Functional Micellar Catalysis. Part 2.[1] Ester Hydrolysis Promoted by Micelles containing the Imidazole Ring and the Hydroxy-group

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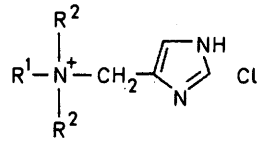
The catalytic effects on the hydrolysis of *p*-nitrophenyl esters due to micelles of quaternary ammonium salts containing the imidazole ring (Ia), the hydroxy-group (IIa), and both functions (IIIa) have been investigated in the pH range 7—9. Both functions have comparable p*K* values when at the head of cationic micelles and their basic forms are the effective nucleophilic sites. Bifunctional micelles made of (IIIa) or of mixed (Ia) and (IIa) are better catalysts than the monofunctional ones, with (Ia) being much more effective than (IIa). Co-operative interaction between the two functional groups is inter- rather than intra-molecular in character. A mechanism is suggested for bifunctional micelles involving acylation of the imidazole ring followed by a relatively rapid acyl transfer to the hydroxy-function in the micellar phase.

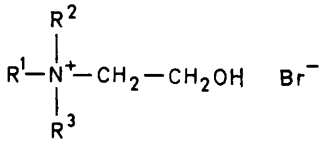
STUDIES of the catalytic properties of functional micelles [2] have been stimulated by the search for models for enzymic catalysts. Thus, the functional groups so far



(I)

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

b ; $R^1 = R^2 = C_2H_5$

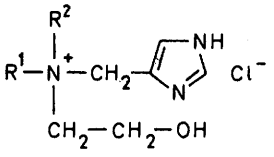


(II)

a ; $R^1 = C_{16}H_{33}$; $R^2 = CH_3$; $R^3 = C_2H_5$

b ; $R^1 = R^2 = C_2H_5$; $R^3 = CH_3$

c ; $R^1 = C_{18}H_{37}$; $R^2 = R^3 = C_2H_5$



(III)

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

b ; $R^1 = C_2H_5$; $R^2 = CH_3$

explored are those used in the active sites of enzymes, the imidazole ring,[3] amino,[4] hydroxy,[5-8] mercapto,[9] and carboxy.

This paper presents a comparative analysis of the catalysis of ester hydrolysis by cationic micelles made of quaternary ammonium ions containing either the imidazole ring or the hydroxy-group or both functions at the polar head. Both functions are involved in the charge-relay mechanism [10] of α-chymotrypsin and related esterases and it seemed interesting to verify possible co-operative interaction between them in micellar systems. Three structures (I)—(III) were investigated. Previously,[1] the behaviour of micelles of (Ia) was discussed in relation to either the non-surfactant analogue (Ib) and to N^{α}-miristoylhistidine in cationic micelles. Surfactants of the choline type (II) have been studied by several investigators: the involvement of the hydroxy-group as a nucleophile in micelles was tentatively suggested by Chevion *et al.*[5b] and Meyer [6] and convincingly shown by Berezin and his co-workers [7] from data obtained for micelles of (IIc) under conditions of saturation kinetics. A preliminary account of a study of the catalytic activity of bifunctional micelles of (IIIa) compared with that of micelles of surfactants of types (I) and (II) was published by Moss *et al.*[8] when our study was already well advanced [11] and their published data as well as some of their more recent results,[12] complementary to those here reported, will also be discussed.

The esters of choice were *p*-nitrophenyl acetate (PNPA) and hexanoate (PNPH) which has the longest

[1] Part 1, U. Tonellato, *J.C.S. Perkin II*, 1976, 771.

[2] For reviews see J. H. Fendler and E. J. Fendler, ' Catalysis in Micellar and Macromolecular Systems,' Academic Press, London, 1975; I. V. Berezin, K. Martinek, and A. K. Yatsimirski, *Russ. Chem. Rev.*, 1973, **42**, 787.

[3] (*a*) A. Ochoa-Solano, G. Romero, and C. Gitler, *Science*, 1967, **156**, 1243; (*b*) C. Gitler and A. Ochoa-Solano, *J. Amer. Chem. Soc.*, 1968, **90**, 5004; (*c*) P. Heitmann, R. Husung-Bublitz, and H. J. Zunft, *Tetrahedron*, 1974, **30**, 4137; (*d*) W. Tagaki, M. Chigira, T. Amada, and Y. Yano, *J.C.S. Chem. Comm.*, 1972, 219; (*e*) J. M. Brown and C. A. Bunton, *ibid.*, 1974, 969; (*f*) J. P. Guthrie and Y. Ueda, *ibid.*, 1973, 898; (*g*) K. Martinek, A. P. Osipov, A. K. Yatsimirski, V. A. Dadali, and I. V. Berezin, *Tetrahedron Letters*, 1975, 1279; (*h*) K. Martinek, A. P. Osipov, A. K. Yatsimirski, and I. V. Berezin, *Tetrahedron*, 1975, **31**, 709; (*i*) D. G. Oakenfull and D. E. Fenwich, *Austral. J. Chem.*, 1974, **27**, 2149.

[4] J. R. Knowles and C. A. Parsons, *Chem. Comm.*, 1967, 755; T. C. Bruice, J. Katzhendler, and L. R. Fedor, *J. Amer. Chem. Soc.*, 1968, **90**, 1333; C. A. Blyth and J. R. Knowles, *ibid.*, 1971, **93**, 3017; D. G. Oakenfull, *J.C.S. Perkin II*, 1973, 1006.

