Catalysis of Ester Hydrolysis by Cationic Micelles of Surfactants containing the Imidazole Ring

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Micelles of cetyl(imidazol-4-ylmethyl)dimethylammonium chloride. (CDAIM) (IIa), are good catalysts for the hydrolysis of *p*-nitrophenyl esters in the pH range 7.2—9.2. The large pH dependence of the catalytic activity is indicated by the enhanced contribution of the anionic form of the imidazolyl ring at the polar head of the surfactant. The catalytic effects of CDAIM are compared with those of N$^{\alpha}$-myristoylhistidine (MirHis) (Ia) in cationic micelles. The two imidazole-containing surfactants differ markedly in several respects such as the micellar activity in neutral solution, the change in catalytic effect with changing pH, and the rate of regeneration of the micellar active site. A cross examination of the effects of CDAIM and MirHis and those of the corresponding non-surfactant analogues (IIb) and (Ib), allows a dissection of the micellar and structural factors responsible for catalytic activity. Much of the difference observed between CDAIM and MirHis is accounted for by structural factors which make the imidazole ring in compounds (II) a much weaker (by *ca.*3 p*K* units) base than that of histidine derivatives (I).

SURFACTANTS containing reactive groups at the polar head form aggregates named functional micelles [1-11] whose surfaces contain, in addition to the usual charged groups, nucleophilic or other reactive sites. Functional micelles have been claimed [3] to be closely analogous to enzymic catalysts: in both micelles and enzymes the non-polar parts are mainly located in the interior regions while the polar functional groups are at the periphery.

Among the several models of functional surfactants so far investigated as micellar catalysts for ester hydrolysis, those containing the imidazole ring have been

[1] E. J. Fendler and J. H. Fendler, *Adv. Phys. Org. Chem.*, 1970, **8**, 271; J. H. Fendler and E. J. Fendler, ' Catalysis in Micellar and Macromolecular Systems,' Academic Press, New York, 1975.

[2] B. M. Dunn and T. C. Bruice, *J. Amer. Chem. Soc.*, 1970, **92**, 6589; W. Tagaki, T. Amada, Y. Yamashita, and Y. Yano, *J.C.S. Chem. Comm.*, 1972, 1131; I. Tabushi and Y. Kuroda, *Tetrahedron Letters*, 1975, 3613.

[3] T. C. Bruice, J. Katzhendler, and L. R. Fedor, *J. Amer. Chem. Soc.*, 1968, **90**, 1333; C. A. Bunton and L. Ionescu, *ibid.*, 1973, **95**, 2912.

[4] For other types and related systems, see (*a*) C. B. Blyth and J. R. Knowles, *J. Amer. Chem. Soc.*, 1971, **93**, 3017, 3021; (*b*) D. G. Oakenfull and D. E. Fenwick, *Austral. J. Chem.*, 1974, **27**, 2149; (*c*) K. Martinek, A. P. Osipov, A. K. Yatsimirski, and I. V. Berezin, *Tetrahedron*, 1975, 709; (*d*) K. Martinek, A. V. Levashov, and I. V. Berezin, *Tetrahedron Letters*, 1975, 1275.

[5] A. Ochoa-Solano, G. Romero, and C. Gitler, *Science*, 1967, **156**, 1243.

[6] C. Gitler and A. Ochoa-Solano, *J. Amer. Chem. Soc.*, 1968, **90**, 5004.

[7] T. E. Wagner, C. Hsu, and C. S. Pratt, *J. Amer. Chem. Soc.*, 1967, **89**, 6355.

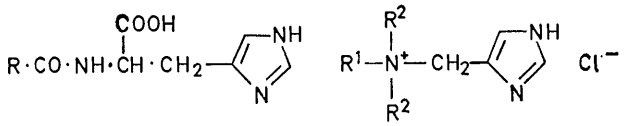
[8] P. Heitmann, R. Husung-Bublitz, and H. J. Zunft, *Tetrahedron*, 1974, **30**, 4137.

[9] J. M. Brown and C. A. Bunton, *J.C.S. Chem. Comm.*, 1974, 969.

[10] W. Tagaki, M. Chigira, T. Amada, and Y. Yano, *J.C.S. Chem. Comm.*, 1972, 219.

