$

Viewed in these terms, the decrease in $\log(k_c/k_u)$ for *m*NPAlk originates in the increase in their pK_1 values (Fig. 3) and the near constancy of pK_{TS} (see below). Similarly, the change in the trend of $\log(k_c/k_u)$ for *p*NPAlk at C_7 arises from the change in dependence of pK_{TS} on *n* after C_6, since pK_1 consistently increases with *n* (Fig. 3).

* Hp-β-CD is much more soluble than β-CD in water.[1d] Another factor why 1:2 binding was not observed earlier for long *p*NPAlk may have been that Bonora *et al.*[2g] analysed their kinetic data using a Lineweaver–Burke approach, which presupposes only 1:1 binding.

† The value of 76 mmol dm^{-3} for the C_8 *m*NPAlk ester may not be very accurate since it is decidedly outside the range of [Hp-β-CD] (=0–20 mmol dm^{-3}) used.

‡ This is not a steric effect: such effects are not evident beyond a chain length of C_4.[17]

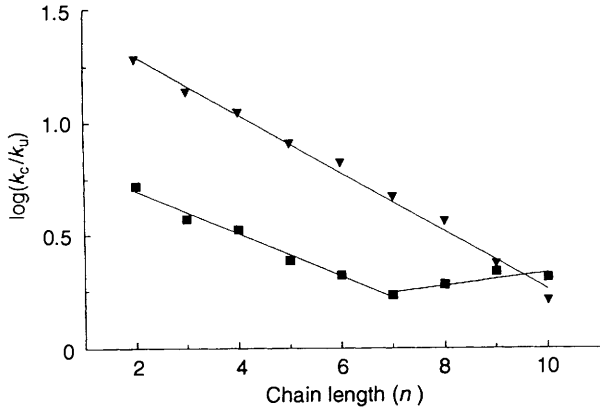


Fig. 4 Chain-length dependence of the maximal rate acceleration (k_c/k_u) for the basic cleavage of *m*- (▼) and *p*-nitrophenyl (■) alkanoates by Hp-β-CD. Data from Table 2.

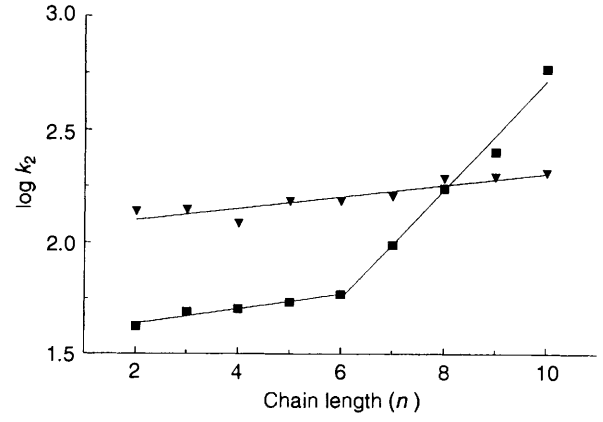


Fig. 5 Chain length dependence of the substrate selectivity (k_2) for the cleavage of *m*- (▼) and *p*-nitrophenyl (■) alkanoates by Hp-β-CD. Data from Table 2.

Substrate Selectivity (k_2).—The second-order rate constants ($k_2 = k_c/K_1$) for the reaction of a catalyst with substrates are measures of the selectivity of the catalyst for different substrates.[2a,b,7-9] With the present esters, Hp-β-CD is more reactive towards the *meta* isomers up to C_8, after which it prefers the *para* isomers (Table 2, Fig. 5). As seen in Fig. 5, log k_2 for *m*NPAlk slowly rises with the chain length (slope = 0.025) from C_2 to C_{10}, whereas for *p*NPAlk the dependence shows two distinct regions: at short chain lengths (C_2 to C_6) log k_2 rises slowly (slope = 0.034), as it does for *m*NPAlk, but from C_6 to C_{10} there is a much sharper increase (slope = 0.24),* so that the graphs for the two series of esters cross at C_8. The data in Fig. 5 suggest that all the *m*NPAlk react with Hp-β-CD through aryl-group inclusion (**3**) while the *p*NPAlk react this way for short chains (C_2 to C_6) and through acyl-group inclusion (**4**) for longer chains.

One potential problem with using k_2 values for comparative purposes is that they take no account of the intrinsic reactivities of substrates.[7,8] It is not a particular problem in the present case because the two series of esters have quite similar reactivities, but in general the problem may be overcome by looking at transition-state binding using $K_{TS} = k_uK_1/k_c = k_u/k_2$, from eqn. (8), which effectively scales k_2 according to k_u.