[5] (*a*) C. A. Bunton and L. G. Ionescu, *J. Amer. Chem. Soc.*, 1973, **95**, 2912; (*b*) M. Chevion, J. Katzhendler, and S. Sarel, *Israel J. Chem.*, 1972, **10**, 795; (*c*) V. Gani, C. Lapinte, and P. Viout, *Tetrahedron Letters*, 1973, 4435; (*d*) I. Tabushi and Y. Kuroda, *ibid.*, 1975, 3613.

[6] G. Meyer, (*a*) *Tetrahedron Letters*, 1972, 4581; (*b*) *Compt. rend.*, 1973, **276C**, 1599.

[7] K. Martinek, A. V. Levashov, and I. V. Berezin, *Tetrahedron Letters*, 1975, 1275.

[8] R. A. Moss, R. C. Nahas, S. Ramaswani, and W. J. Sanders, *Tetrahedron Letters*, 1975, 3379.

[9] W. Tagaki, T. Amada, Y. Yamashita, and Y. Yano, *J.C.S. Chem. Comm.*, 1972, 1131.

[10] (*a*) W. P. Jenks, ' Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969; (*b*) B. S. Hartley in ' Structure and Activity of Enzymes,' eds. T. W. Goodwin, J. I. Harris, and B. S. Hartley, Academic Press, London, 1964.

[11] F. Casagrande, Doctoral Thesis, University of Padova, 1975.

[12] R. A. Moss, R. C. Nahas, and S. Ramaswani, *J. Amer. Chem. Soc.* in the press, and presented in part at the International Symposiun on Micellization, Solubilization and Microemulsions, 7th Amer. Chem. Soc. Northeast Regional Meeting, Albany, 1976; R. A. Moss, personal communication.

carbon chain without complications [13] arising from self-aggregation of the substrate.

RESULTS

C.m.c. and pK Values.—The critical micelle concentration (c.m.c.) values of the three surfactants * CDMAIM (Ia), CEMAH (IIa), and CMAHIM (IIIa) have been determined by conductivity measurements at 25° and are shown in Table 1. The c.m.c. is expected to be lowered in buffer

TABLE 1

C.m.c. and apparent pK values of micellar surfactants (Ia)—(IIIa)

	CDMAIM (Ia)	CEMAH (IIa)	CMAHIM (IIIa)
10^5C.m.c./M [a]	10.5 ± 0.5	2.9 ± 0.2	1.95 ± 0.1
pK_1(IM) [b]	3.5 ± 0.2		3.7 ± 0.3
pK_2(IM) [c]	<11.5		<11.5
pK_{OH} [d]		*ca.* 10.5	*ca.* 10.5

[a] In pure water. [b] Determined, as described,[1] from the pH value at which the degree of dissociation of the polyelectrolytic micelle, α, equals 0.5. [c] The upper value is that of TEAIM (Ib). [d] The value is that reported [7] for micellar (IIc).

solutions due to the presence of electrolytes.[2] In the case of CMAHIM, the value in 0.02M-borate is 1.2×10^{-5}M as judged [7] from the dependence of the *p*-nitrophenol burst

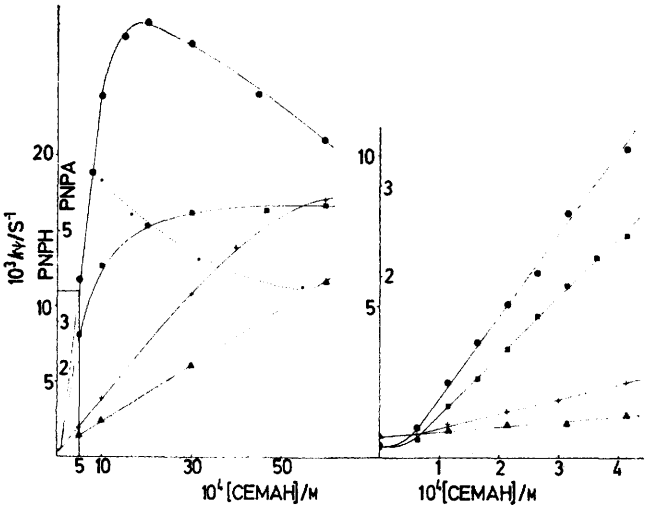


FIGURE 1 Rate–[CEMAH] profiles: with added 0.02M-borate at pH 8.85, ● PNPH (dotted line shows the inhibiting effect of [KBr] for a 7×10^{-4}M solution of CEMAH), ×, PNPA; with added Tris (I = 0.05) at pH 8.65, ■ PNPH, ▲ PNPA. Co-ordinate expansion for low [CEMAH] is shown on the right-hand side

(see Figure 3) on the surfactant concentration. On the other hand, with the condition [ester] ≪ [surfactants] the apparent ' kinetic ' c.m.c., *i.e.*, the intercept of the pre- and post-micellar regions of k_ψ data measured in both Tris and borate buffers (see Figures 1 and 4) are higher than in pure water. It is not clear what makes the aggregates formed at lower surfactant concentrations than the ' kinetic ' c.m.c. unable to display fully their catalytic properties. As a trend common to all types of micelles, the ' kinetic ' c.m.c. increases with increasing [surfactant] : [ester] ratios, on going from PNPH to PNPA, and from Tris to borate buffers.