[11] J. P. Guthrie and Y. Ueda, *J.C.S. Chem. Comm.*, 1973, 898.

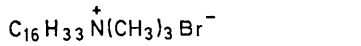
more widely explored for reasons related to its biochemical function.[12] Two structural types have attracted particular[4] attention: N$^{\alpha}$-acylhistidine derivatives (I) and tetra-alkylammonium salts containing the imidazol-4-yl residue (II).



(I) (II)

a; R = $C_{13}H_{27}$
b; R = CH_3

a; $R^1 = C_{16}H_{33}$, $R^2 = CH_3$
b; $R^1 = R^2 = C_2H_5$



(III)

The catalytic effects of N$^{\alpha}$-myristoylhistidine (MirHis) (Ia), in micelles of cetyltrimethylammonium bromide (CTABr) (III), have been extensively investigated by Ochoa-Solano and his co-workers;[5,6] studies on the reactivity of N$^{\alpha}$-stearoylhistidine[7] and, more recently, on that of N$^{\alpha}$-lauroylhistidine[8] have also been reported. Moreover, micelles of a cationic surfactant containing L-histidine methyl ester were found to promote rapid and enantioselective ester hydrolysis by Brown and Bunton.[9] On the other hand, only limited information is so far available for type (II) surfactants;[10,11] a report by Tagaki and his co-workers[10] on the micellar effects of (II; $R^1 = C_{18}H_{37}$, $R^2 = CH_3$) indicated promising catalytic properties for this compound in terms of both rate enhancement and catalytic turnover.

The present study is a detailed kinetic analysis of the hydrolysis of *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl hexanoate (PNPH) in the presence of surfactant CDAIM (IIa) and of the structurally analogous non-surfactant TEAIM (IIb). The results are compared with those observed in the presence of MirHis (Ia) and of the non-surfactant AcHis (Ib). Differences and similarities in the catalytic properties of surfactants (I) and (II) are rationalized in terms of micellar and structural effects.

RESULTS

The critical micelle concentration (c.m.c.) value of CDAIM in pure water is $1.05 \pm 0.05 \times 10^{-4}$M as determined by conductivity measurements[13] at 25°. The c.m.c. in 0.05M-Tris buffers in the pH range 7.2—9.2 is higher, 1.5—1.8×10^{-4}M as shown (see below) by rate–concentration profiles. On the other hand, TEAIM does not show any of the changes characteristic[1,14] of micelle formation up to 2.5×10^{-2}M.

The acid–base behaviour of the ampholytic imidazole residue which is of prime importance in understanding its catalytic effect can be defined by measuring the two pK values. In the case of TEAIM, pK_1 is 4.3 ± 0.1 and pK_2 11.2 ± 0.3. On the other hand micelles of CDAIM carrying a large number of ionizable groups behave as polyelectrolytes since the apparent dissociation constant varies with the degree of dissociation α. The apparent pK_1 value of 3.5 was obtained from the value of pH for which $\alpha = 0.5$; in the pH range 2.5—3.7 the modified Henderson equation[15] $pH = pK_{1,app} - a \log(1 - \alpha/\alpha)$ was obeyed, giving $pK_{1,app} = 3.5 = pH + 0.8 \log(1 - \alpha/\alpha)$. Large deviations from the above law above pH 4.5 can be related to the presence of the anionic form of imidazole. The apparent pK_2 of micellar CDAIM is inaccessible due to the decomposition of the surfactant at high pH values.

Rate of Hydrolysis of PNPA and PNPH.—(a) *In the presence of TEAIM and imidazole* (*Im*). The rate of hydrolysis of the esters in 0.05M-Tris buffers at pH 7.2—9.2 is enhanced by the addition of TEAIM as well as by imidazole here used as a reference compound. A plot of the observed pseudo-first-order rate constants k_{ψ} against concentration at any given pH is first order in catalyst and allows the second-order rate constants k_c to be calculated (Table 1).

