Functional Micellar Catalysis. Part 4.[1] Catalysis of Activated Ester Hydrolysis by Surfactant Systems as Chymotrypsin Models

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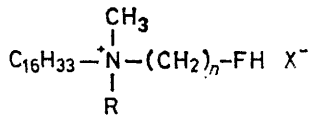
Micelles made up of each of five surfactants of general structure $C_{16}H_{33}\overset{+}{N}(CH_3)_2(CH_2)_nFH$ [(I), n = 2, FH = OH; (II), n = 1, FH = C(Ph)=NOH; (III), n = 1, FH = CO·C(Ph)=NOH; (IV), n = 1, FH = imidazol-4-yl (Im); (V), n = 2, FH = NH·CO·Im], of the bifunctional surfactant $C_{16}H_{33}\overset{+}{N}(CH_3)(CH_2Im)(CH_2CH_2OH)$ (VI), of N^{α}-myristoylhistidine (VII), and of *N*-methylmyristohydroxamic acid (VIII) have been investigated as catalysts of *p*-nitrophenyl acetate (PNPA) and hexanoate (PNPH) hydrolysis in the pH range 7—8. The activity of α-chymotrypsin toward PNPA at pH 7.95 has also been measured. The kinetic analysis was carried out under the same conditions in each instance and rate data are directly compared. A nucleophilic mode of action is indicated for each type of micelle and differences in the rate of acylation and turnover are discussed with reference to related micellar systems and to the enzyme α-chymotrypsin.

MICELLAR catalysis of synthetic functional surfactants as a model for enzymic catalysis has been extensively investigated in recent years.[2] Functional surfactants containing hydroxy [3-5] (also in the activated form of oxime and hydroxamic acid derivatives [5]), amino,[6] and mercapto groups,[7] the imidazole ring,[4,5d,8] and other reactive sites [9] have been studied and their catalytic properties tested in the hydrolysis of activated esters, generally *p*-nitrophenyl carboxylate or phosphate esters. In many cases, large kinetic effects have been observed and several systems come near enzyme reactions in giving large rate enhancements.[2b]

Published data are still fragmentary and a comparison of the micellar catalytic efficiency of various types of functional surfactants from available reports is difficult due to the different conditions used in the kinetic studies and also to the different criteria by which rate data are evaluated. We have recently measured the catalytic effects in the hydrolysis of activated esters and amides of several micellar functionalized reagents under common conditions in order to compare their effectiveness directly. Kinetic measurements were made for co-micelles of cetyltrimethylammonium bromide (CTABr) and the functional surfactants in Tris buffers of constant ionic strength: the operational advantages of these conditions and the quantitative treatment of kinetic data for these co-micellar systems have been discussed elsewhere.[1] We have also indicated that the apparent catalytic rate constants obtained for co-micelles are close to those obtained for homogeneous micelles under identical conditions.

We here present the results of the kinetic analysis for a series of surfactants, containing the hydroxy group (also in the form of oxime and hydroxamic acid derivatives) or/and the imidazole ring, which can be taken as models of trypsin-type enzymes.[10] Most of them (I)—(VI) are quaternary ammonium salts; two of them (VII) and (VIII) are myristoyl derivatives. The results here reported concern the catalytic activity of (I)—(VIII) in the hydrolysis of *p*-nitrophenylacetate (PNPA) and hexanoate (PNPH).



(I) — (VI)

(I) R = C_2H_5, n = 2, FH = OH, X = Br

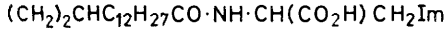
(II) R = CH_3, n = 1, FH = C(Ph) = NOH, X = Br

(III) R = CH_3, n = 1, FH = CO·C(Ph) = NOH, X = Cl

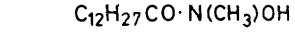
(IV) R = CH_3, n = 1, FH = Im, X = Cl

(V) R = CH_3, n = 2, FH = NH·CO·Im, X = Cl

(VI) R = $(CH_2)_2OH$, n = 1, FH = Im, X = Cl



(VII)



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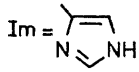


RESULTS

The synthesis and general properties of most of the surfactants here investigated have been reported, those of (I), (III), (IV), and (VI) in previous Parts,[4b,5h,8f] those of (VII) by Gitler and Ochoa-Solano [8a] and those of (VIII) by Kunitake *et al.*[5a] Table 1 reports the available apparent pK_a values of the functional groups in micelles, partly directly measured and partly estimated, together with the c.m.c. values in pure water.