The c.m.c. of CDMAIM reported by Moss *et al.*[8] (0.01M-phosphate; pH 8) is 7.9×10^{-5}M, in good agreement with that of Table 1 when the medium effect is taken into account, whereas their value for CMAHIM, 6.9×10^{-5}M, is considerably higher. The c.m.c. of CEMAH compares well with that of (IIc) obtained by Berezin and his co-workers [7] and is much lower than that (6.5×10^{-4}M) reported by Meyer [6a] for (II; $R^1 = C_{16}H_{33}$; $R^2 = R^3 = CH_3$); the latter value appears to be overestimated also from the rate–concentration profile obtained from the kinetic data reported by the author.

Table 1 also shows pK data for the function groups in micelles. For CDMAIM and CMAHIM only the first dissociation constant, pK_{1IM}, of the ampholytic imidazole ring could be measured, whereas pK_{1IM} could not be directly determined due to decomposition of the surfactants at high pH values. The upper limit of pK_{1IM} indicated in Table 1 is that measured [1] for the non-surfactant model TEAIM (Ib); the actual value in micelles is likely to be at least one unit lower. This can also be assumed in view of the data reported by Berezin and his co-workers.[7] The pK_{OH} of the non-surfactant (II; $R^1 = R^2 = R^3 = C_2H_5$) was found to be 12.8 ± 0.3, much higher than that shown in Table 1 for micellar (IIc).

Rate of Hydrolysis of PNPA and PNPH.—Measurements were made at 25° for Tris buffers of constant ionic strength (I 0.05) of pH 7.2—9.05 and in 0.02M-borate buffer of pH 8.86 (unless otherwise specified) containing, in each case, 1% acetonitrile.

(a) *In the presence of CDMAIM* (Ia). Rate data for 0.05M-Tris buffers containing 2.5% acetonitrile have been reported.[1] To compare the effect of this surfactant to that of others under the same conditions, the apparent catalytic rate constants have been redetermined as described [1] and are shown in Table 4. They are higher than those published

TABLE 2

Rate constants $10^4 k_\psi/s^{-1}$ for the hydrolysis of PNPA [a] and PNPH [a] in the presence of CEMAH

		PNPA		PNPH	
10^4[CEMAH]/M	pH [b]	7.9	9.05	7.9	9.05
		0.42	4.4	0.56	2.9
1.0			5.0	1.40	8.1
1.5				1.90	14.5
2.0			6.2	2.54	20.3
2.5				3.31	
3.0			8.0	3.85	33.6
4.0		0.56	9.05	5.1	44.1
10.0		1.30	17.5	7.8	
20.0		2.75			
30.0		5.05			

[a] $[ester]_0 = 0.8$—1.5×10^{-5}M. [b] Tris buffers (I = 0.05). Rate data at pH 8.65 and 8.86 are in Figure 1.

due both to change in buffer composition and to the lower amount of acetonitrile added (responsible for 25—30% of the observed increase). Other general features were similar to those described.

(b) *In the presence of CEMAH* (IIa) *and DEMAH* (IIb).

* The following rationale is used for abbreviations: A (ammonium) is preceded by the initials of the ' inert ' nitrogen substituents (C = cetyl, M = methyl, DM = dimethyl, *etc.*) and followed by those of substituents containing the function (IM = imidazol-4-ylmethyl; H = 2-hydroxyethyl).

[13] J. P. Guthrie, *J.C.S. Chem. Comm.*, 1972, 897.

The rate–concentration profiles for solutions of CEMAH and also the magnitude of the catalytic effect were dependent on the type of buffer. This is shown in Figure 1. In borate buffer, the $k\psi$ values for PNPH increase to a maximum value ($k\psi_{max.}/k_0 = 750$) at [CEMAH] = *ca.* 2×10^{-3}M and then drop rather sharply. One factor responsible for the drop in the observed rate is electrolyte inhibition.[14]

TABLE 3

Rate constants $10^4 k_\psi/s^{-1}$ for the hydrolysis of PNPA and PNPH [a] in the presence of CMAHIM

10^4[CMAHIM]/M		PNPA			PNPH		
	pH [b] 7.2	7.9	8.65	7.2	7.9	8.65
1.0	0.40	1.9	8.2	18.6	70	350
1.5			15.3	38.5	137	720
2.0	0.93	5.18	23.5	59.3	204	1 070
2.5			33.0	74	266	1 438
3.0	1.63	9.60	39.1	90	339	1 850
3.5				113		
4.0	2.35	14.0	59.7	132	473	
5.0	3.03	18.4				

[a] $[ester]_0 = 0.8$—1.5×10^{-5}M. [b] Rate data at pH 8.86 are in Figure 4.

Addition of KBr to a solution of [CEMAH] = 7×10^{-4}M decreases the observed rate as shown. However, in the range [CEMAH] = 0.5—4×10^{-4}M the observed rate constants increase virtually linearly with surfactant concentration as shown in Figure 1 and the apparent catalytic rate constants were derived from the slope of the linear tract following the ' kinetic ' c.m.c. Data not shown in Figure 1 are in Table 2 and the catalytic rate constants are collected in Table 4. The observed rate constants for the hydrolysis of both esters increase linearly with increasing concentrations of DEMAH: the effect is quite small as revealed by the catalytic rate constants of Table 4.

