Hydrolysis of 3-Nitrophenyl Acetate by β-Cyclodextrin in Substituted Imidazole Buffers

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In order to examine the effects of substituted imidazoles upon cyclodextrin esterolysis,† the title hydrolysis has been carried out at 30 °C using imidazole, 2-methyl-, 2-isopropyl-, 2,4,5-trimethyl-, and 2-(1,1-dimethyl-2-hydroxyethyl)-imidazole. Observed pseudo-first-order rate constants (k_{obs}) are analysed in terms of a Lineweaver–Burk-type equation to give the first-order rate constant (k_{cat}) for a cyclodextrin-ester complex (CD-S) and an apparent dissociation constant (K_{app}). The latter gives the dissociation constants (K_d and K_i) for CD-S and a cyclodextrin–imidazole complex. Using the equation $k_{cat} = k_{cat-OH}[OH] + k_{cat-Im}[Im]_f$, the second-order rate constants for CD-S due to hydroxide ion (k_{cat-OH}) and due to an imidazole base (k_{cat-Im}) are determined. A plot of k_{cat-Im} *versus* pK_a of the imidazoles gives β 0.62, and solvent D_2O effects upon k_{cat-Im} for 2-isopropyl- and 2,4,5-trimethyl-imidazole are estimated to be *ca.* 2. From these results, it is suggested that the imidazoles used here act as a general base catalyst for the cyclodextrin esterolysis.

Cyclodextrins have been utilized as enzyme models because of their ability to form inclusion complexes.[1,2] It is known that esterolytic reactions catalysed by cyclodextrins proceed through nucleophilic attack by their alkoxide ions and that the reactions are highly dependent on the concentration of hydroxide ion in solution.[3,4] In attempts to accelerate these reactions, modifications of cyclodextrins or substrates as well as the synthesis of imidazole-attached cyclodextrins have been carried out.[1,4,5] In view of discussions about the charge-relay system for enzymes,[6] it was desirable to construct a proper model which works by a general base mechanism through a covalently attached base. Before synthesizing appropriate models, however, it was still useful to examine the effects of added bases on cyclodextrin reactions. In this respect, Komiyama *et al.* have reported the hydrolysis of 3-t-butylphenyl acetate by α-cyclodextrin in the presence of benzimidazoles as catalyst. They concluded that these imadazoles act as nucleophilic catalysts.[7]

We have shown previously that the nucleophilic reactivity of substituted imidazoles is markedly diminished by bulky substituents,[8] while general base activity is little affected.[9] This paper describes the effects of these substituted imidazoles upon cyclodextrin-mediated cleavage of an aryl acetate.

Results

The hydrolysis of 3-nitrophenyl acetate was carried out in the absence or presence of β-cyclodextrin with substituted imidazoles as buffer at 30 °C and ionic strength 0.20. The buffer ratio was adjusted to 5.0 for imidazole, 2-methyl-, 2-isopropyl-, and 2-(1,1-dimethyl-2-hydroxyethyl)-imidazole, and to 2.5 for 2,4,5-trimethylimidazole. The initial concentrations of the imidazole [Im], the cyclodextrin [CD], and the ester [S] were of the order of 10^{-1}, 10^{-3}, and 10^{-4}M, respectively.

Observed pseudo-first-order rate constants (k_{obs}) are collected in Table 1. A free base concentration up to 0.3M was used, since aggregation-like behaviour was observed at higher concentrations.‡ As shown in Figure 1, saturation-type kinetics are seen for most of the imidazoles. However, in the case of imidazole itself, k_{obs} decreases with increasing cyclodextrin concentration.