TABLE 1

Catalytic rate constants k_c/l mol^{-1} s^{-1} of TEAIM and Im with PNPA and PNPH

	PNPA		PNPH	
pH	TEAIM	Im	TEAIM	Im
7.2	0.025	0.28	0.015	0.185
7.7	0.09	0.35	0.07	
8.9	0.38	0.43		0.28
9.2	0.55		0.52	

The catalytic rate constant of imidazole increases with increasing pH in the manner predicted by its pK_1 value (6.9)[16] assuming that the protonated form is inactive. In the case of TEAIM, the protonated form is absent in the acidity range explored as its pK_1 value is 3—5 units lower than the pH used. The high dependence of the catalytic rate constant of TEAIM on pH indicates formation of the anionic form of the imidazolyl residue.

(b) *In the presence of CDAIM.* The hydrolysis rate is strongly enhanced by addition of CDAIM in 0.05M-Tris buffers for surfactant concentrations above 1.5—1.8×10^{-4}M. The pseudo-first-order rate constants for the condition [CDAIM] > [ester] are in Table 2 and a typical rate–concentration profile is shown in Figure 1.

From the slope of the linear part of the plot of k_{ψ} against [CDAIM] for surfactant concentrations definitely above the c.m.c., the apparent * catalytic rate constants of Table 3 were evaluated. Here again, as discussed in the previous

* The problem of evaluating the 'true' rate constants of imidazole derivatives in the micellar phase has been recently discussed by Martinek and his co-workers.[4e]

[12] B. S. Hartley in 'Structure and Activity of Enzymes,' eds. T. W. Goodwin, J. I. Harris, B. S. Hartley, Academic Press, London, 1964, p. 47; M. L. Bender and B. W. Turnquest, *J. Amer. Chem. Soc.*, 1957, **79**, 1952; T. C. Bruice and G. L. Schmir, *ibid.*, p. 1663; W. P. Jencks, *Biochim. Biophys. Acta*, 1957, **24**, 227.

[13] K. J. Mysels and P. Mukerjee, 'National Standard Reference Data System Compilation of Critical Micelle Concentrations,' U.S. Government Printing Office, Washington, D.C., 1969.

[14] E. H. Cordes and R. B. Dunlap, *Accounts Chem. Res.*, 1969, **2**, 329.

[15] A. Katchalsky and P. Spitnik, *J. Polymer Sci.*, 1957, **2**, 432. A. Katchalsky, N. Shavit, and H. Eisenberg, *ibid.*, 1954, **13**, 69.

[16] T. C. Bruice and G. L. Schmir, *J. Amer. Chem. Soc.*, 1958, **80**, 148.

section, the strong dependence of k_c on pH suggest that the basic form of the imidazolyl ring is likely to be involved.

(c) *In the presence of mixed micelles of CTABr and CDAIM.* The catalytic properties of MirHis (Ia) were measured in mixed micelles with CTABr (c.m.c. *ca.* 5×10^{-5}M in 0.05M-Tris buffers). To compare surfactants (Ia)

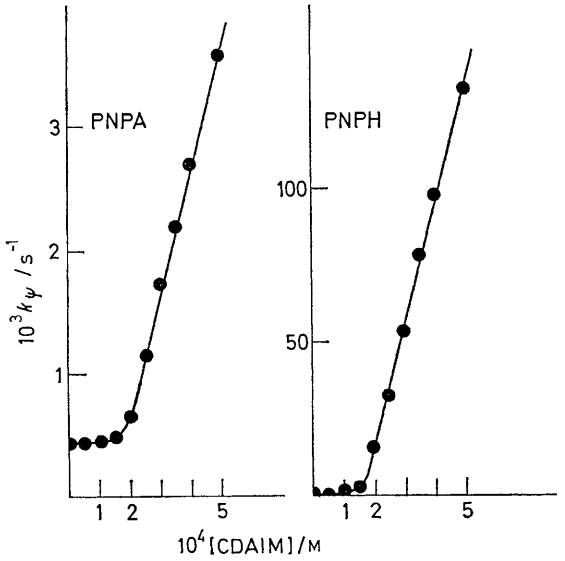


FIGURE 1 Pseudo-first-order rate constants for the hydrolysis of PNPA and PNPH in the presence of CDAIM at pH 9.2 and 25°

and (IIa) under as similar conditions as possible, the reactivity of CDAIM in mixed micelles with CTABr was also measured. Moreover the measurements in mixed micelles provide a way of determining the catalytic rate constants of CDAIM by a method different from that of the previous