(c) *In the presence of CMAHIM* (IIIa) *and EMAHIM* (IIIb). The pseudo-first-order rate constants for the conditions [CMAHIM] ≫ [ester] are in Table 3 and a typical rate concentration profile is shown in Figure 4. At pH 7.9 at least, the rate increase is linear with surfactant concentrations up to 1.5×10^{-3}M for PNPA and 6—7×10^{-4}M for PNPH. The apparent catalytic rate constants are in Table 3 together with those measured for the non-surfactant EMAHIM. For solutions of the latter salt, the $k\psi$ values increase linearly with [EMAHIM] up to at least 4×10^{-3}M. The catalytic rate constants at pH 7.9 have been confirmed by measurements made in the presence of mixed micelles of CMAHIM and cetyltrimethylammonium bromide (CTABr) of different ratios ([CTABr] : [CMAHIM] = 2, 4, and 20). By the use of equation (1),[3b] discussed elsewhere,[1,15] plots of the quantity [CMAHIM]/$k\psi$

$$[CMAHIM]/k\psi = 1/k_c + [CTABr]/k_cK \qquad (1)$$

versus [CTABr] (see Figure 2) allowed to determine the following k_c 1 mol^{-1} s^{-1} values, 4.8 ± 0.7 (PNPA) and 190 ± 25 (PNPH), and K/mol l^{-1}, 2.8 ± 0.30 × 10^{-2} (PNPA) and 5.2 ± 0.3 × 10^{-4} (PNPH).

Under the condition [PNPH] > [CMAHIM] although over a very narrow range of concentrations due to the limited solubility of the ester and the relatively large turnover rate, saturation kinetics were observed as shown in Figure 3. A plot of the *p*-nitrophenol burst *versus* surfactant concentration indicates an approximate c.m.c. value of 1.2×10^{-5}M. The slope of the linear stationary phase following the initial burst gives a rate value of *ca.* 7×10^{-3} mol s^{-1} of liberated *p*-nitrophenol per mol of micellar CMAHIM.

(d) *In the presence of mixed surfactants.* Micellar solutions of mixed CEMAH and CDMAIM are better catalysts than those of each single surfactant in terms of both catalytic rate constants and kinetic c.m.c. (relative to CDMAIM) as illustrated in Figure 4. The value of k_c/l mol^{-1} s^{-1} of a 1.2 : 1 mixture of CEMAH and CDMAIM is similar to that of CMAHIM (2 300 and 2 600 in the case of PNPH) or better (157 and 90 in the case of PNPA). On the other hand, mixing of CEMAH to CMAHIM in the ratio 1.2 : 1 does not appreciably affect the efficiency of the latter in the case of PNPH but increases it in the case of PNPA. Mixed micelles of CEMAH and CDMAIM and mixed micelles of

TABLE 4

Catalytic rate constants k_c/l mol^{-1} s^{-1} of micellar surfactants (Ia)—(IIIa) and of non-surfactant salts (IIb) and (IIIb)

Catalyst		PNPA				PNPH			
	pH 7.2	7.9	8.65	8.86	7.2	7.9	8.65	8.86
CDMAIM (Ia)	0.37	2.9	12.1	51	12.9	92	480	1 100
CEMAH (IIa) [a]		0.09	0.57	1.6		1.3	13.5	27
DEMAH (IIb)				0.002 5			0.001 5	0.002 3
CMAHIM (IIIa)	0.65	4.5	18	90	38	135	800	2 600
EMAHIM (IIIb)		0.035	0.10		0.031	0.045	0.10	

[a] At pH 9.05: 1.4 (PNPA) and 15.1 (PNPH).

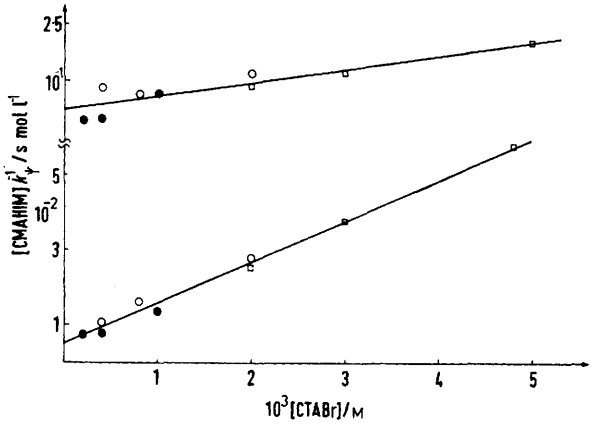


FIGURE 2 Plot of rate data following equation (1). [CTABr] : [CMAHIM] ratios: ● 2; ○ 4; □ 20

[14] C. A. Bunton in ' Reaction Kinetics in Micelles,' ed. E. H. Cordes, Plenum, New York, 1973, p. 73, and references therein.

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

CEMAH and CMAHIM are virtually equal towards each ester. Rate–concentration profiles are in Figure 4.