The reaction sequence for the initial stage of the hydrolysis is shown in the Scheme. Here, the substrate is converted into products *via* two pathways represented by the first-order rate constants, k_{un} and k_{cat}. The overall rate is given by equation (1), where equation (2) holds. From equations (1) and (2),

$$\text{Rate} = k_{obs}[S]_0 = k_{un}[S] + k_{cat}[\text{CD-S}] \quad (1)$$

$$[S]_0 = [S] + [\text{CD-S}] \quad (2)$$

$$(k_{obs} - k_{un})[S]_0 = (k_{cat} - k_{un})[\text{CD-S}] \quad (3)$$

equation (3) is easily obtained. The k_{un} pathway is more precisely represented by equation (4). The k_{cat} pathway is

$$k_{un} = k_0 + k_{Im}[Im]_f \quad (4)$$

expressed by equation (5) if the imidazole used as buffer has catalytic activity. k_{cat-OH} and k_{cat-Im} are the second-order

$$k_{cat} = k_{cat-OH}[OH] + k_{cat-Im}[Im]_f \quad (5)$$

rate constants for CD-S due to hydroxide ion and the imidazole base, respectively. At a given constant pH, $k_{cat-OH}[OH]$ is constant. The initial concentrations of the cyclodextrin $[CD]_0$ and imidazole $[Im]_0$ are given by equations (6) and (7).

$$[\text{CD}]_0 = [\text{CD}] + [\text{CD-Im}] + [\text{CD-S}] \simeq [\text{CD}] + [\text{CD-Im}] \quad (6)$$

$$[Im]_0 = [Im] + [\text{CD-Im}] \quad (7)$$

The dissociation constants, K_i and K_d, are expressed by equations (8) and (9), when equilibrium is attained. By using

$$K_i = [\text{CD}][Im]/[\text{CD-Im}] \quad (8)$$

$$K_d = [\text{CD}][S]/[\text{CD-S}] \quad (9)$$

equation (7) and the right-hand part of equation (6), equation (10) may be derived from equation (8). Similarly, from

† The term esterolysis is used to mean fission *of* an ester.
‡ A break such as reported for 2-isopropylimidazole [9] was observed for 2,4,5-trimethylimidazole by an n.m.r. technique.

Table 1. Hydrolysis of 3-nitrophenyl acetate in substituted imidazole buffers with varying concentrations of β-cyclodextrin [a]

Buffer free base (M)	10^3[β-cyclodextrin]/M: 0.00 [b]	3.33	5.00	6.67	8.33	10.0	$10^3k_{cat}/s^{-1}$	10^3K_{app}/mol l^{-1}
	$10^3k_{obs}/s^{-1}$							
Imidazole (pH 7.75) [c]								
0.10	25.7	21.5	19.9	18.4	17.4	16.7	10.0	16.9
0.20	49.0	43.1	41.4	38.8	36.3	34.4	12.5	24.4
2-Methylimidazole (pH 8.68) [c]								
0.10	3.88	5.25	5.70	6.11	6.33	6.56	9.01	9.08
0.20	7.54	8.25	8.50	8.72	8.88	8.99	10.6	10.8
0.30	11.0	11.4	11.4	11.5	11.6	11.6		
2-Isopropylimidazole (pH 8.54) [c]								
0.10	0.407	1.79	2.21	2.55	2.94	3.17	5.96	10.1
0.20	0.758	1.80	2.20	2.56	2.84	3.18	6.82	15.9
0.30	0.976	1.84	2.20	2.55	2.80	3.04	7.64	22.2
In D_2O (pD 8.65) [c]								
0.10	0.347	1.43	1.71	2.07	2.24	2.43	4.19	8.46
0.30	0.768	1.52	1.89	2.09	2.31	2.54	5.12	14.9
2-(1,1-Dimethyl-2-hydroxyethyl)imidazole (pH 8.15) [c]								
0.10	0.0621	0.405	0.545	0.662	0.776	0.868	2.56	20.8
0.20	0.0676	0.318	0.438	0.542	0.635	0.698	2.99	35.0
2,4,5-Trimethylimidazole (pH 9.36) [d]								
0.10	0.532	9.58	12.6	15.5	17.2	18.9	39.0	10.8
0.20	0.602	7.98	10.6	13.2	15.2	17.2	42.3	15.6
0.30	0.684	6.59	8.64	10.9	13.0	14.5	46.1	22.7
In D_2O (pD 9.56) [d]								
0.10	0.360	8.27	10.4	12.7	14.1	15.5	28.4	8.49
0.20	0.449	6.75	9.10	10.9	12.6	13.5	30.0	12.3

[a] At 30 °C and ionic strength 0.20 (KCl). [b] k_{obs} at zero cyclodextrin concentration = k_{un}. [c] Buffer ratio 5.0. [d] Buffer ratio 2.5.

