Research Article

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A study on a primitive artificial esterase model: reactivity of a calix[4]resorcinarene bearing carboxyl groups

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The host molecule octacarboxymethyl calix[4]resorcinarene 1 catalyses the hydrolysis of substituted phenyl *N*-methylpyridinium-4-carboxylate esters 3a–f by complexation followed by intracomplex reaction *via* an anhydride intermediate. The reactivity in the presence of 1 is higher than that of the background at low pH; at high pH an inversion of reactivity occurs, the background becomes predominant since the host inhibits hydrolysis of the esters. The reactivity of esters 3a–f complexed with the host suffers little change in effective charge on the phenolic oxygen (−0.15 units) in contrast with the changes observed in alkaline hydrolysis (−0.28 units) and in the hydrolysis of the model monoaryl glutarate esters (-1.02 units). The less negative effective charge in the transition state for host 1 catalysis compared with that in the glutarate case is ascribed to stronger solvation by water molecules in the complex compared with that due to water molecules in the bulk solvent. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: calixresorcinarenes; molecular receptors; catalysis; ester hydrolysis

INTRODUCTION

Carboxylate ions, in aqueous solution, catalyse the hydrolysis of substituted phenyl esters via an anhydride intermediate or by general base catalysis (Scheme 1).[1–7]

Since carboxylate ions can be readily incorporated into macrocycles possessing cavities suitable as hosts, the system would be interesting to study as a primitive model of an enzyme. Calix[4]resorcinarenes would be particularly useful because they have a binding site, and the rim of the macrocycle can be functionalized easily to possess carboxylate ions which would be suitable for reaction with aryl esters as in Scheme 1. Previous work has shown that resorcinarenes bearing aliphatic chains terminated by $(CH_3)_2N$ groups at their upper rim can be good catalysts for ester hydrolysis.[8] In this study, we investigate the effect of a carboxy containing resorcinarene **1** on the hydrolysis of some selected substituted phenyl esters.

Esters derived from *N*-methylpyridinium-4-carboxylic acid are particularly useful in a preliminary study because they bear a positive charge and therefore are expected to complex more efficiently than neutral esters to a host possessing carboxylate anion groups. Moreover, the reactivity of such esters is higher than that of corresponding neutral aliphatic esters and the effective charge characteristics of acyl group transfer reactions with these positively charged esters have been elucidated previously.[9,10]

The purpose of this work is to investigate the selectivity and influence of the host molecule **1** on the reactivity of some carboxylic acid esters, either neutral (**2a–c**) and positively charged (**2d** and **3a–f**), as shown in Chart 1. Linear free energy relationships giving rise to effective charge distributions[11–13] will also provide information about the micro-environment of the guest ester (substituted phenyl *N*-methylpyridium-4-carboxylate tosylate salts **3**) in the complex with resorcinarene derivative **1**.

RESULTS

Kinetics of hydrolysis of the esters nicely fit first-order rate laws up to 90% of the full reaction. Addition of **1** inhibits the hydrolysis of both neutral and charged esters at pH 7.05 and above (Fig. 1 and Fig. 2, respectively).

On the other hand, the hydrolysis of the ester **3a** at lower pH's is catalysed by the addition of **1** (Figure 2). Rate constants (k_{obs}) depend on the concentration of the host and follow the rate law of Eqn (1), derived from Scheme 2, where k_b is the background rate constant, K_S is the dissociation constant of the host–guest complex and k_c is the rate constant for the complexed ester.

$$k_{obs} = \frac{(k_b \times K_s + k_c \times [\text{host}])}{(K_s + [\text{host}])} \quad (1)$$

Rate constants for the hydrolysis of esters **2a–d** in the absence or presence of varying concentrations of host **1** are given in Table 1.

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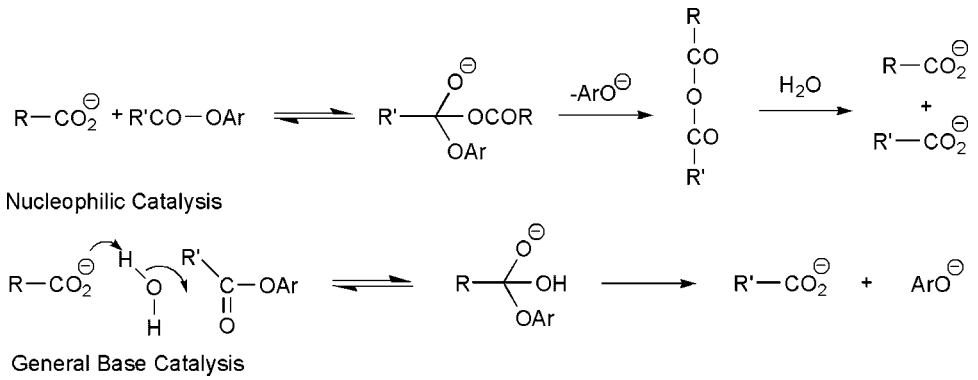