Transition-state Binding (K_{TS}).—For the cleavage of *m*NPAlk and *p*NPAlk by α-CD, a clear distinction in the modes of transition-state binding was evident in the values of $pK_{TS} = -\log K_{TS}$.[9] In the case of the *meta* isomers, pK_{TS} varied little with the acyl chain length, *n* (slope = 0.04 ± 0.03), consistent with aryl-group inclusion in the transition state (**3**). In sharp contrast, pK_{TS} for the *para* isomers increased linearly with *n*, and the slope was 0.25 ± 0.01, close to those of ~0.20 for plots of pK_1 *vs.* *n* for substrate binding (see above). This dependence was taken as excellent evidence of acyl-group inclusion (**4**).[7,9]

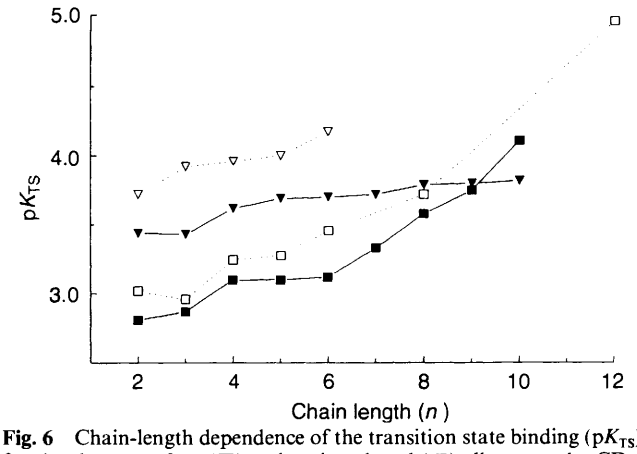


Fig. 6 Chain-length dependence of the transition state binding (pK_{TS}) for the cleavage of *m*- (▼) and *p*-nitrophenyl (■) alkanoates by CDs. Solid symbols and lines are for Hp-β-CD (data from Table 2); open symbols and dotted lines are for β-CD [based on C_2–C_6 data, from ref. 9, and on C_8 and C_{10} data, from ref. 2(*g*)].

With β-CD, the distinction was less clear, as seen in Fig. 6; values of pK_{TS} for *m*NPAlk (C_2 to C_6) drift upwards, as do those for *p*NPAlk (C_2 to C_6). However, for two longer *para* isomers (C_8 and C_{12}) pK_{TS} rises sharply, using data from Bonora *et al.*[2g]

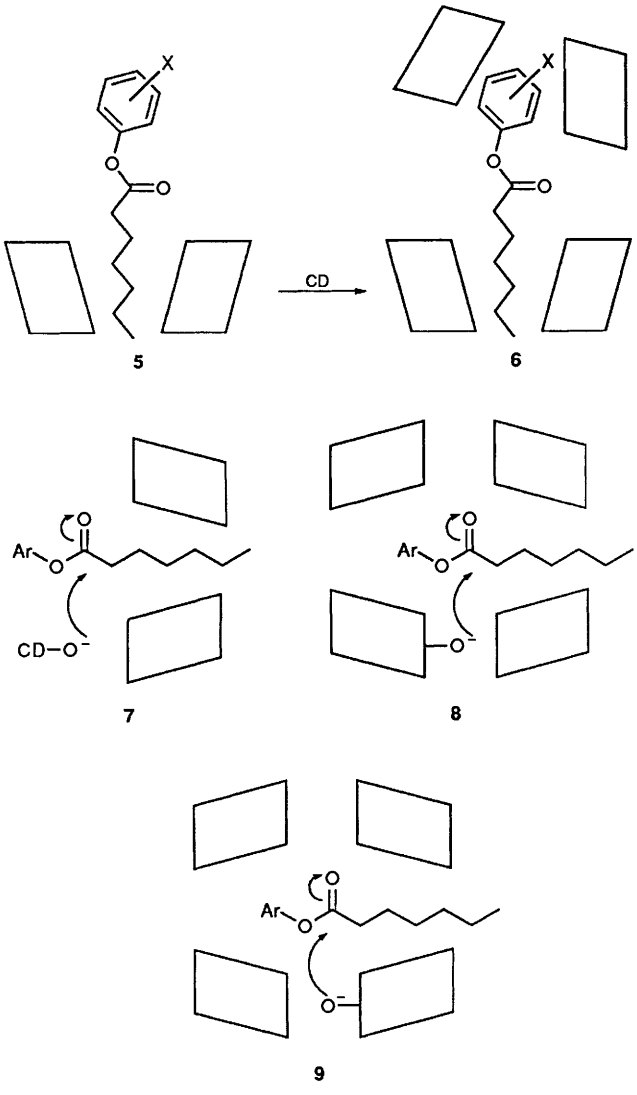
For the reaction of *m*NPAlk with Hp-β-CD, pK_{TS} varies little with *n*, particularly from C_4 to C_{10} (Fig. 6). The variations for *p*NPAlk are similar for C_2 to C_6 but thereafter pK_{TS} increases steeply (Fig. 6). Thus, just as with log(k_c/k_u) and log k_2, there is an abrupt change in dependence of pK_{TS} on chain length after the *p*NPAlk C_6 ester. We suggest that the *meta* isomers (C_2 to C_{10}) employ aryl-group binding (**3**) and so do the *para* isomers from C_2 to C_6. Beyond C_6, binding of the long hydrophobic alkanoate chain of *p*NPAlk affords greater transition-state stabilization and so esterolysis takes place through acyl group inclusion (**4**).