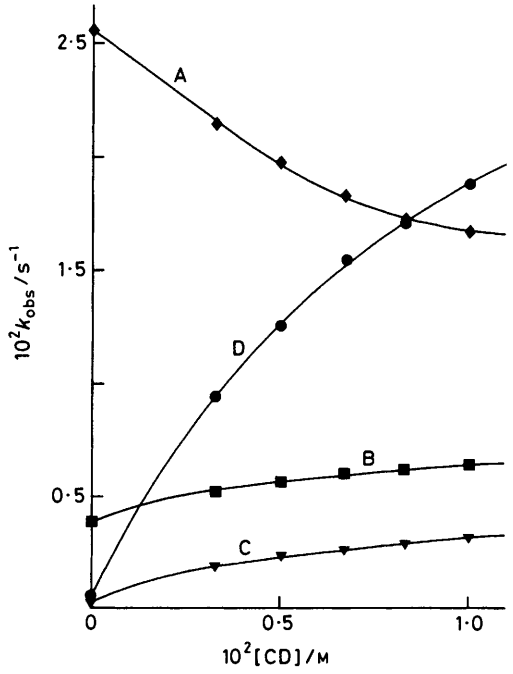
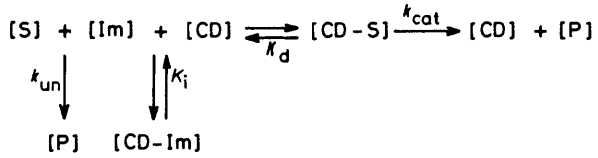


Figure 1. Observed first-order rate constants *versus* β-cyclodextrin concentration in substituted imidazole buffers of 0.10M free base concentration: A, imidazole; B, 2-methylimidazole; C, 2-isopropylimidazole; D, 2,4,5-trimethylimidazole. Solid lines are produced by computer using the constants listed in Table 2



Scheme

equation (9), equation (11) is obtained by using equation (2) and the right-hand parts of equations (6) and (10). Combin-

$$[\text{CD-Im}] = \frac{[\text{CD}]_0[\text{Im}]_0 + [\text{CD-Im}]^2}{K_i + [\text{Im}]_0 + [\text{CD}]_0} \simeq \frac{[\text{CD}]_0[\text{Im}]_0}{K_i + [\text{Im}]_0} \quad (10)$$

$$\frac{[\text{CD-S}]}{[\text{S}]_0} = \frac{K_i[\text{CD}]_0}{K_d(K_i + [\text{Im}]_0) + K_i[\text{CD}]_0} \quad (11)$$

ation and rearrangement of equations (3) and (11) give equation (12), where the apparent dissociation constant, K_{app}, is defined by equation (13).

$$\frac{1}{k_{obs} - k_{un}} = \frac{K_d(K_i + [\text{Im}]_0)}{[\text{CD}]_0K_i(k_{cat} - k_{un})} + \frac{1}{k_{cat} - k_{un}} \quad (12)$$

$$K_{app} = \frac{K_d(K_i + [\text{Im}]_0)}{K_i} = K_d + \frac{K_d[\text{Im}]_0}{K_i} \quad (13)$$

Thus the observed rate constants (k_{obs}) are plotted in terms of a Lineweaver–Burk-type equation (12) as shown in Figure 2,

Table 2. Various constants for hydrolysis of 3-nitrophenyl acetate by β-cyclodextrin in substituted imidazole buffers

Imidazole substituent	pK_a [a]	$10^3k_0/s^{-1}$	10^3k_{Im}/ l mol^{-1} s^{-1}	10^3[OH$^-$] k_{cat-OH}/s^{-1}	10^3k_{cat-Im}/ l mol^{-1} s^{-1}	10^3K_d/mol l^{-1}	K_I/mol l^{-1}
1 2-H	7.19	2.4	233	0.75	2.5	9.4	0.15
2 2-Me	8.10	0.22	36.6	7.4	16.0	7.3	0.51
3 2-Pr^i (H_2O)	8.01	0.16	2.8	5.1	8.4	4.1	0.081
4 2-Pr^i (D_2O)		0.14	2.1	3.7	4.6	5.2	0.20
5 2-$HOCH_2C(Me)_2$	7.54	0.55	0.066	2.1	4.3	6.8	0.058
6 2,4,5-Me_3 (H_2O)	9.03	0.45	0.75	35.3	35.5	5.5	0.14
7 2,4,5-Me_3 (D_2O)		0.27	0.89	26.8	16.0	4.7	0.17

[a] Ref. 8.