The pK value of surfactant (I) was previously [4b] estimated as 10.5, based on earlier reports [3e] for a micellar analogue. This value is an underestimate by at least two units [2a] and the value shown in Table 1 is that measured for a structurally close analogue by Pillersdorf and Katzhendler.[3f] The pK values of the oxime-functionalized surfactants (II) and (III) and that of the hydroxamic acid (VIII) have been determined spectrophotometrically for solution of co-micelles of

the same compositions used for the kinetic measurements in Tris and borate buffers; those of (II) and (VIII) compare reasonably well with those reported by Kunitake and his co-workers [5a,d] and obtained under somewhat different conditions and also with recent data by Pillersdorf and Katzhendler [5g] for a hydroxamic acid-functionalized surfactant.

TABLE 1

Apparent pK_a and c.m.c. values

Surfactant	pK (OH)	pK_1 (Im) [a]	pK_2 (Im) [b]	10^4C.m.c. (mol l^{-1})
(I)	12.8 [c]			0.3
(II)	9.3			1.8
(III)	8.0			0.6
(IV)		3.5	*ca.* 11	1.0
(V)			10.4	5.1
(VI)	*ca.* 13	3.7	*ca.* 11	0.2
(VII)		6.2 [d]		
(VIII)	9.1			

[a] First dissociation constant of Im. [b] Second dissociation constant of Im. [c] From ref. 3*f*. [d] From ref. 8*a*.

The second ionization constants of the imidazole group of surfactants (IV) and (VI) have been estimated [4b] from the value (pK_a 11.5) measured on a non-micellar model of (IV), whereas that of (V) is that reported by Kunitake *et al.*[5d] for *N*-laurylimidazol-4-ylcarboxamide in CTABr micelles (1×10^{-3}M) (μ 0.01; 3% v/v ethanol 30°). The pK_2(Im) of (VII) is not available: it is likely to be in the range 13.5—14 as pK_1(Im) [8a] is 3 pK units larger than that of (IV) or (VI).

Table 1 also shows the available c.m.c. values of the soluble surfactants as determined in pure water by conductivity measurements. The c.m.c. of (II) is close to the solubility limits of the surfactant and must therefore be taken as an approximate value. Surfactants (VII) and (VIII) are virtually insoluble in water. Co-micelles made up of CTABr and the functional surfactant, under the conditions used for the kinetic analysis (see below), have quite a low c.m.c. (3—5×10^{-5}M total detergent concentration) and also ensure solubility for (II), (VII), and (VIII) up to a sufficiently high concentration of micellar functional surfactants for a proper kinetic analysis.

Kinetic Measurements.—Rate measurements were made for solutions of co-micelles of CTABr and the functional surfactant, D^f, in the molar ratio *ca.* 7 : 1 in Tris buffers of constant ionic strength (μ 0.1) adjusted with KCl with added 1% v/v CH_3CN at pH 7.15 and 7.95 and 25°.

With the condition [ester] ≪ $[D^f]$, the observed pseudo-first-order rate constants k_ψ may be expressed [1] by equation (1) where k_c is the second-order apparent catalytic rate

$$k_\psi = (k_0 + k_c[D^f]_m)/(1 + K_s[D^t]_m) \qquad (1)$$

constant, K_s the binding constant of the substrate to the micellized surfactants, $[D^f]_m$ the concentration of the micellar functional detergent, and $[D^t]_m$ the total detergent concentration in micelles. The k_0 term of equation (1) is the pseudo-first-order rate constant observed for the substrate of choice in micellar solutions of CTABr alone at [CTABr] = $[D^t]$; at least in the case of *p*-nitrophenyl ester hydrolysis, this constant is only slightly lower than that in the absence of CTABr and the possible reasons for such an inhibitory effect have recently been offered.[11] The k_c and k_s constants were evaluated from the raw kinetic data by recently described methods.[1]

The K_s values were in the range 30—50 l mol^{-1} in the case of PNPA and (1.4—2.3) $\times 10^3$ l mol^{-1} in the case of PNPH with no evident dependence on the structure of the functional surfactant or pH of the solution.