Cleavage Involving two Molecules of Hp-β-CD.—Such reactions are evident with both series of esters at chain lengths beyond C_6 [Fig. 1(*b*) and 2(*b*)]. With the longest esters, the kinetic data are curved at high [Hp-β-CD], implicating 1:2 (guest:host) binding [eqn. (6)]. Presumably, the simultaneous binding of two CD molecules is only feasible when the alkanoate chain is sufficiently long that the aryl group protrudes well outside the CD cavity enclosing the alkyl chain of the acyl group (**5**→**6**).

Rate constants for cleavage by two CD molecules ($k_{c2} = k_{cc}/K_2$, Table 1) increase with chain length, probably for similar reasons: only with a long acyl chain is the ester carbonyl reasonably accessible to an external nucleophile,[10b] which in this case is the anion of a second molecule of the CD (**7**). However, in view of the clear evidence of 1:2 binding, it seems likely that reaction takes place within a discrete S·CD_2 complex, either as in **8** or as in **9**. Choosing between these three possibilities (**7**, **8**, **9**) is not easy, of course, because they are kinetically equivalent, but we will try.

We note that k_{c2} values for *m*NPAlk are greater than those for *p*NPAlk, which is the reverse of their intrinsic reactivities

* The steeper slope (=0.24) is comparable to that found for substrate binding of both esters [eqns. (9a) and (9b)] through their acyl chains. With β-CD and 4-carboxy-2-nitrophenyl alkanoates the slope for substrate binding is 0.32.[10]



(Table 1). Conceivably, this could be due to the *meta* isomers reacting as in **8**, making use of the superior reactant geometry afforded by the *meta* substituent.[2a–d,6–9] Consistent with this view, the values of k_{cc}, for reaction within the 1:2 complexes, are essentially constant for the C_8, C_9 and C_{10} esters.

By analogy with eqn. (8), we can define an apparent constant for binding of the second CD to the cleavage transition state, eqn. (11).[10] Values of this constant for *m*NPAlk decrease

$$K_{TS'} = \frac{[TS{\cdot}CD][CD]}{[TS{\cdot}CD_2]} = \frac{k_c}{k_{c2}} \qquad (11)$$

substantially with chain length (Table 2), which is not surprising if k_c corresponds to aryl inclusion (**3**), as argued above, so that the second CD necessarily binds to the acyl chain. The decrease in $K_{TS'}$ converts into a systematic increase in $pK_{TS'}$ with n (r = 0.983) with a steep slope of 0.55, a further indication of the great importance of binding the acyl chain (**8** or **9**); the slope matches that for the binding of linear alcohols to α- and β-CD,[9] and to Hp-β-CD.[13] In contrast, values of $K_{TS'}$ for *p*NPAlk vary less with n, presumably because k_c entails acyl binding (**4**) and so the second CD must bind to the aryl group.

One further aspect of the $S{\cdot}CD_2$ complexes demands comment: these ternary complexes *are more reactive* than their binary (S·CD) counterparts (Table 1). With the three long *m*NPAlk esters the factors are 6, 8 and 14; for the lone *p*NPAlk example the factor is only 3. Again, the superiority of the *m*NPAlk systems may denote that they can simultaneously employ the better positioning that accrues from the *meta* substituent *and* a sizeable hydrophobic effect that comes from removing the alkyl chain from bulk water,[20] as in the mechanism outlined in **8**. In fact, the reactivities of the ternary complexes of the long esters are almost as large as those of the binary complexes of the two, more reactive acetates (Table 1).

Comparison of Hp-β-CD *and* β-CD.—As discussed above, there seems to be very little difference between the binding of the esters *m*NPAlk and *p*NPAlk (and other alkyl-bearing guests)[13] to Hp-β-CD and to β-CD. However, there do appear to be some differences in the transition-state binding for *m*NPAlk and *p*NPAlk, as seen in Fig. 6. In particular, values of K_{TS} are consistently higher for Hp-β-CD than for β-CD: for the *meta* isomers the factor is 2–3 while for the *para* isomers the factor is 1.5–2. Similar modest effects on ester cleavage were found by earlier workers studying β-CD functionalized with groups on the primary side.[4a,c,5b] These small effects barely warrant discussion: suffice it to say that they may result from subtle differences in the solvation of Hp-β-CD and β-CD.