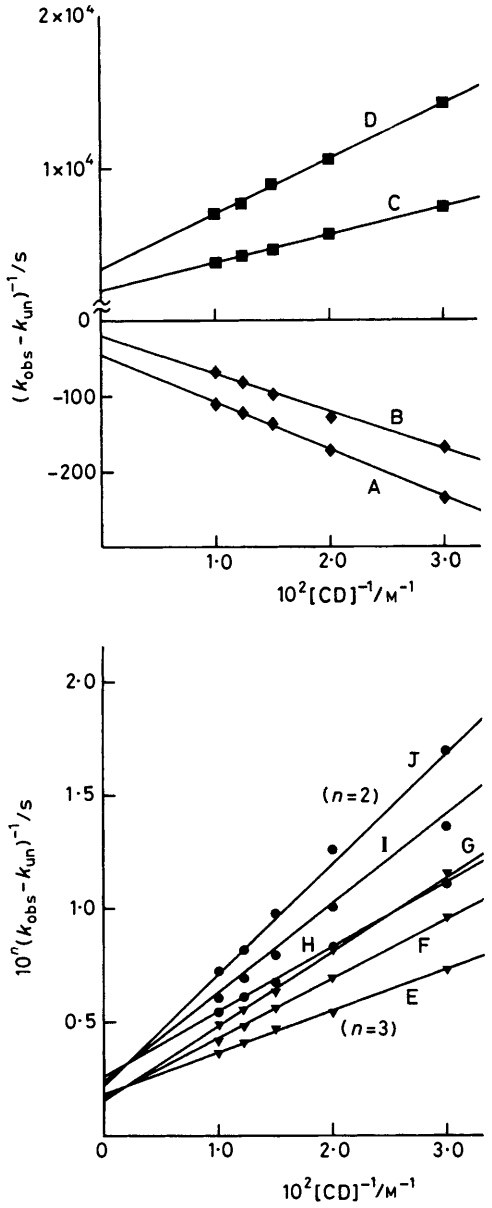


Figure 2. Lineweaver–Burk-type plots for different buffer free base concentrations to give k_{cat} and K_{app} values: for imidazole, A 0.10M, B 0.20M; for 2-methylimidazole, C 0.10M, D 0.20M; for 2-isopropylimidazole, E 0.10M, F 0.20M, G 0.30M; for 2,4,5-trimethylimidazole, H 0.10M, I 0.20M, J 0.30M. The buffer ratio is given in Table 1. Solid lines are drawn by computer using the constants listed in Table 2

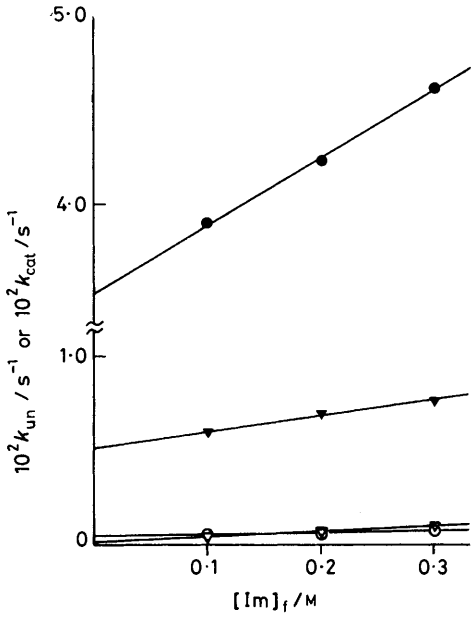


Figure 3. Plots of k_{cat} and k_{un} *versus* $[Im]_f$: for 2,4,5-trimethylimidazole, ● k_{cat} and ○ k_{un}; for 2-isopropylimidazole, ▼ k_{cat} and ▽ k_{un}

and the values of k_{cat} and K_{app} obtained are included in Table 1.