The k_c constants are shown in Tables 2 and 3. The estimated error of the k_c values is rather large (mostly within but in some cases larger than 30%). The bifunctional surfactant (VI) was previously reported to be slightly more reactive than (IV); the k_c values of Table 2 and 3 give the opposite indication in accord with the observations by Moss *et al.*[4a]

TABLE 2

Apparent catalytic rate constants, k_c/1 mol^{-1} s^{-1}, for the hydrolysis of PNPA [a]

pH	(I)	(II)	(III)	(IV)
7.15	0.025 [b]	50	920	1.3
7.95	0.15 [b]	305	2 420	7.9
pH	**(V)**	**(VI)**	**(VII)**	**(VIII)**
7.15	4.8	1.2	6.5	48
7.95	29	7.1	6.8	300

[a] For conditions see text. [b] Approximate values.

With the condition [ester] ≫ $[D^f]_m$, following the appearance of *p*-nitrophenol, ' burst ' kinetics were observed at pH 7.95 for co-micelles of (II), (III), and (VIII) in the hydrolysis of PNPA and for co-micelles of most of the surfactants investigated in the hydrolysis of PNPH. From the slope of the ' burst ' kinetics, corrected for the ' spontaneous ' hydrolysis of the ester, the turnover rate constants, $k_{turnover}$, were evaluated from literature methods.[1,12] The $k_{turnover}$ term is related to the second-order acylation rate constant, k_a (~ k_c),[1] and to the pseudo-first-order deacylation rate constant through equation (2), where $[S]_0$ is the initial ester concentration. In the case of co-micelles of (IV), (V), and (VII) and PNPA hydrolysis ' burst ' kinetics could not be observed probably due to the fact that $k_a[S]_0 < k_d$ because of the limited solubility of the ester used. The $k_{turnover}$ values of PNPH hydrolysis are in Table 3. These constants are also affected by a rather large error, those of (II), (III), and (VIII) because of uncertainties in the correction for the spontaneous hydrolysis of the ester and those of (IV)—(VI) because of the limited solubility of the ester [additional CH_3CN (see Table 3) was used in these cases]. Table 2 also shows $k_{turnover}$ data for co-micelles of (VI) measured for the hydrolysis of PNPH and PNPA in borate buffers of low ionic strength (see Discussion section).

$$k_{turnover} = [S]_0 k_a k_d/([S]_0 k_a + k_d) \qquad (2)$$

TABLE 3

Apparent catalytic, k_c/1 mol^{-1} s^{-1}, and turnover, $k_{turnover}/s^{-1}$,[a] rate constants for the hydrolysis of PNPH [b]

	pH	(I)	(II)	(III)	(IV)
k_c	7.15	0.3	470	8 500	54
k_c	7.95	1.8	2 500		290
$10^4 k_{turnover}$	7.95		<1	<1	35 [c]
		(V)	(VI)	(VII)	(VIII)
k_c		135	38	115	440
k_c		1 250	210	150	2 600
$10^4 k_{turnover}$		50 [c,d]	38 [c]	3.6	<1

[a] Moles of liberated *p*-nitrophenol per mole of micellar functional surfactant per second. [b] For conditions see text, unless otherwise indicated. [c] 3.5% v/v CH_3CN. [d] Other $k_{turnover}$ values {borate 0.01M; μ 0.01 (KCl); [CTABr] 1×10^{-3}M; 3% v/v EtOH; 30 °C} for the hydrolysis of PNPH 120 s^{-1} at pH 8.0; for the hydrolysis of PNPA, 90 s^{-1} at pH 8.0 and 405 s^{-1} at pH 8.8.