TABLE 2

Rate constants for the hydrolysis of PNPA and PNPH in the presence of CDAIM at 25° [a]

	$10^5 \times k_\psi$/s^{-1}					
10^4[CDAIM]/M	PNPA			PNPH		
pH:	7.2	7.7	8.65	7.2	7.7	8.65
	1.04	3.95	30.1	3.47	7.0	16.4
1.0	1.12	5.41	38.6	9.7	43.7	172
2.0	2.18	9.2	50.1	48	307	2 095
3.0	4.65	25.9	90.2	109	765	5 010
3.5	5.55					
4.0	7.04	35.6	119	179	1 350	7 970
5.0	9.63	47.9	163	247	1 880	11 100

[a] Rate data at pH 9.2 are reported in Figure 1. $[Ester]_0 = 1.2 \times 10^{-5}$M.

TABLE 3

Catalytic rate constants k_c/l mol^{-1} s^{-1} of micellar CDAIM

Ester pH:	7.2	7.7	8.65	9.2
PNPA	0.25	1.29	3.8	8.3
PNPH	6.5	52.5	300	395

section when allowance is made for the following mechanistic assumptions. According to Gitler and Ochoa-Solano [6] this type of mixed micelles is made of active (M_a) and inactive (M_i) regions although such separation in the highly mobile micellar system is to be taken as an over-simplification as pointed out by Maugh and Bruice.[17] The functional surfactant resides in the active and the CTABr molecules in the inactive zone which lead only to non-productive binding of substrates. Assuming the operation of equations (1)—(3) where $S{\cdot}M_i$ is the inactive

$$S + M_i \overset{K_i}{\rightleftharpoons} S{\cdot}M_i \quad (1)$$

$$S + M_a \xrightarrow{k_c} Ac{\cdot}M_a + POH \quad (2)$$

$$Ac{\cdot}M_a + H_2O \xrightarrow{k_d} M_a + AcOH \quad (3)$$

adsorption complex, POH *p*-nitrophenol, $Ac{\cdot}M_a$ the intermediate resulting from acylation of the imidazolyl ring of the catalyst, k_c the actual catalytic rate constant, and k_d the rate constant for deacylation, the experimental first-order rate constant, under the conditions $[M_i] > [M_a] \gg [S]$, is given by equation (4) which can be written as (5).

$$k_\psi = k_c[M_a]/(1 + [M_i]/K_i) \quad (4)$$

$$[M_a]/k_\psi = 1/k_c + [M_i]/k_cK_i \quad (5)$$

From equation (5) the values of k_c and K_i can be derived from the intercept and slope of a plot of $[M_a]/k_\psi$ against $[M_i]$. The pseudo-first-order rate constants measured

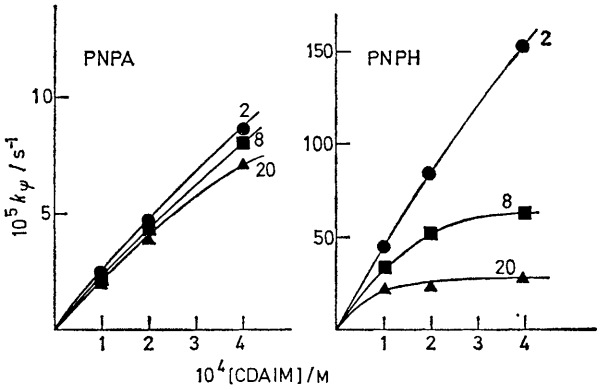


FIGURE 2 Net pseudo-first-order rate constants ($k_{obs} - k_{CTABr}$) for the hydrolysis of PNPA and PNPH in the presence of mixed micelles of CTABr and CDAIM. The ratio [CTABr] : [CDAIM] is indicated in the Figure