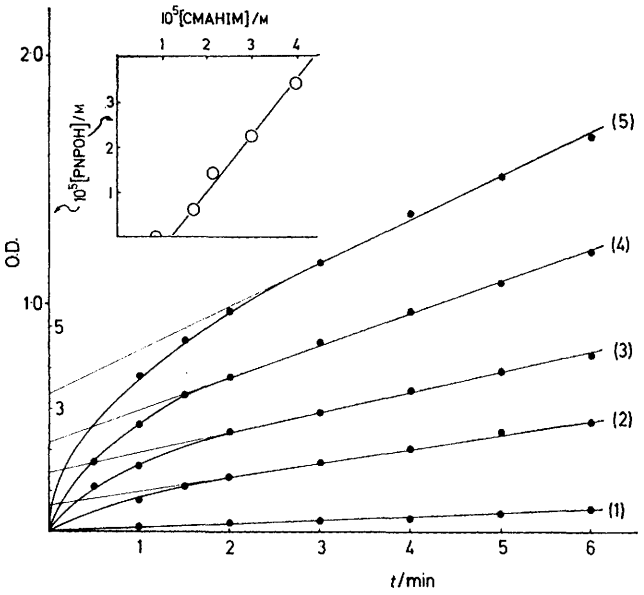


FIGURE 3 Product–time diagram for the liberation of *p*-nitrophenol (PNPOH) under the conditions: 0.02M-borate, pH 8.1, [PNPH] = 1.8×10^{-4}M; 10^5[CMAHIM] (1) = 0.86; (2) = 1.7; (3) = 2.13; (4) = 3.0; (5) = 4.0M. O.D. at 400 nm. Upper left: plot of PNPOH burst *versus* [CMAHIM]

Search for the Acylated Intermediate.—To obtain information on the acylation site (N or O) of bifunctional micelles (either of CMAHIM or of mixed CEMAH and CDMAIM) spectral changes were recorded in the region 230—270 nm under the conditions indicated in Figure 5. *N*-Acetylimidazole derivatives are known [3b,d,10a,16] to absorb at $\lambda_{max.}$ 245—248 nm and build-up of this intermediate would result in an increase of absorbance in this spectral region followed by a slower decrease. This has been verified, as

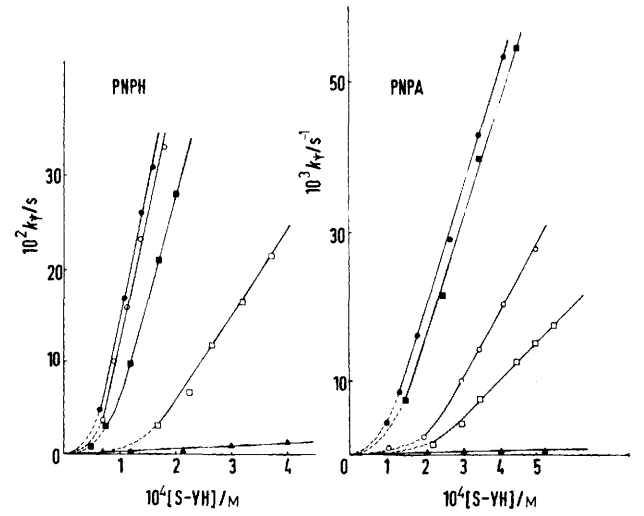


FIGURE 4 Rate–concentration profiles for PNPH (left) and PNPA (right) hydrolysis in 0.02M-borate, pH 8.85. S-YH = ▲ CEMAH; □ CDMAIM; ○ CMAHIM; ■ CDMAIM (mixed with CEMAH in the ratio 1 : 1.2); ● CMAHIM (mixed with CEMAH in the ratio 1 : 1.2)

reported,[1,3d] for solutions of CDMAIM (Figure 5A) but not for solutions of CMAHIM or for a 1.2 : 1 mixture of CEMAH and CDAIM. For solutions of bifunctional micelles as well as for those of CEMAH a clean isosbestic point at 247 nm was observed (Figure 5B). On the other hand when CEMAH was added to a solution of CDMAIM and PNPA after the time needed to reach the maximum optical density at 245 nm a very rapid decrease in absorbance was observed and the isosbestic point was again recorded (Figure 5C). These observations have been independently confirmed by Moss and his co-workers [12] who also isolated *O*-acetylated CMAHIM from a reaction of PNPA and CMAHIM.

On the other hand, the above authors [12] did observe changes at 245 nm when PNPH was used ([PNPH] = 2.0×10^{-4}M; [CMAHIM] = 5.0×10^{-3}M; pH 8.0; 0.4M-phosphate) corresponding to rapid build-up and decay of