Scheme 1. Mechanisms for the carboxylate ion-catalyzed hydrolysis of esters

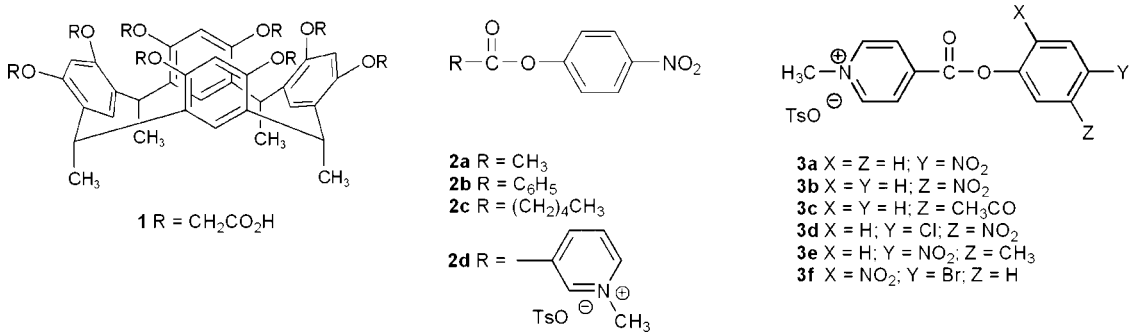


Chart 1. Structures of macrocyclic host **1** and carboxylates **2** and **3**

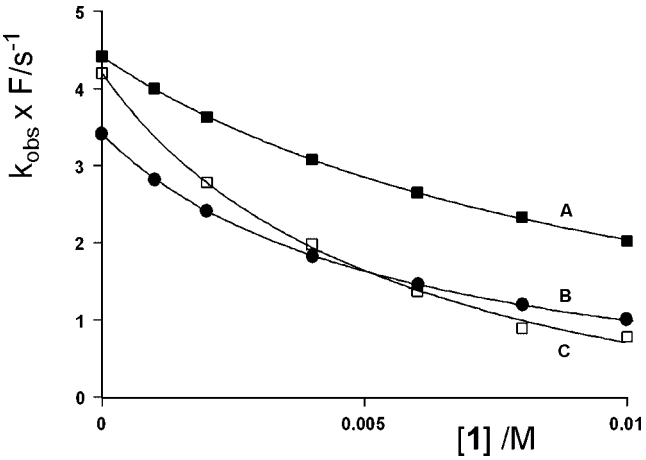


Figure 1. Dependence of rate constants on host **1** concentrations for the hydrolysis of **2a** (■, curve A, pH 10.38, 0.02 M borate, $F = 10^2$) and **2b** (●, curve B, pH 12.05, 0.10 M phosphate, $F = 10^2$), and the ethanolaminolysis of 2b (□, curve C, pH 8.32, 0.5 M ethanolamine, $F = 10^3$) at 25 °C. Data are from Table 1 and lines are calculated from Eqn (1) using parameters from Table 1

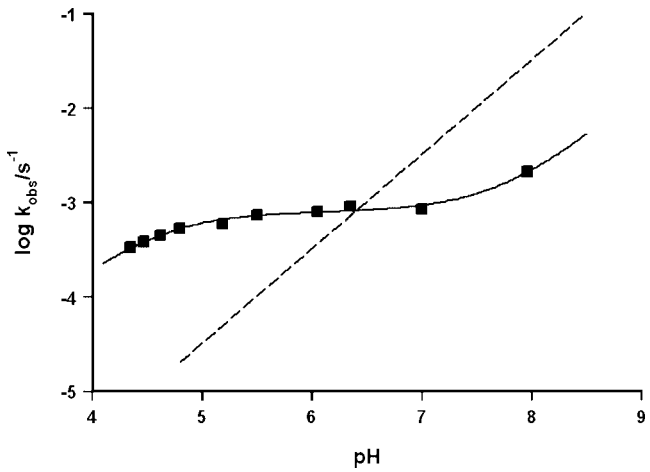
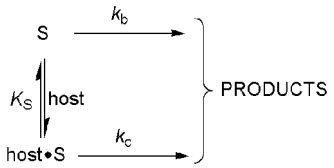


Figure 2. Dependence on pH of the hydrolysis of ester **3a** in the presence of 5×10^{-3} M host **1** (solid line, closed squares) or in its absence (dashed line) at 25 °C. Data are from Table 3 and the solid line is calculated from Eqn (2); dashed line is calculated from $k = 3.24 \times 10^4 \times [OH^-]$ (from Table 4)

The reaction of 4-nitrophenyl benzoate **2b** with the nucleophile ethanolamine was also examined to see if the inhibition could be simply due to electrostatic repulsion between ionized **1**, which is negatively charged, and hydroxide ion (see Fig. 1 and Table 1). The rate constants for the hydrolysis of the charged esters **3a–f** in the absence and in the presence of increasing concentrations of the host are recorded in Table 2 together with the derived kinetic parameters K_S (the dissociation constant of



Scheme 2.