In order to compare the catalytic effectiveness of the micellar models to that of α-chymotrypsin we have measured the apparent catalytic rate constant and the turnover rate of the enzyme under the same standard conditions used for the micellar reagents for the hydrolysis of PNPA at pH 7.95. In the presence of CTABr {[CTAB] : [enzyme] 8.1 : 1; [PNPA] 4.5×10^{-6}M; [enzyme] $(1.4—2.8) \times 10^{-5}$M} the mean (four measurements) second-order rate constant was 2 010 ± 50 l mol^{-1} s^{-1} (3 080 ± 150 l mol^{-1} s^{-1}, in the absence of CTABr, same conditions) and with the conditions [enzyme] $(0.7—2.7) \times 10^{-5}$M and [PNPA] $(1—2.5) \times 10^{-4}$, the $k_{turnover}$ (average of six kinetic runs) was $(138 \pm 15) \times 10^{-4}$ s^{-1}. The second-order rate constant is larger than that reported by Bender and Nakamura [13] (560 l mol^{-1} s^{-1}; 0.1M-phosphate; pH 7.94; 10% v/v CH_3CN) which is often quoted as a reference value, while the rate of turnover is similar to that (150×10^{-4} s^{-1}) measured by Gutfreund and Sturtevant [14] (20% v/v Pr^{i}OH; pH 8.0; 25 °C).

DISCUSSION

From a preliminary scrutiny of Tables 2 and 3, the functional micellar reagents here investigated appear to differ widely in their esterolytic reactivity in the pH range 7—8, a factor larger than 10^4 being involved between the k_c values of (I) and (III). In one case (III), the apparent catalytic rate constant at pH 7.95 is larger than that of α-chymotrypsin. On the other hand, the rate of turnover is generally small and only in the case of the imidazole-functionalized surfactants (IV)—(VI) is the $k_{turnover}$ value of the same order of magnitude as that observed for the enzyme.

On the whole, although odd combinations in the magnitude of the k_c and $k_{turnover}$ constants make the systems here investigated not as efficient as the enzyme for the same type of reaction, the rate enhancements observed are quite remarkable for such simple, mostly monofunctional models.

Mechanism of Esterolysis.—The available evidence concerning the mechanism of ester cleavage of the functional surfactants under study, points to a common nucleophilic pathway involving acylation and deacylation of the micellar function ~FH as shown in the Scheme. The main indications of this mode of action

$$\sim F(H) + RCO{\cdot}OAr \longrightarrow \sim F{\cdot}COR + ArO(H)$$

$$\sim F{\cdot}COR + HO^- \longrightarrow \sim F(H) + RCO_2^-$$

SCHEME

come from (a) the reported identification or isolation of the acylated intermediate in the case of micellar surfactants (IV),[8c,f] (VI),[4b,8g] (VII),[8a] and of analogues of (VIII) [8i] and (b) the observation of ' burst ' kinetics for the above systems as well as for those made up of (II), (III), and (V). The case of choline-type surfactants such as (I) has been largely debated following earlier suggestions of other mechanisms.[3c,d] Work from Berezin's,[3c] Bunton's,[3a,b] and Katzhendler's laboratories clearly showed that these reactants do also react by way of a nucleophilic mechanism in mildly alkaline solution.

Acylation.—With the exception of the histidine derivative (VII) which reacts [8a] with the neutral, free base form of the imidazole as a nucleophile, at least [8b,f] in the pH range 7—8, the effective nucleophilic species involved in the acylation step of the esterolysis are the anionic forms of the functions of the micellar reagents here investigated. This has been deduced [4b,8f] from the pH–rate profiles in the case of (IV), (VI), and (I) and may *a fortiori* be inferred to be the case for other surfactants containing stronger acidic functions than (I) and (IV). The changes in the k_c constants on going from pH 7.15 to 7.95 in the hydrolysis of both esters provide further, though limited, support to the above assumption.