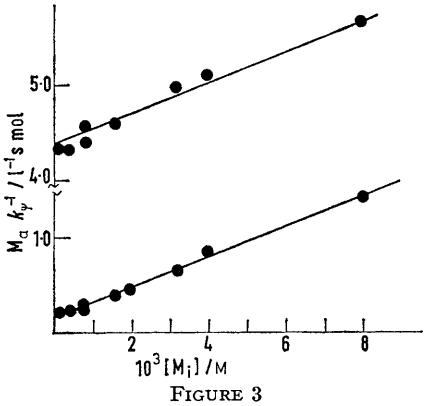


FIGURE 3

in the presence of mixed micelles of three different [CTABr] : [CDAIM] ratios at pH 7.2 are shown in Figure 2. From the plots of $[M_a]/k_\psi$ against $[M_i]$ of Figure 3, assuming

[17] T. Maugh, jun., and T. C. Bruice, *J. Amer. Chem. Soc.*, 1971, **93**, 6584.

$[M_a]$ = [CDAIM] and $[M_i]$ = [CTABr], the k_c values are 0.23 ± 0.01 for PNPA and 6.2 ± 0.6 l mol^{-1} s^{-1} for PNPH. These values compare remarkably well with those of Table 3 thus indicating that the microenvironment of the imidazolyl ring in homogeneous and mixed micelles is substantially identical. The K_i values are 2.6 ± 0.2 × 10^{-2} for PNPA and 9.5 ± 0.9 × 10^{-4} for PNPH and can be compared with the values of 3.3 ± 0.6 × 10^{-2} and 5 ± 0.4 × 10^{-4} mol l^{-1} evaluated for mixed micelles of CTABr and MirHis. The different K_i constants obtained in the two cases, if any precise meaning can be attached to them in view of the critical discussion by Maugh and Bruice,[17] may also be due to the roughly approximate assumption [7] in the evaluation of $[M_i]$.

Under conditions [CTABr] > [Ester] > [CDAIM] the appearance of *p*-nitrophenol does not follow saturation kinetics, as observed for mixed micelles of CTABr and MirHis (see Figure 4) over the range of CDAIM and PNPA concentrations.

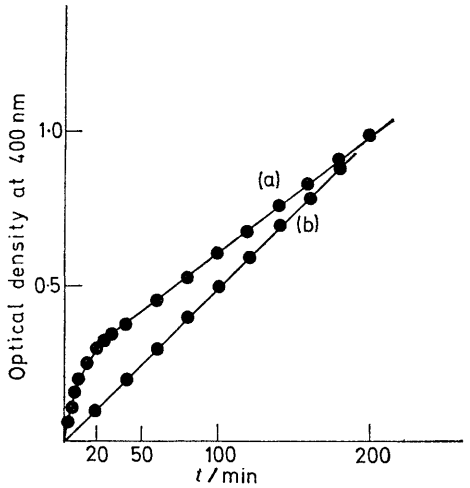


FIGURE 4 Rate of formation of *p*-nitrophenol in 0.05M-Tris buffer at pH 7.2 and 25° (a) [CTABr] 1 × 10^{-3}M; [PNPA] 5 × 10^{-4}M; [MirHis] 5 × 10^{-5}M; (b) [CTABr] 2 × 10^{-3}M; [PNPA] 1 × 10^{-3}M; [CDAIM] 1 × 10^{-4}M

Build-up of the Intermediate.—Following equations (2) and (3), the imidazole ring is acylated and the resulting *N*-acylated intermediate is then hydrolysed. In the case of MirHis the intermediate was isolated by column chromatography and showed a peak at 248 nm. As observed by Tagaki and his co-workers,[10] addition of PNPA (1.5 × 10^{-4}M) to a solution of CTABr (3 × 10^{-2}M) and MirHis (3 × 10^{-3}M) in 0.05M-phosphate buffer at pH 8.0, causes a very rapid and persistent increase of optical density (from 0.55 to 1.05) at 248 nm indicating accumulation of an intermediate.

On the other hand, addition of the same amount of PNPA to a solution of 5 × 10^{-3}M-CDAIM in the same buffer produces only a small and temporary increase in optical density (from 0.55 to 0.63 after 3 min) at 248 nm. It appears therefore that the intermediate does not appreciably accumulate during the reaction in the case of CDAIM.