Table 1. Dependence of the rate constant k_{obs} and the derived constants k_b, k_c and K_s, on the concentration of host **1** for the hydrolysis of some 4-nitrophenyl esters at 25 °C in water solvent[a]

	$10^2 \times k_{obs}/s^{-1}$				
[**1**]/M($\times 10^3$)	**2a**[b]	**2b**[c]	**2b**[d]	**2c**[e]	**2d**[f]
0.00	4.42	3.42	0.420	0.710	0.343
1.00	4.00	2.82		0.645 (0.005)[e]	0.317
2.00	3.63	2.42	0.278	0.610 (0.010)[e]	0.267
4.00	3.08	1.85	0.198	0.558 (0.020)[e]	0.230
6.00	2.65	1.45	0.137	0.540 (0.025)[e]	0.210
8.00	2.33	1.18	0.089	0.527 (0.030)[e]	0.192
10.0	2.02	1.00	0.078		0.182
20.0					0.148
pH	10.38	12.05	8.32	10.95	7.05
$k_b/s^{-1}(\times 10^3)$	44.0 ± 0.5	34.1 ± 0.1	4.21 ± 0.08	7.10 ± 0.04	3.48 ± 0.06
$k_c/s^{-1}(\times 10^3)$	—[g]	—[g]	—[g]	4.51 ± 0.16	1.01 ± 0.14
K_S/M ($\times 10^3$)	10.8 ± 6.0	5.51 ± 0.20	5.71 ± 0.84[i]	3.03 ± 0.47	4.68 ± 0.80

[a] The initial concentration of esters is 1×10^{-5} M and the wavelength for kinetics is 400 nm.
[b] 0.02 M borate.
[c] 0.1 M K_2HPO_4.
[d] Reaction of ester **2b** with 0.10 M ethanolamine.
[e] Borate (0.02 M); the numbers within the brackets show the concentration of the host.
[f] K_2HPO_4 (0.05 M) and the host concentration is in the range of 0–10^{-3} M.
[g] Zero within experimental errors.

Table 2. Dependence of rate constants on the concentration of host **1** for the hydrolysis of tosyl salts of substituted phenyl *N*-methylpyridinium-4-carboxylates at pH 7.05 (0.05 M KH_2PO_4) and 25 °C[a]

	$10^2 \times k_{obs}/s^{-1}$					
[**1**]/M ($\times 10^3$)	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**
0.00	1.54	0.790	0.466	1.44	1.57	3.28
0.20	1.12	0.530	0.305	0.891	1.08	2.00
0.40	0.850	0.410	0.241	0.681	0.821	1.45
0.60	0.770	0.376	0.225	0.582	0.729	1.24
0.80	0.671	0.327	0.206	0.511	0.630	1.04
1.00	0.641	0.319	0.201	0.473	0.601	0.972
2.00	0.515	0.259	0.182	0.366	0.449	0.709
pK_a[b]	7.14	8.35	9.19	7.75	7.26	6.36[c]
k_b/s^{-1} ($\times 10^3$)[d]	15.5 ± 0.2	7.91 ± 0.09	4.67 ± 0.05	14.4 ± 0.4	10.5 ± 0.2	33.0 ± 0.3
k_c/s^{-1} ($\times 10^3$)[e]	3.33 ± 0.39	1.94 ± 0.12	1.54 ± 0.06	2.41 ± 0.05	2.78 ± 0.26	3.87 ± 0.34
K_S/M ($\times 10^4$)[f]	3.30 ± 0.37	2.47 ± 0.21	1.73 ± 0.16	2.35 ± 0.04	3.11 ± 0.23	2.43 ± 0.12
k_c/K_S ($s^{-1}M^{-1}$)[g]	10.9	7.85	8.9	10.2	8.94	15.9