The values of k_{cat} for imidazole are too small relative to the k_{un} values to be determined accurately. The k_{cat} value for 0.3M-2-methylimidazole was not obtained due to small differences in k_{obs} values. The constants K_d and K_I were determined according to equation (13), where the imidazole free base concentration $[Im]_f$ was used for $[Im]_0$.

In Figure 3, plots of k_{cat} and of k_{un} *versus* $[Im]_f$ are shown for some of the imidazoles. The slope and intercept of the k_{cat} plot yield the values of k_{cat-Im} and $k_{cat-OH}[OH]$, and, similarly, the k_{un} plot gives k_{Im} and k_0, respectively, as described in equations (4) and (5). The various constants obtained are summarized in Table 2.

Hydrolysis in D_2O was conducted in a few cases and these data are also presented in Tables 1 and 2.

The u.v. maximum of imidazole in water was shifted from 206 to 207.5 nm by addition of the cyclodextrin, but no such shift was observed for 2,4,5-trimethylimidazole.

Discussion

As cyclodextrins form complexes with a variety of substances,[10,11] a large amount of an imidazole base as buffer might be thought to hamper the formation of a cyclodextrin–

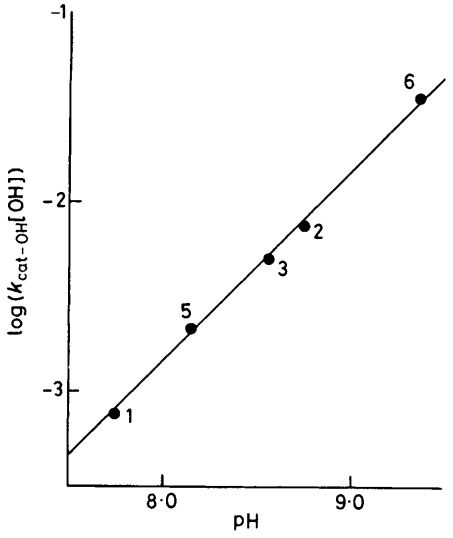
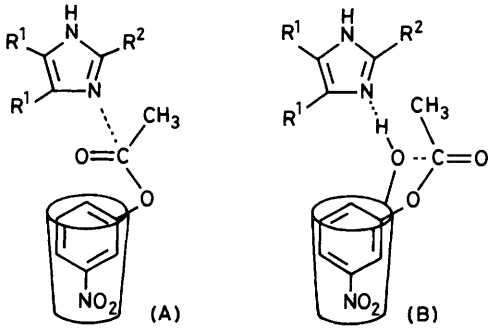


Figure 4. Plot of log(k_{cat-OH}[OH]) *versus* pH of the solution. A straight line with slope 1.0 is provided: numbers refer to the imidazoles given in Table 2



ester complex. The saturation-type kinetics observed (Figure 1), however, indicate that interaction between the substrate and cyclodextrin is still present. Under these conditions the reaction is shown in the Scheme. The equilibrium conditions postulated for the use of K_d and K_I are reasonable, since many cyclodextrin reactions have been proved to act in this manner.[1]

Equation (4) is the usual expression for nucleophilic catalysis of aryl esters by imidazoles.[8,12] In Table 2 the values of k_0 appear rather scattered, though it should vary little in the pH range 7.75—9.36. This is unavoidable in the present treatment where k_0 is determined as a value within the limit of errors of k_{un}.

In the k_{cat} pathway, when a second reactant is involved, its concentration is included in the rate constant,[13] so that k_{cat} is expressed by equation (5). The term k_{cat-OH}[OH] has been confirmed by Breslow *et al.*,[4] though experimental verification of the term k_{cat-Im}[Im][CD-S] is difficult because of the interdependence of [CD-S] on other variables. Therefore, various k_{cat} values for different buffer concentrations at a constant $[S]_0$ were determined with the condition [CD] > [S], so as to observe exclusively a substrate-deacylation step.