Conclusions

(*a*) Both *m*-nitro and *p*-nitrophenyl alkanoates (C_2–C_{10}) bind to Hp-β-CD in a 1:1 manner by inclusion of the alkyl chains of their acyl groups. (*b*) The *m*-nitro isomers (C_2–C_{10}) undergo basic cleavage through aryl inclusion (**3**). (*c*) The short *p*-nitrophenyl esters (C_2–C_6) react less efficiently by aryl inclusion but longer esters (C_7–C_{10}) react with acyl inclusion (**4**), and after C_8 they are more reactive than their *meta* isomers. (*d*) Some long esters show 1:2 (ester:CD) complexation where the second CD must bind to the aryl group. (*e*) With longer esters cleavage involving two CD molecules is observable and reaction within the ternary complexes ($S{\cdot}CD_2$) is faster than in the binary equivalents (S·CD). For the *meta* isomers, at least, this cleavage probably occurs as depicted in structure **8**.

Experimental

'Hydroxypropyl-β-cyclodextrin' is not a pure compound, being available at different degrees of substitution. We used the material supplied by Aldrich which has a stated average molecular weight of 1500, corresponding to the replacement of about six of the seven primary hydroxy groups of β-CD by 2-hydroxypropyl groups. We appreciate that such material is a mixture so that values of measured parameters might vary from sample to sample. However, we feel that trends in parameters are meaningful, especially in the present case where the values of K_1 for Hp-β-CD and β-CD binding to various species are virtually identical.[13] Moreover, since we have found good reproducibility in behaviour, we believe the material has a consistent composition.

Most of the *p*-nitrophenyl esters (C_2–C_6, C_8, C_{10}) were obtained from Sigma; the C_7 and C_9 esters were synthesized by a DCC[21] method (see below). All of the *m*-nitrophenyl esters were prepared by classical methods, as previously,[9] or using DCC, as follows.

The phenol (1.0 equiv.), the carboxylic acid (1.15 equiv.) and DCC (1.0 equiv.) were dissolved in freshly distilled CH_2Cl_2, and the resultant solution was refluxed overnight. The product solution was reduced to 25% of its volume and solid dicyclohexylurea was filtered off. The remaining solvent was evaporated from the filtrate and the residual oil was filtered to remove any more of the urea that precipitated; the oil was then purified by column chromatography on silica gel, using CH_2Cl_2 as the eluent. The desired ester was in the first, pale yellow band to elute from the column. The final product (pale yellow oil or

solid) was isolated by evaporation of the solvent. The solid *m*NPAlk C_{10} ester was recrystallized from methanol to give pale yellow needles. The identity and purity of the esters were checked by TLC, their NMR spectra, and by the spectral change that occurred on hydrolysis in aqueous base.

Reactions were carried out by 1:1 stopped-flow mixing of a 0.4 mol dm^{-3} phosphate buffer (pH 11.6) with the ester (20–100 μmol dm^{-3} for *p*NPAlk; 100–400 μmol dm^{-3} for *m*NPAlk) dissolved in water or Hp-β-CD solutions, so that final concentrations were half these. Substrate solutions were made by dilution of strong stock solutions in spectral grade methanol.

Kinetics procedures largely followed previous practice,[8-10] except for the equipment. The reactions were followed by monitoring the first-order production of the nitrophenolate anions at 390–410 nm,[9] using a stopped-flow apparatus from Tri-Tech Dynamic Instruments (Winnipeg, Manitoba, Canada). The output voltage of the photomultiplier was captured with a Metrabyte DASH 16F A/D card, installed in an Olivetti M24 microcomputer, and then converted into transmittance and absorbance (A). Normally, 100 absorbance values, covering 10 half-lives, were collected. First-order rate constants were estimated from the slope of ln ($A_\infty - A$) against time, for 20–30 absorbance values spanning 2–3 half-lives. In cases where the overall absorbance change was small owing to the very low $[ester]_0$, so that the traces were noisy, A_∞ was estimated by the Swinbourne method.[22] Recorded rate constants (k_{obs}) were the averages of 5–10 determinations. The observation cell of the apparatus was kept at 25 °C.

Fitted constants for eqns. (3), (5) and (7) were obtained by non-linear least-squares methods, using in-house software or commercial programs (Inplot, SigmaPlot), all based on the Marquardt algorithm.[23] Results from the various programs generally agreed to more than three significant figures.

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