[a] Tosyl salt of substituted phenyl *N*-methylpyridinium-4-carboxylate. Concentrations of substrates and wavelengths for kinetic studies: **3a** (4×10^{-5} M, $\lambda_{max} = 400$ nm); **3b** (2.5×10^{-4} M, $\lambda_{max} = 340$ nm); **3c** (4×10^{-5} M, $\lambda_{max} = 325$ nm); **3d** (2.5×10^{-4} M, $\lambda_{max} = 340$ nm); **3e** (2.5×10^{-4} M, $\lambda_{max} = 370$ nm) and **3f** (4×10^{-5} M, $\lambda_{max} = 410$ nm).
[b] The ionizations constants of leaving groups are from Reference[14], unless stated otherwise.
[c] pK_a value from Reference[15].
[d] First-order rate constant for the reaction of esters in the buffer (including buffer and hydroxide ion terms).
[e] First-order rate constant for the ester complex with host **1**.
[f] Dissociation constant of esters from their complex with host **1**.
[g] Apparent second-order rate constant for the reaction of substituted phenyl *N*-methylpyridinium-4-carboxylate esters with free host **1**.

Table 3. The effect of pH on the hydrolysis of **3a** in the presence of host **1** (5×10^{-3} M) at 25 °C. The host molecule itself provides buffering capacity

pH	4.35	4.47	4.62	4.80	4.80	5.19	5.50	6.05	6.35	7.00	7.96
$k_{obs} \times 10^4/s^{-1}$	3.37	3.83	4.45	4.72	5.35	5.93	7.30	8.03	9.07	8.55	21.2

the ester–host complex) and k_c/K_S (the apparent second-order rate constant for the reaction of *N*-methylpyridinium 4-carboxylates through the ester–host complex).

The pH-dependence of the reactivity of **1** with the 'charged' 4-nitrophenyl ester **3a** was determined for a 5 mM constant concentration of host (higher concentrations were not attainable due to insufficient solubility of host **1** at low pH's); although this concentration does not completely complex the ester, a significant part of the reaction flux is via the complex and if K_S is insensitive to pH, the pH-dependence will be a reasonably good reflection of that for k_c. The rate constants possess a plateau region between pH's 5 and 7 which falls off at low pH and increases at higher pH. The rate constants fit Eqn (2) (see Table 3), where k_c^{max} is the first-order rate constant for the host-mediated reaction, k_{OH} is the second-order rate constant for alkaline hydrolysis of the anionic form of the complexed ester, and K_a and K_W are the ionization constants of the host and water, respectively.

$$k_{obs} \approx k_c = \frac{\left(k_c^{max} + K_{OH} \times K_W \times 10^{pH}\right)}{\left(1 + 10^{-pH}/K_a\right)} \qquad (2)$$

The derived parameters in Eqn (2) are as follows: $pK_a = 4.51 \pm 0.05$, $k_c^{max} = (7.96 \pm 0.18) \times 10^{-4}\ s^{-1}$, $k_{OH} = (1.42 \pm 0.15) \times 10^3\ M^{-1}\ s^{-1}$ with $pK_W = 14.0$. The value of the apparent pK_a is lower than that determined titrimetrically for the host in a different solvent system (5.95, see Section 'Experimental'), but this is not significant as change in the solvent is likely to change the pK_a substantially.

With reference to the reactivity of **3a** in the presence of host **1**, the pH (7.05) of the measurements (Table 2) is within the plateau region as shown in Fig. 2, and hence the parameters k_c actually can be taken as k_c^{max} (in Eqn (2), the term $k_{OH} \times K_W \times 10^{pH}$ can be assumed as negligible in comparison with k_c^{max}). Rate constants for the hydrolysis of substituted phenyl *N*-methyl pyridinium-4-carboxylates are linearly related to phosphate (HPO_4^{2-}) concentrations ($k_{obs} = k_{HPO_4}[HPO_4^{2-}] + k_{OH}[OH^-]$) and the derived second-order rate constants for phosphate and hydroxide catalyzed reactions are shown in Table 4 (it is assumed that the contribution of water to the overall rate is negligible), where k_{HPO_4} and k_{OH} are second-order rate constants for HPO_4^{2-} and hydroxide ion catalyzed hydrolysis of 4-nitrophenyl *N*-methylpyridinium-4-carboxylates. The dependence of the rate and equilibrium parameters on the pK_a of the leaving phenolate ion for the hydrolysis of substituted phenyl *N*-methylpyridinium-4-carboxylate in the absence and in the presence of the host molecule **1** fit Eqns (3–6).

$$\log k_{HPO_4} = -(0.42 \pm 0.07)pK_a^{ArOH} + (2.7 \pm 0.5) \qquad (3)$$

$$\log k_{OH} = -(0.28 \pm 0.03)pK_a^{ArOH} + (0.31 \pm 0.19) \qquad (4)$$

$$\log k_c^{max} = -(0.15 \pm 0.01)pK_a^{ArOH} - (1.47 \pm 0.09) \qquad (5)$$

$$\log K_S = -(0.068 \pm 0.037)pK_a^{ArOH} - (3.1 \pm 0.3) \qquad (6)$$

A small difference is found between the β_{LG} in Eqn (4) (−0.28) and that previously reported (−0.35)[10] for a set of esters bearing (partly) different substituents. In this work we used the presently assessed value.