DISCUSSION

Simple cationic micelles like those of CTABr reduce the rate of ester hydrolysis in Tris buffers.[5,6] The large rate enhancements observed in the presence of micelles of CDAIM and the behaviour of mixed micelles of CTABr and CDAIM shown in Figure 2 clearly indicate functional micellar catalysis, the imidazole ring at the polar head of the surfactant being involved in promoting the esterolysis of PNPA and PNPH. The mechanism has been assumed to be that suggested for MirHis as well as for the other imidazole derivatives [3] and includes two essential steps: (i) nucleophilic attack on ester which results in the acylation of the imidazolyl ring and (ii) hydrolysis of the acylated intermediate leading to regeneration of the catalytic site.

Assuming the presence of a common mechanism, rate data of CDAIM, MirHis, and their non-surfactant analogues TEAIM and AcHis can be directly compared.

Catalytic Activity at pH 7.2.—The relative catalytic reactivities at pH 7.2 in 0.05M-tris buffer are shown in Table 4. Clearly, CDAIM is a much poorer * catalyst

TABLE 4

Relative catalytic reactivities

Ester	$\frac{k_c(\text{CDAIM})}{k_c(\text{MirHis})}$ [a]	$\frac{k_c(\text{CDAIM})}{k_c(\text{TEAIM})}$	$\frac{k_c(\text{MirHis})}{k_c(\text{AcHis})}$ [a]
PNPA	0.04	10	19
PNPH	0.05	440	810

[a] Rate constants taken from ref. 8, corrected for the basic form of the imidazole ring. pK_1 of AcHis is 7.04.[16]

than MirHis for reasons which are not related to micellar efficiency. In fact the activity of both micellar surfactants relative to that of the corresponding non-surfactant models is similar within a factor of two. Micellar efficiency for both systems increases on going from PNPA to PNPH as expected from the increased hydrophobic interactions between substrate and micelle.[1,14]

Therefore, the different activity of the two surfactants is essentially related to the greater nucleophilicity of the imidazole residue of MirHis than that of CDAIM, as indicated by the difference of 3 pK units in basicity. On the other hand, neither saturation kinetics nor substantial accumulation of the intermediate were observed under proper conditions in the case of CDAIM and, hence, its deacylation is faster than that of MirHis. This can be related to the fact that the imidazolyl head of CDAIM is a better leaving group than that of MirHis in hydrolysis, without necessarily invoking micellar effects in terms of hydrophobic [6] or electrostatic [10] interactions.

Effect of pH.—In discussions of the data in Table 4, the imidazole ring of CDAIM was assumed to be entirely in the neutral form at pH 7.2. There appears to be an oversimplification as judged from Figure 5 which shows the effect of pH on the observed catalytic rate constants. A sigmoidal curve is obtained for MirHis in the range pH 4—8 related to the apparent pK_1 of 6.2, and only

* Tagaki and his co-workers reported [10] that surfactant (II; $R^1 = C_{18}H_{37}$, $R^2 = CH_3$) is only 1.3 times less reactive towards PNPA at pH 7 than MirHis in mixed micelles of CTABr with equal concentrations of functional surfactants. The longer (by two methylene groups) tail of their surfactant than that of CDAIM can hardly be expected to account for this discrepancy.

above pH 8.5 is the contribution of the imidazolyl anion expected to become significant as observed by Heitmann and his co-workers [8] for mixed micelles of CTABr and N^{α}-lauroylhistidine. In the case of CDAIM, the increase of rate in the pH range 7.2—9.2 is likely to show as the lower part of a sigmoid related to the unknown pK_2. It is also evident from Figure 5 that the kinetic contribution of the anionic form of the imidazolyl residue of CDAIM is substantial even at pH 7.2 and therefore the k_c(CDAIM)/k_c(MirHis) values (Table 4) are to be taken as the upper limits of the relative activities of the two surfactants as free neutral bases. Recent studies of the pH dependence of esterolytic processes have clearly shown the great nucleophilic effectiveness of the imidazolyl anion in micellar systems.[4c,d,8] At pH