As described in equation (12), a plot of $1/(k_{obs} - k_{un})$ *versus* $1/[CD]_0$ yields $1/(k_{cat} - k_{un})$ as the intercept and k_{cat} is obtained independently of K_{app}. Owing to the limited solubility of the cyclodextrin, extrapolation to 1/[CD] = 0 is necessary in the k_{cat} determination, and the values thus

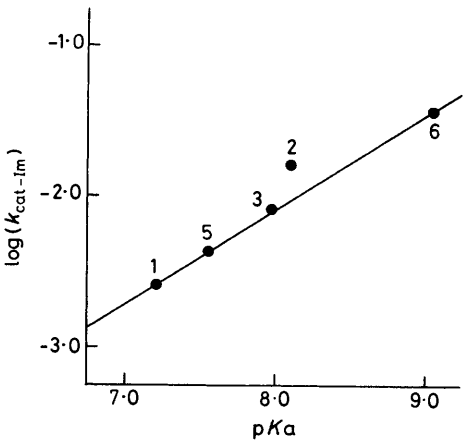


Figure 5. Brönsted plot of log(k_{cat-Im}) values *versus* pK_a of the imidazoles. The slope is estimated to be 0.62: numbers refer to the imidazoles given in Table 2

obtained contain errors. Therefore, the values should be checked for their reliability from different points of view.

A plot of log(k_{cat-OH}[OH]) values for the substituted imidazoles *versus* pH of these buffer solutions provides a straight line with a slope of unity as shown in Figure 4. Extrapolation of the line to pH 10.60 meets a point which has been determined by VanEtten *et al.* in carbonate buffer with the same ionic strength.[3] The straight line shown in Figure 4 implies that each of the k_{cat-OH} values and hence each of the k_{cat} values are reasonable, since hydroxide ion-promoted cyclodextrin reactions produce just such a straight line.[3,4,14]

As Table 2 shows, 2,4,5-trimethylimidazole has a significant kinetic association with cyclodextrin (K_I), although no spectral shift is observed. The association of these imidazoles with cyclodextrin is generally weak, as is seen in the much larger value of K_I than K_d. This suggests that most of the imidazole is not complexed with cyclodextrin. For a single substrate K_d should be constant and a value of 0.008 mol l^{-1} in carbonate buffer [3] has been obtained for the complex in the present case. The values here could be thought to be close to this value after making allowances for the errors involved, since K_d and K_I are obtained as a pair *via* a secondary plot of the observed data.

In view of the results, it is conceivable that the role of the imidazole is catalysis for k_{un} and k_{cat} pathways and inhibition toward the formation of a cyclodextrin–ester complex. These complicated features may be seen as an apparently mixed type of inhibition kinetics [13] as shown in Figure 2, though an imidazole acting as an inhibitor is competitive in nature.

In order to test further the constants obtained (Table 2), computer-produced curves were provided for the plots of k_{obs} *versus* $[CD]_0$ (Figure 1) and of $1/(k_{obs} - k_{un})$ *versus* $1/[CD]_0$ (Figure 2), using the individual values of k_0, k_{Im}, k_{cat-OH}, k_{cat-Im}, K_d, and K_I. Agreement of these curves with the observed points is reasonably good. Therefore, the Scheme and the equations describe the essential features of the hydrolysis.

An increase in k_{cat} with increasing imidazole concentration is attributable to an interaction between a complexed ester and an imidazole base. It is possible for an imidazole to compete with hydroxide ion for attack upon CD-S by virtue of its overwhelming concentration in the neutral pH region.

An imidazole is considered to act either as a nucleophilic or as a general-base catalyst as depicted in structure (A) or (B). It is shown in (A) that the complexed ester is still attacked

by an imidazole directly. The situation in (B) is partly analogous to an enzyme process in which a captured substrate is attacked by the hydroxy-group of an adjacent serine residue with general base assistance by the imidazolyl group of a histidine residue.[6] Retardation by cyclodextrin of the direct attack of imidazole upon the ester is clearly seen from the decrease in k_{obs} with increasing cyclodextrin concentration (curve A in Figure 1). It appears that structure (A) is unfavourable and makes a small contribution, if any.