The rate constants for hydrolysis of the ester **3a** in acetate buffers at pH 5.85 vary linearly as a function of acetate ion concentration (0–0.050 M) yielding a second-order rate constant of $1.09 \times 10^{-3}\ M^{-1}\ s^{-1}$ for attack of acetate ion on **3a**.

A trapping experiment was carried out by reacting **1** (5.0 mM) with 0.02 M tosyl 4-nitrophenyl *N*-methylpyridinium-4-carboxylate **3a** at pH 8.36 in the presence of excess morpholine

Table 4. Effect of phosphate (HPO_4^{2-}) and hydroxide ion on the hydrolysis of substituted phenyl *N*-methylpyridinium-4-carboxylate esters at pH 7.00 and 25 °C[a,b]

Esters	N[c]	$k_{HPO_4} \times 10^{-2}/M^{-1}\ s^{-1}$[d]	pK_a^{ArOH}	$k_{OH} \times 10^{-4}/M^{-1}\ s^{-1}$[e]
3a	4	36.7 ± 3.0	7.14	3.24 ± 0.2
3b	4	11.5 ± 1.0	8.35	1.68 ± 0.1
3c	5	7.28 ± 0.4	9.19	0.90 ± 0.02
3d	4	31.4 ± 2.9	7.75	3.61 ± 0.15
3e	4	26.1 ± 1.0	7.26	2.00 ± 0.08
3f	4	136 ± 10	6.36	7.79 ± 0.07

[a] Ester concentration and wavelengths for kinetic studies given in Table 1.
[b] Concentration range of phosphate buffer between 0.02 and 0.1 M.
[c] Number of data points not including duplicates.
[d] Second-order rate constants for HPO_4^{2-}-catalyzed hydrolysis of substituted esters calculated from the slope of k_{obs} against $[HPO_4^{2-}]$.
[e] Calculated from the intercept of the plot of k_{obs} against $[HPO_4^{2-}]$ assuming $K_w = 10^{-14}$.

(0.4 M). After completion of the reaction (as judged by monitoring the release of 4-nitrophenol) the solution was brought to pH 2 with dilute HCl and the resultant precipitate was isolated by filtration and washed with water (to remove the residual unreacted amine as well as all products derived from the ester which are soluble in water at acidic pH's). The ^{1}H NMR spectrum of the carefully dried residue was analysed, revealing that an aliquot of the trapping agent had undergone incorporation into **1**, consistent with a final mixture of host modified as the monoamide **1a** (as shown in Scheme 3) and the starting material **1** in a ratio of 1:3.