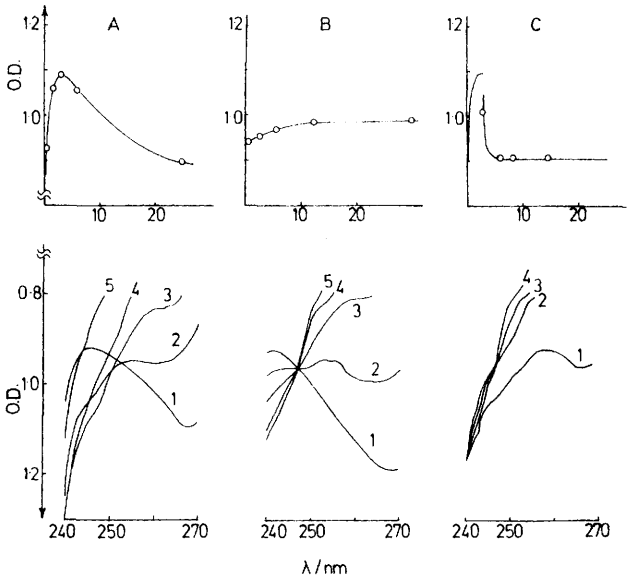


FIGURE 5 Absorbance at 245 nm *versus* time (upper) and corresponding spectral changes in the region 230—270 nm (lower) recorded progressively at times shown (open circles) in the upper part. pH 8.1, 0.02M-borate, $[PNPA]_0 = 1.3 \times 10^{-4}$M, [S-IM] = 2.5×10^{-3}M. A, CDMAIM; B, CMAHIM [mixed CEMAH and CDMAIM (1.2 : 1 molar ratio) display identical behaviour on a slightly different time scale]; C, CDMAIM after 3 min. CEMAH was added (2.5% volume change) to make a 1.2 : 1 molar ratio with CDMAIM. Absolute O.D. values shown in diagrams are only indicative

an *N*-acylated intermediate. This observation has been confirmed in this laboratory.

DISCUSSION

The effects of micellar surfacants (Ia), (IIa), and (IIIa) on the hydrolysis of *p*-nitrophenyl esters PNPA and PNPH, when compared with those of the non-surfactant salts (Ib), (IIb), and (IIIb) (see Part 1 and Table 4) or with those of non-functional surfactants [3b,d,5c,6a,17] like

[16] M. L. Bender and V. W. Turnquest, *J. Amer. Chem. Soc.*, 1957, **79**, 1656.

[17] L. R. Romsted and E. H. Cordes, *J. Amer. Chem. Soc.*, 1968, **90**, 4404.

CTABr, are clearly diagnostic of micellar functional catalysis. A common general mechanism can therefore be outlined for the micelles under investigation [equations (2)—(4)]. Micellar systems are shown within square brackets and -YH is the function (imidazolyl or hydroxy, undissociated) which is acylated [equation (3)] and deacylated [equation (4)].

$$[\text{S-YH}] + \text{RCO}_2\text{Ar} \overset{K}{\rightleftharpoons} [\text{S-YH,RCO}_2\text{Ar}] \quad (2)$$

$$[\text{S-YH,RCO}_2\text{Ar}] \xrightarrow{k_2} [\text{S-YCOR}] + \text{ArOH} \quad (3)$$

$$[\text{S-YCOR}] + \text{H}_2\text{O} \xrightarrow{k_3} [\text{S-YH}] + \text{RCO}_2\text{H} \quad (4)$$

The c.m.c. values of Table 1 and the ' kinetic ' c.m.c. from rate–concentration profiles at any pH indicate that micelles of CEMAH and CMAHIM containing the hydroxy-group are formed at lower surfactant concentrations than those of CDMAIM containing only the imidazole ring. Strong hydrogen bonds due to the hydroxy-functions at the polar heads are likely [5c] to be responsible for binding of the surfactants. At any rate, this is one factor, besides the apparent rate constants discussed below, which makes the overall catalytic effect of bifunctional micelles better than that of CDMAIM at least at low surfactant concentrations and particularly for PNPH (see Figure 4).

A precise determination of the binding constant K for micelle–substrate association is a prerequisite to any detailed kinetic analysis.[3g] Attempts to measure [18] K values for micellar solutions of (Ia)—(IIIa) at low pH values in acetate buffer failed to give reproducible results. Scattered data from the application [1] of equation (1) to mixed micelles of CTABr with CDMAIM and CMAHIM and recent values reported by Berezin and his co-workers [7] for micelles of (IIc) lend credit to the basic assumption made here: the K values of micelles of the three surfactants (Ia)—(IIIa), mixed micelles of them and with CTABr, being all made of cetylammonium ions, are rather similar under equal conditions and towards the same ester. Taking as typical K/l mol^{-1} values those reported [3b] for CTABr micelles, 30 and 2 000 for PNPA and PNPH, much of the increase in efficiency of the micellar catalysis on going from PNPA to PNPH can be accounted for.[1]

The Acylation Stage.—Within the limits of the assumptions made above, the acylation rate can be deduced, although on a relative scale, from the apparent catalytic rate constants of Table 4. The following rate sequence results: CMAHIM > CDMAIM ≫ CEMAH by ratios 30—50 : 20—30 : 1 in the case of PNPA and 40—100 : 35—70 : 1 in the case of PNPH at any pH and buffer investigated.

Such analysis indicates that although the pH dependence of the k_c values is quite large, the pH–rate profile of the three types of micelles is substantially similar. As discussed in detail in Part 1 for CDMAIM micelles, the observed pH dependence indicates that the anionic form of the imidazole ring is involved as nucleophile. This appears to be true also for the hydroxy-group of micellar CEMAH, as suggested by Berezin and his co-workers [7] and expected from the pK values of Table 1.

Bifunctional CMAHIM is better than CDMAIM although the difference in k_c is small. This is a point of discrepancy with the report by Moss *et al.*,[8] although less serious than it appears. These authors indicate the following order of effectiveness: CDMAIM > CMAHIM ≫ (II; $R^1 = C_{16}H_{33}$; $R^2 = R^3 = CH_3$; Cl^-) based on $k\psi_{max.}$ observed in pH 8 phosphate buffers. Obviously the criteria for judging the effectiveness are quite different and complementary. The one used here provides a better approach to a quantitative estimate of the reaction rate since the limited range of surfactant concentration is not much affected by electrolyte effects and all those factors which strongly influence the shape of the rate–concentration profiles and the position of $k\psi_{max.}$ (see Figure 2).