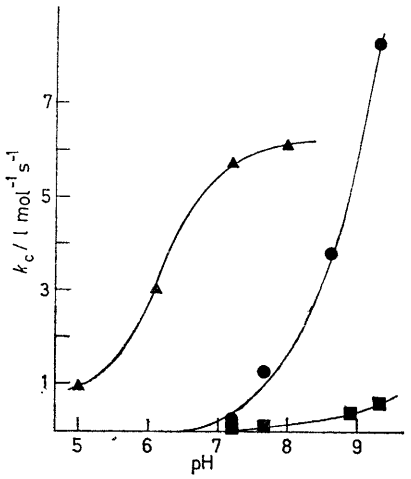


FIGURE 5 Apparent catalytic rate constants for the hydrolysis of PNPA as a function of pH at 25°. ▲ MirHis in mixed micelles with CTABr. Evaluated from ref. 8 on the basis of k_c = 6.28 l mol^{-1} s^{-1} reported for MirHis, imidazole neutral base form. ● CDAIM from Table 3. ■ TEAIM from Table 1

>9, the contribution of the anionic form makes CDAIM a better catalyst than MirHis.

On the other hand, the pH–rate profiles of CDAIM and TEAIM are rather similar. This is better shown by the reactivity ratio k_c(CDAIM)/k_c(TEAIM) calculated from Tables 1 and 3. This changes little on going from pH 7.2 to 7.7 and 9.2, *e.g.* 10, 14.3, and 15 for PNPA; 440, 750, and 760 for PNPH, thus indicating that the effect of pH is not related to micelles but to structural factors.

The pH effect on the catalytic activity of CDAIM is analogous to that observed by Overberger and his co-workers [18] for poly-5(6)vinylbenzimidazole. In that case, however, the pH–rate profile of the polymer was found to be different from that of the monomer and, following these authors, this can be explained by assuming an interaction between anionic and neutral imidazole residues of the polymer, the neutral one acting as a weak acid in esterolysis. Such bifunctional catalysis which requires the juxtaposition of the catalytic groups in a manner which can be attained [18b] in a rigid polymeric chain in the proper conformation is apparently not effective on the surface of micelles.

Conclusions.—From the results of this study it seems apparent that imidazole containing surfactants of type (I) and (II) in cationic micelles have quite complementary properties in terms of rate acceleration and catalytic turnover. Differences are essentially related to changes in the acid–base behaviour of the imidazole ring and hence to structure. On the whole, the large micellar catalytic effects in the hydrolysis of simple esters such as PNPA and PNPH are predictable from the behaviour of non-surfactant structurally analogous models when allowance is made for the following major micellar effects: (i) the basicity of the imidazolyl ring in the charged surface of cationic micelles relative to that of the model is decreased due to the strong field effect; [2,19] the apparent pK_1 is 0.8 units lower in the case of MirHis and CDAIM. Thus both the free neutral and the anionic form of the imidazole ring are present in micelles at lower pH. (ii) Hydrophobic interactions between substrate and micelles account for much of the catalytic efficiency. Lengthening of the acyl group of esters results in additional energy, estimated [9,20] to be *ca.* 400 cal mol^{-1} per methylene group, used on the micellar surface to lower the free energy of activation of the catalysed reaction.

EXPERIMENTAL

p-Nitrophenyl acetate (PNPA), m.p. 77—78° (lit.,[21] 77.3°), *p*-nitrophenyl hexanoate (PNPH), b.p. 172—174° at 6 mmHg (lit.,[22] 173—175° at 6 mmHg), 4-hydroxymethylimidazole hydrochloride, m.p. 108° (lit.,[23] 107—109°), and 4-chloromethylimidazole hydrochloride, m.p. 143—144° (lit.,[24] 144—145°), τ (CD_3OD) 1.05 (1 H, s), 2.35 (1 H, s), 4.9br (2 H, s), and 5.17 (2 H, s), were synthesized and purified according to literature procedures. Cetyldimethylamine, L-histidine, and myristoyl chloride were commercial products.

N^{α}-Myristoyl-L-histidine (MirHis) (Ia) was obtained as described by Gitler and Ochoa-Solano.[6]

Triethyl(imidazol-4-ylmethyl)ammonium Chloride Hydrochloride (TEAIM) (IIb).—4-Chloromethylimidazole hydrochloride (10.6mM) was added to a solution of freshly distilled triethylamine (21.2mM) in dry methanol (10 ml). After 5 min, dry sodium carbonate (28mM) was added and the suspension was stirred for 3 min and then filtered. Longer contact with carbonate may cause decomposition of the desired product. The solution was evaporated to a small volume (1—2 ml), again filtered, and the solvent

[18] (*a*) C. G. Overberger, T. St. Pierre, N. Vorheimer, J. Lee, and S. Yaroslavsky, *J. Amer. Chem. Soc.*, 1965, **87**, 296; (*b*) C. G. Overberger and M. Morimoto, *ibid.*, 1971, **93**, 3222.