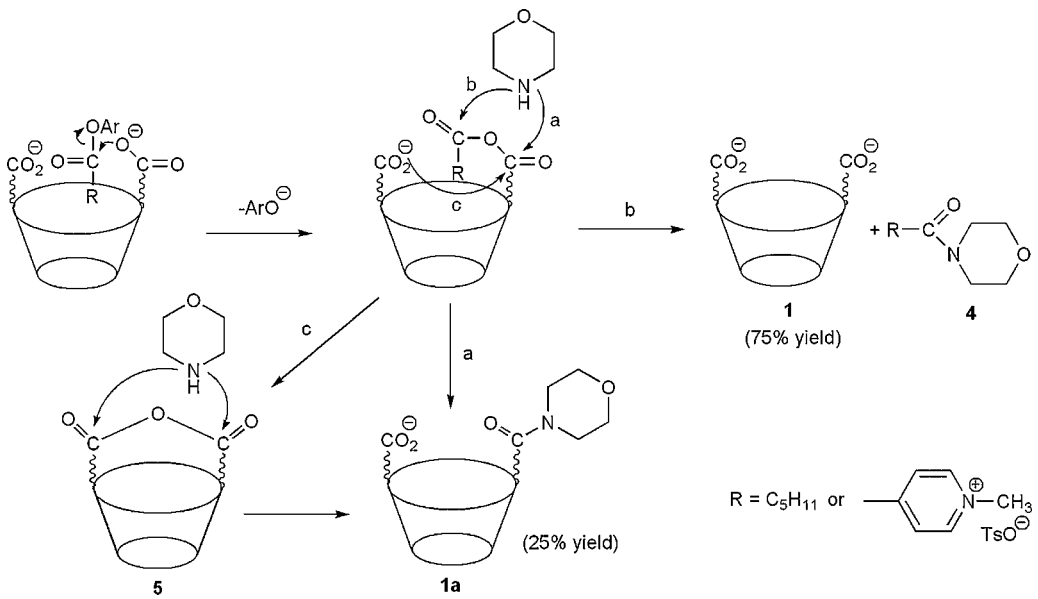
DISCUSSION

The pH-dependence of the hydrolysis of 4-nitrophenyl *N*-methyl pyridinium-4-carboxylates (shown in Figure 2) is consistent with the involvement of a carboxyl group of the host molecule ($pK_a^{apparent} = 4.51 \pm 0.05$) in the reaction. The second-order rate constant for the acetate-catalyzed reaction of 4-nitrophenyl *N*-methylpyridinium-4-carboxylate is $1.09 \times 10^{-3}\,M^{-1}\,s^{-1}$ and this is probably an upper limit because previous studies on acetate-catalyzed hydrolysis of 4-nitrophenyl esters indicate that the 4-nitrophenyl esters possess both general base and nucleophilic components.[1–7] This value can be directly compared with the value of the parameter k_c^{max}/K_S for **3a** ($10.9\,M^{-1}\,s^{-1}$), which indicates a significant catalytic effect even if the number of carboxylic groups in **1** is taken into account ($([k_c^{max}/K_S)/8]/k_{nuc} = 1250$).

Figure 2 shows that the host acts as a catalyst for ester hydrolysis at low pH's but as an inhibitor at higher pH's; this observation results from the hydroxide ion-catalyzed hydrolysis of the ester being low at low pH's but increasing at high pH until it takes the majority of the reaction flux. This change in reactivity from catalysis to inhibition is of interest from a practical point of view; theoretically the value of k_c determined under conditions of inhibition reflects the reactivity of the ester in the ester–host complex and is independent of the accelerative or inhibitory nature of the system.

Tables 1 and 2 indicate that host **1** complexes with 4-nitrophenyl *N*-methyl pyridinium-4-carboxylate ($K_S = 0.33$ mM) better than with 4-nitrophenyl *N*-methylpyridinium-3-carboxylate ($K_S = 4.7$ mM), possibly due to a more favourable structure of the ester enabling it to be accommodated within the cavity of the host. The improved binding (as expressed by $1/K_S$) of charged esters compared with that of the neutral ones (Table 1) may be attributed to favourable electrostatic interactions between carboxylate and *N*-methyl residue of the pyridine ring. Electrostatic interactions are often weak in bulk water as charges are dispersed by solvation. In this system it is likely that the aromatic rings provide a hydrophobic micro-environment, thus strengthening the electrostatic interaction between the carboxylate groups of the host and the positive charge of the ester. As a result, the positively charged esters are bound to the host more strongly than are neutral esters. Benzoate ester associates with the host twofold better than acetate, which may be attributed to a more favourable interaction of benzoate within the cavity by means of π–π interactions between the phenyl component of the benzoate and the aromatic cavity of **1**.

Addition of host **1** induces complete inactivation of the benzoate and acetate esters ($k_c = 0$), whereas esters of hexanoic acid and of the charged acids retain a significant reactivity. Chawla and Pathak[16] report that derivatives of calix[n]arenes ($n = 4$, 5, 6 and 8) slow down the release of phenol of *p*- and *m*-substituted phenyl benzoates. This is ascribed by these authors to the encapsulation of the released phenoxide ion into the cavity of the host during the reaction. Ionic repulsion may not be the reason for the rate inhibition because reaction of benzoate ester with ethanolamine (Figure 1 and Table 1) in the presence of the host molecule also suffers complete inhibition; since at the pH of the experiment ethanolamine is not negatively charged, it will not be electrostatically repelled by the host and therefore hindrance of negatively charged nucleophiles (e.g., buffer and hydroxide ions) ensuing from electrostatic repulsion may not simply account for the rate retardation. Thus, it is more likely that



Scheme 3. The mechanism of action of host **1** for the intramolecular cleavage of 4-nitrophenyl esters

benzoate and acetate moieties are located in the cavity so that their reaction centres are entirely blocked from intermolecular attack (buffer, hydroxide and water) or intramolecular nucleophilic attack by the carboxylate groups of the host.