The greater effect of CMAHIM micelles than of those of CDMAIM indicates a co-operative mode of intervention of the second group. This interaction is however inter- rather than intra-molecular in character as judged from the fact that mixed micelles of CEMAH and CDMAIM are just as effective or more effective than homogeneous micelles of CMAHIM (Figure 4). On the other hand, intramolecular interaction arising, for instance, from hydrogen bonds between N and O appeared *a priori* unlikely from inspection of models.

The interesting question is however which group actually acts as nucleophile in bifunctional micelles. The absorbance changes in the region 245—248 nm with PNPA under the conditions indicated (Figure 5) suggest that an *N*-acetylimidazole derivative accumulates [3] during the reaction in the case of CDMAIM and that this does not occur in the case of CMAHIM or of mixed CDMAIM and CEMAH. These observations and the isolation of *O*-acetylated CMAHIM by Moss and his co-workers [12] lead to the conclusion that *O*-acylation occurs in bifunctional micelles and the process is quite effectively promoted by the imidazole ring of a neighbouring surfactant molecule in the micellar phase. On the other hand, the build up of an *N*-acylated intermediate has been observed with PNPH and CMAHIM. Moreover the catalytic kinetic effects of CDMAIM and CMAHIM are substantially similar indicating that the imidazole group is the effective nucleophilic site in both cases, only weakly assisted by the hydroxy-group of bifunctional micelles in the acylation stage.

The Deacylation Stage.—The above apparently conflicting facts as well as the others examined below may be explained by the two-step mechanistic hypothesis suggested by Moss and his co-workers.[12] The assumption is that in bifunctional micelles *N*-acylation initially occurs followed by relatively rapid acyl transfer to the hydroxy-group. The results of experiments carried out by adding CEMAH to a solution of reacting CDMAIM and PNPA

[18] A. K. Yatsimirski, K. Martinek, and I. V. Berezin, *Tetrahedron*, 1971, **27**, 2855.

when the build-up of the *N*-acetylated intermediate had reached its peak (Figure 5C) support the hypothesis. It presumably indicates that *N*- to *O*-transacetylation occurs much faster than the overall deacylation process.

The build-up of an *N*-acylated intermediate is thus determined by the relative rate of the *N*-acylation and transacylation processes. Under the conditions of Figure 5, in the case of PNPA transacylation is faster than acylation, and the *N*-acyl intermediate does not accumulate. In the case of PNPH the reverse is true and *N*-deacylation becomes rate limiting. Moreover under the saturation conditions of Figure 3, the steady-state part of the diagram is likely to be controlled by the rate of *N*-deacylation of the *O*-acylated micelle.

Conclusions.—To sum up, although definite conclusions on the mechanism need further work, available evidence concerning bifunctional micelles indicates a mechanism involving the imidazole nitrogen as the nucleophilic site and the hydroxy-group as an effective catalyst in the *N*-deacylation process rather than co-operative charge-relay[10,19] chymotrypsin-like behaviour. At any rate the catalytic properties of the functional micellar ' chymotrypsin analogues ' here investigated are remarkable in terms of rate acceleration and catalytic turnover. The apparent catalytic rate constants are of the order of magnitude observed [20] for the enzyme itself (for acetylation of α-chymotrypsin is 560 l mol^{-1} s^{-1} at pH 8) at least in non-specific esterolytic processes.

EXPERIMENTAL

The *p*-nitrophenyl esters PNPA and PNPH, cetyl(imidazol-4-ylmethyl)dimethylammonium chloride hydrochloride (CDMAIM) (Ia) and its precursor 4-chloromethylimidazole hydrochloride were synthesized as described.[1] 2-(*N*-ethyl-*N*-methylamino)ethanol was obtained by reaction between 2-methylaminoethanol and ethyl bromide in benzene. It had b.p. 148—150° (lit.,[21] 145—150°), τ (neat) 5.15br (1 H), 6.5 (2 H, m, CH_2O), 7.6 (4 H, m, CH_2N), 7.85 (3 H, s), and 9.05 (3 H, t).

2-(N-*Cetyl*-N-*methylamino*)*ethanol.*—Cetyl chloride (52.2 g) and 2-methylaminoethanol (35 g) were dissolved in benzene (75 ml) and the solution heated at reflux for five days. The upper layer thus formed was separated and fractionally distilled. The title *amine* (81%) had b.p. 155—158° at 3 mmHg, m.p. 26.5° (Found: C, 75.95; H, 13.6; N, 4.6. $C_{19}H_{41}NO$ requires C, 76.15; H, 13.8; N, 4.7%); τ (neat) 5.75 (1 H), 6.45 (2 H, m, CH_2O), 7.65 (4 H, m), 7.8 (3 H, s), 8.7 (*ca.* 28 H), and 9.1 (3 H, t).