[19] C. Tanford, *Adv. Protein Chem.* 1962, **17**, 69.

[20] R. Lumry in ' The Enzymes ' eds. P. D. Boyer, H. Lardy, and K. Myrback, Academic Press, New York, 1959, vol. 1, pp. 183—190 and references therein.

[21] L. Faller and J. M. Sturtevant, *J. Biol. Chem.*, 1966, **241**, 4825.

[22] S. Kreisky, *Acta Chem. Scand.*, 1957, **11**, 913.

[23] J. Parrod, *Bull. Soc. chim. France*, 1932, **51**, 1424; ' Organic Syntheses ' E. C. Horning, Wiley, New York, 1962, Coll. Vol. III, pp. 460—462.

[24] J. Pyman, *J. Chem. Soc.*, 1899, **99**, 764.

removed under vacuum. Further treatment with sodium carbonate for a short time may be needed to remove the excess of amine (as hydrochloride). The residue was taken up with *ca.* 20mM-HCl in methanol and the solvent removed under reduced pressure. The crude product was twice crystallized from methanol–ether and dried over P_2O_5 under vacuum. The *solid* (63%) had m.p. 161—163° (Found: C, 46.95; H, 7.95; N, 16.5; Cl, 27.6. $C_{10}H_{21}Cl_2N_3$ requires C, 47.3; H, 8.25; N, 16.55; Cl, 27.9%), τ (D_2O) 0.72 (1 H, s), 1.74 (1 H, s), 5.07 (2 H, s), 6.10 (6 H, q), and 8.18 (9 H, t).

Cetyl(imidazol-4-ylmethyl)dimethylammonium Chloride Hydrochloride (*CDAIM*) (IIa).—Cetyldimethylamine (3.05 g) was added during 10 min under stirring to 4-chloromethylimidazole hydrochloride (0.867 g). Sodium carbonate (1.0 g) was added, the mixture stirred for 5 min, and filtered. The solution was then evaporated to a small volume, again filtered, and the solvent removed. Water was added to the residue and the free amine extracted several times with ether. The aqueous solution was evaporated to dryness and the residue was treated with HCl (2 equiv.) in methanol. The solvent was removed under reduced pressure and the residue crystallized from methanol–ether. The *product* (36%), hygroscopic, melts at 73—75° forming liquid crystals which decompose at 185° (Found: C, 62.1; H, 10.95; N, 9.75; Cl, 16.35. $C_{22}H_{45}Cl_2N_3$ requires C, 62.55; H, 10.75; N, 9.95; Cl, 16.75%), τ (D_2O) 0.78 (1 H, s), 1.68 (1 H, s), 5.08 (2 H, s, N^+CH_2Im), 6 53 (6 H, s), 8 70br (*ca* 30 H), and 9 1 (3 H, t).

The pK determinations in the case of TEAIM were made for solutions of 0.05M-KCl at 25° following the procedure described by Bruice and Schmir.[16] The pK_1 of CDAIM was determined for 5.4—5.2 $\times$ 10^{-3}M solutions of surfactant in 0.1M-KCl; the solution was titrated with concentrated sodium hydroxide with stirring by using a microburette and a E510 Metrohm pH meter.

Kinetic Measurements.—As a general procedure, 0.05 ml of a solution of ester (1.2 $\times$ 10^{-3}M) in acetonitrile were added to 0.05M-Tris buffer (2.0 ml) of given pH, containing the catalyst, if any, at the desired concentration, and the appearance of *p*-nitrophenol was followed by recording the change in absorbance at 400 nm using a 2400 Gilford spectrophotometer. Rate constants were calculated using the absorbance at infinite time determined for each experiment [5] by applying the integrated first-order equation which was obeyed well up to at least 80% reaction.

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