A large increase in k_{cat} relative to k_{un} in Figure 3 or a larger k_{cat-Im} than k_{Im} in Table 2 for the cases of 2-isopropyl-, 2-(1,1-dimethyl-2-hydroxyethyl)-, and 2,4,5-trimethyl-imidazole clearly favours situation (B). A Brönsted plot of $\log(k_{cat-Im})$ *versus* pK_a for the imidazoles yields a straight line with a slope of 0.62 (Figure 5). Since the nucleophilic activity of substituted imidazoles is much more affected by their substituents than is general base activity [9] and k_{Im} and k_{cat-Im} are quite different functions of pK_a, β 0.62 is a good indication as general base assistance.[15] Solvent D_2O isotope effects obtained for 2-isopropyl- and 2,4,5-trimethyl-imidazole (Table 2) are also in line with this result. The values of *ca.* 2 for k_{cat-Im} suggest general base catalysis, whereas those of *ca.* 1 for k_{Im} indicate nucleophilic catalysis.

In micellar systems with both hydroxy and imidazolyl groups, the imidazolyl group first attacks on the carbonyl group of aryl esters, followed by acyl group transfer to the hydroxy-group.[16] However, in tighter micelles where an imidazolyl group is much more immobilized, general base catalysis by the imidazolyl group for hydrolysis of aryl carboxylates is observable.[17] In the hydrolysis of phenyl 4-hydroxybutyrate, direct nucleophilic catalysis by imidazole has been seen instead of intramolecular hydroxy-group participation with assistance by the imidazole, although for weak nucleophilic catalysts the participation with general base assistance has been observed.[18]

On the other hand, general base catalysis by imidazole has been detected in intramolecular acyl group transfer in 2-hydroxymethyl-4-nitrophenyl trimethylacetate.[19] These different examples of aryl ester cleavage show that general base catalysis occurs where there is a restriction on the nucleophilic activity of the attacking base. The present system where a substrate is bound into the cyclodextrin cavity may be compared to the case of the functional tight micelles [17] or of the sterically hindered intramolecular trimethylacetyl group transfer.[19]

For a similar cyclodextrin-mediated hydrolysis of aryl carboxylates, a different conclusion was reached, where imidazole nucleophilic catalysis is believed to prevail,[7] but there are differences from our procedures in the kinetic experiments and data analysis.

In conclusion, our results favour general base catalysis by an imidazole base in cyclodextrin–aryl ester cleavage, but the experimental limitations in the present case necessitate further investigations.

Experimental

3-Nitrophenyl acetate was recrystallized from n-hexane, m.p. 54—55 °C. Sources of imidazoles were described previously.[8] β-Cyclodextrin was a commercial product and purified by repeated recrystallization from water and dried *in vacuo*, $[\alpha]_D$ 162° (lit.,[10] 162.5°). Deuterium oxide (Merck, Uvasol) had a minimum 99.75% D. Water and deuterium oxide were glass distilled before use. Spectroscopic grade acetonitrile was used. Imidazole buffers were carefully prepared by partial neutralization of concentrated solutions of free bases with hydrochloric acid. The ionic strength was kept constant (0.20) by addition of KCl. Lower concentration buffers were obtained by serial dilution with 0.20M-KCl aqueous solution. The D_2O buffer was obtained from deuterium-exchanged imidazoles and their deuteriochloric acid salts. The pH of solutions was measured on a Toadenpa model HM 20B pH meter.

Kinetic Method.—The hydrolysis reaction was followed by monitoring the liberation of 3-nitrophenol on a Hitachi 124 spectrophotometer. A constant temperature was maintained. To a buffer solution (3.0 ml) in a cuvette containing the cyclodextrin (CD) was added the ester in acetonitrile (0.020 ml) with vigorous stirring. The initial concentration of the ester was 1.0×10^{-4}M. Each rate was measured three times in water and twice in D_2O. The observed first-order rate constants (k_{obs}) were obtained as the slope of plots of $\ln (OD_0 - OD_\infty)/(OD_t - OD_\infty)$ *versus* time. Usually, a good first-order dependence was observed for at least three half-lives. Errors were estimated at $\pm 5\%$ for most k_{obs} values. The values of k_{cat} and K_{app} were obtained from plots of k_{obs} and $[CD]_0$. The pH of solutions was measured before and after the kinetic runs and found to remain constant ($\pm$ 0.01 pH unit).

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