Cetylethyl-(2-hydroxyethyl)methylammonium Bromide (*CEMAH*) (IIa).—2-(*N*-Cetyl-*N*-methylamino)ethanol (2 g) and ethyl bromide (0.76 g) were dissolved in benzene (30 ml) and were stirred at room temperature for four days. The solvent was then evaporated under reduced pressure and the residue was dissolved in methanol. The solution was treated with activated charcoal and then with sodium carbonate, filtered, and evaporated. The residue was repeatedly washed with ether, dissolved in benzene, and lyophilized. The *solid* (80%), moderately hygroscopic, melts at 64° forming liquid crystals and decomposes at 195° (Found C, 61.5; H, 11.0; N, 3.3; Br, 19.55. $C_{21}H_{46}BrNO$ requires C, 61.75; H, 11.25; N, 3.45; Br, 19.6%), τ (D_2O) 5.8 (2 H, m), 6.4 (6 H, m), 6.75 (3 H, m), 8.65br (*ca.* 28 H), and 9.05 (3 H, t).

Diethyl-(2-hydroxyethyl)methylammonium Bromide (*DEMAH*) (IIb).—2-(*N*-Ethyl-*N*-methylamino)ethanol (1.05 g) and ethyl bromide (0.49) were dissolved in benzene (20 ml) and after one day worked up as described above for CEMAH. The product, recrystallized from methanol–ether was kept under vacuum over P_2O_5; if was very hygroscopic and gave poor elemental analyses (Found: C, 39.05; H, 8.85; N, 6.25; Br, 37.2. Calc. for $C_7H_{18}BrNO$: C, 39.65; H, 8.5; N, 6.6; Br, 37.7%), τ (D_2O) 5.66 (2 H, m, CH_2O), 6.24 (6 H, m, CH_2N), 6.63 (3 H, s), and 8.35 (6 H, t).

Cetyl-(2-hydroxyethyl)(imidazol-4-ylmethyl)methylammonium Chloride Hydrochloride (*CMAHIM*) (IIIa).—2-(*N*-Cetyl-*N*-methylamino)ethanol (2 g, 6.6mM) was dissolved in methanol (4 ml) together with 4-chloromethylimidazole hydrochloride (0.51 g, 3.3mM) at room temperature. After 10 min finely divided, dry sodium carbonate (0.5 g) was added, the mixture stirred for 5 min and filtered. The solvent was evaporated under reduced pressure and the residue washed twice with 20 ml portions of anhydrous ether. The residue had to be treated again twice with sodium carbonate and ether to remove the amine (hydrochloride). The residue was then dissolved in methanol, filtered, and treated with methanolic HCl (2 equiv.). The solvent was removed under reduced pressure and the residue recrystallized twice from methanol–ether. The product (39%), moderately hygroscopic, melts at 110° giving liquid crystals which decompose at 191—192° (Found: C, 59.8; H, 10.2; N, 9.35; Cl, 15.95. Calc. for $C_{23}H_{47}N_3OCl$: C, 61.05; H, 10.45; N, 9.3; Cl, 15.65%), τ (CD_3OD) 0.95 (1 H, s), 2.0 (1 H, s), 5.05 (2 H, m, NCH_2IM), 5.95 (2 H, m, CH_2O), 6.7 (4 H, m), 6.9 (3 H, s), 8.7br (*ca.* 28 H), and 9.1 (3 H, t).

Ethyl-(2-hydroxyethyl)(imidazol-4-ylmethyl)methylammonium Chloride Hydrochloride (EMAHIM) (IIIb).—The product was obtained (22%) from 2-(*N*-ethyl-*N*-methylamino)ethanol (0.95 g) and 4-chloromethylimidazole hydrochloride (0.69 g) in methanol (3 ml) following the procedure described for CMAHIM. The very hygroscopic product gave a poor elemental analysis (Found: C, 41.7; H, 7.8; N, 16.25; Cl, 28.15. Calc. for $C_9H_{19}Cl_2NO$: C, 42.2; H, 7.45; N, 16.4; Cl, 27.7), τ (D_2O) 0.9 (1 H, s), 1.9 (1 H, s), 5.05 (2 H, m), 5.9 (2 H, m), 6.4 (4 H, m), 6.75 (3 H, s), and 8.5 (3 H, t). The pK_1 of EMAHIM in 0.05M-KCl and that of CMAHIM for surfactant solutions (4.9—4.7 × 10^{-3}M) in 0.1M-KCl were determined by described procedures.[1]

Kinetic Measurements.—As a general procedure, a solution of ester (0.5—2.1 × 10^{-3}M) in spectrograde acetonitrile (0.02 ml) were added to the pre-thermostatted solution (2.0 ml) of ammonium salts in the given buffer and the appearance of *p*-nitrophenol monitored at 400 nm using a Gilford 2400 spectrophotometer. The pseudo-first-order rate constants were obtained as described; for very slow runs (with DEMAH) $k\psi$ were obtained by the initial rate methods and very fast runs ($k\psi > 1 \times 10^{-1}$ s^{-1}) were occasionally checked by using a Durrum stopped-flow

[19] D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature*, 1969, **221**, 337 and references therein.

[20] M. L. Bender and K. Nakamura, *J. Amer. Chem. Soc.*, 1962, **84**, 2577.

[21] I. F. Halverstadt, W. R. H. Hardie, and A. R. Williams, *J. Amer. Chem. Soc.*, 1959, **81**, 3625.

apparatus. Solutions of CDMAIM and CMAHIM in buffers of pH > 8 were freshly prepared before each run to avoid any significant decomposition of the surfactants.

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