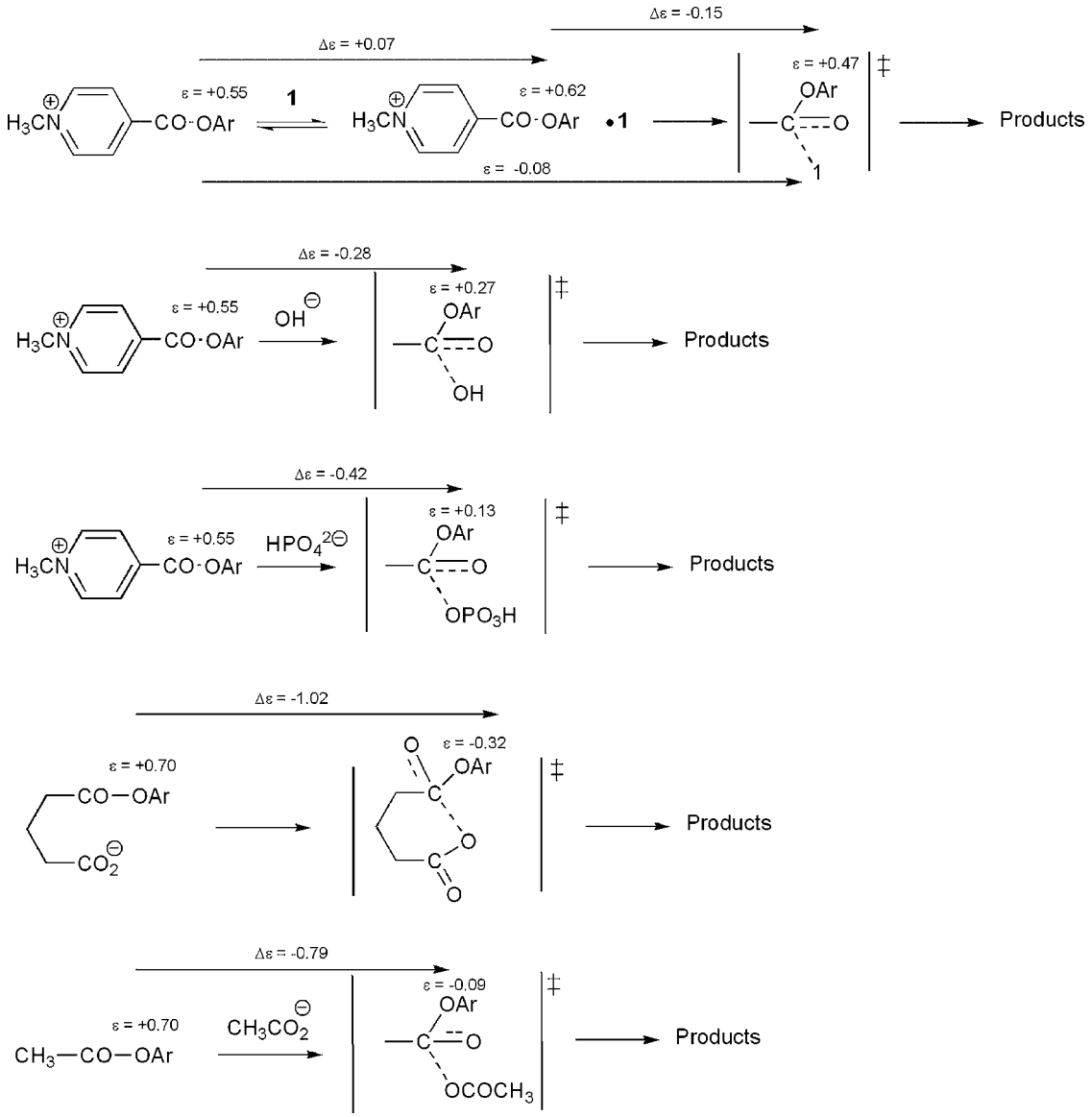
The parent calix[4]resorcinarene, with all eight phenolic groups of the host ionized, shows similar saturation kinetics in the hydrolysis of acetylcholine where a 10-fold rate retardation is observed.[17]

If carboxylate groups participate intramolecularly in the reaction via an anhydride (see Scheme 3), the intermediate could be trapped by an amine to give an amide which could then be analysed.

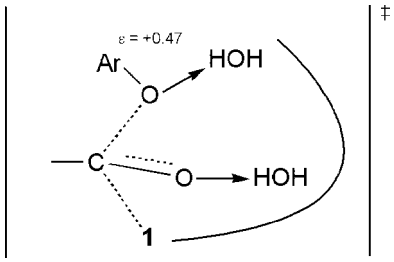
The trapping of the intermediate in the hydrolysis reaction of 4-nitrophenyl *N*-methylpyridinium-4-carboxylate in the presence of the host **1** with morpholine confirms that the reaction involves intramolecular nucleophilic attack of carboxylate of the host with the guest ester. The ^{1}H NMR analysis of the host isolated after catalysis is consistent with asymmetrical bond cleavage (a and b) yielding host-amide **1a** and *N*-methylpyridinium-4-carboxamide **4**. The preference for the pathway (b) with respect to (a) could be rationalized on the basis of the different reactivity of the two moieties of the asymmetric anhydride, the less reactive one being that on the side of the host, due to electronic and (possibly) steric reasons. An additional pathway contributing (in part) to the formation of amide **1a** could be that indicated as pathway (c), where the 'internal' anhydride **5** is formed which then reacts with morpholine to give host-amide. Since the product analysis shows that just *ca.* 25% of the host has been converted to the host–amide product, the pathways (a) and (b) must lie along the reaction path, otherwise the host would be converted exclusively to **1a**.

The effective charge map of the reaction of host **1** with esters **3** can be derived from the Brønsted correlations (Eqns 3–6) and is shown in Scheme 4, compared with those of model reactions. It is interesting to note that the aryl oxygen of the ester has slightly more positive effective charge in the complex (+0.62) than in the free ester (+0.55).

The transition structure of the reaction exhibits an effective charge on the ether oxygen of the ester (+0.47) which is significantly more positive than that found in the transition state



Scheme 4. Effective charge map[11] for the reaction of host **1** with *N*-methylpyridinium-4-carboxylate esters compared with those of model reactions



Scheme 5. Cartoon of the transition structure of the intramolecular reaction of ester with host **1** illustrating the electrophilic interaction via solvation with water molecules in the host–guest complex

of the reaction with hydroxide ion of the uncomplexed substrate (+0.27), and is *dramatically* more positive than those observed for nucleophilic catalysis by carboxylate ion in the model reactions (intramolecular, −0.32[7] and intermolecular, −0.09[6]). In both the latter model reactions, the transition structure is likely to possess substantial ArO—C bond fission and to reflect a concerted displacement by the carboxylate anion because the carboxylate ion is a weak nucleophile. The reaction of carboxylate ion in the ester complex is unlikely to differ markedly in its fundamental mechanism from the model; the most plausible explanation of the substantially more positive effective charge is that there is solvation of the developing negative charge (on the aryl oxygen) by groups or solvent molecules residing in the host complex. There is no electrophilic group in the host's structure which could be responsible for such a solvation process but water molecules present in the host–guest complex (Scheme 5) could solvate the developing negative charge.

Since the water molecules would not be the part of a water structure, the solvation interaction could be stronger than that which occurs in bulk solution. This would have a greater effect on the developing negative charge in the transition state compared with that in the bulk solvent where the solvating power of a water molecule would be reduced by its interaction with other water molecules. A similar positive effective charge was observed in the ester hydrolysis catalyzed by the structurally related resorcinarene derivative with eight *N*,*N*-dimethylamino functions attached to its upper rim.[8]

EXPERIMENTAL

Materials

All materials for buffer solutions were of analytical reagent grade. Water was double-distilled from glass and degassed *in vacuo*. 4-Nitrophenyl acetate and benzoate were from previous work from these laboratories. 4-Nitrophenyl hexanoate was purchased from the Sigma company. Tosylate salts of substituted phenyl *N*-methylpyridinium-3-carboxylate and *N*-methylpyridinium-4-carboxylate were synthesized as described previously.[9] The macrocycle, octacarboxymethyl calix[4]resorcinarene (**1**) was prepared from the corresponding octaethyl ester.[8] The octaethyl ester (5 g, 50 mmol) was dissolved in THF (50 ml) and mixed with KOH (1 M, 50 ml). The solution was stirred for 24 h, then evaporated *in vacuo*, diluted with water (100 ml) and neutralized with HCl to give a precipitate (**1**) which was recrystallized from water/ethanol to yield needles, m. p. > 300°. Found C, 53.62; H, 5.14% Formula $C_{48}H_{48}O_{24}\cdot 4H_2O$ requires C, 53.34; H, 5.22%. ^{1}H NMR (200 MHz, DMSO-d_6, ppm): $\delta = 6.43$ (s, 8H, Ar—**H**), 4.57–4.55 (q, 4H, $J = 7$ Hz, —C**H**CH$_3$), 4.52 (s, 8H, —O—C**H**$_2$—CO$_2$H), 4.31 (b, 8H, —O—C**H**$_2$CO$_2$H) and 1.42–1.39 (d, 12H, $J = 7$ Hz —CHC***H***$_3$). Owing to the low solubility of neutral host **1** in pure water, the pK_a was determined by pH-titration in 40/60 v/v water/DMF at 25° and is 5.95 ± 0.04, suggesting that no significant interaction among the carboxy groups is taking place during ionization. Equivalent weight from the pH-titration: 128.3; Formula $C_{48}H_{48}O_{24}\cdot 4H_2O$ requires: 135.1.

Methods

The kinetic methods employed in this study have been described previously.[8]

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