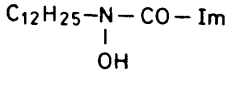
The apparent catalytic rate constants k_c of Tables 2 and 3 must therefore be related to the fraction of dissociated functions and hence to their apparent pK_a values. The catalytic rate constants corrected for the fraction of anionic forms, k_c^-, may be evaluated [1] by means of equation (3) assuming, as a first approximation, that the activity of the undissociated functions may be negligible. Further approximation are related to the

$$k_c^- = k_c(1 + [H^+]/K_a\text{app}) \qquad (3)$$

uncertainty of the p$K_a$app values of Table 1, as mentioned above. Some values have been estimated or determined for conditions, such as the ionic strength of the solution or the degree of dissociation of the functions, different from those used in the kinetic experiments: the relevance of these factors has been recently discussed.[3f] The corrected apparent catalytic rate constants thus evaluated from the data in Tables 1—3 are the following (average of two pH values) for each reagent and for the hydrolysis of PNPA (10^{-3} k_c^-/1 mol^{-1} s^{-1}) and of PNPH (10^4 k_c^-/1 mol^{-1} s^{-1}): (I), 11.2, 13; (II), 7.1, 6.3; (III), 6.3, 6.8; (IV), 9.1, 35; (V), 8.3, 29; (VI), 8.3, 25; (VIII), 4.4, 3.9. These values are surprisingly similar (within a factor of three in the case of PNPA): the differences in the k_c constants seems to be accounted for by the fraction of dissociated functions of the micellar reagents in spite of the fact that the anionic nucleophiles cover a range of basicity of 4.8 pK units. One cannot expect any Brønsted log k_c^- *versus* pK_a linear relationship out of such widely different types of oxygen and nitrogen nucleophiles, including some ' α ' nucleophiles [15] such as the oximate and hydroxamate functions. Differential desolvation [5a,d] of the anionic groups in micelles can hardly be involved as a levelling factor in view of recent evidence reported [1,16] against the desolvation hypothesis. The equalization of the k_c^- constants may be partly the result of coincidental factors. On the whole, however, the small sensitivity of the acylation rate to the basicity of the attacking nucleophiles indicates that this process occurs *via* a rate-limiting formation of a tetrahedral intermediate which favourably partition to products and is in line [17] with the fact that the anionic functions here considered [8c] are much more basic nucleophiles than the leaving *p*-nitrophenolate group (pK_a 7.02 [18]).

Deacylation. Catalytic Efficiency.—(a) *Monofunctional reagents.* The deacylation process has not been investigated in detail and is here assumed to be a hydroxide-ion promoted acyl transfer to water for all the monofunctional surfactants. As indicated by the turnover rate constants of Table 3 (PNPH hydrolysis), deacylation is much slower for all the oxygen-functionalized than for the activated imidazole-containing surfactants (IV) and (V). Among the factor which favour the rapid deacylation of micelles of (IV), Tagaki and his co-workers [8d] indicated co-operative assistance by the positively charged ammonium residue favourably located for binding a hydroxide ion close to the *N*-acylated imidazole group of (IV). This argument, however, does not apply to (V) where the imidazole ring is far removed from the cationic head or it should be equally applied to all other cases of quaternary ammonium salts. Clearly, the imidazole residues of (IV), (V) [and (VI), see below], under the conditions explored and for structural reasons, are such a good compromise between effective nucleophiles and good leaving groups as to make the turnover rates of these reagents of the same order of magnitude as that of α-chymotrypsin in Tris buffers at pH 7.95 and μ 0.1.

Changing the ionic strength and, perhaps, the buffer, has been reported by Kunitake *et al.*[5d] to have a dramatic effect on the reactivity of the bifunctional reagent (IX)



(IX)

in CTABr micelles toward PNPA. The deacylation rate was found to be particularly affected, as indicated by a 150-fold increase on going from μ 0.5 (KCl, 0.1M Tris) to 0.01 (KCl, 0.01M-borate) at pH 9.0 and 30°. We ran some kinetic experiments on surfactant (V) which contains the same -CO-IM functions as (IX) under the same low μ (0.01) conditions used by the above authors, with excess ester over (V). Table 3, footnote *d* shows the conditions and data. In the case of PNPH hydrolysis at pH 8 (30°), the turnover rate is *ca.* 2.5 times larger than that observed at μ 0.1 and pH 7.95 (25°), not such a dramatic change in view of the higher reaction temperature. The benefits of the low ionic strength are more evident in the case of PNPA hydrolysis: 'burst' kinetics could be observed and the $k_{turnover}$ values for (VI) at pH 8 and 8.8 were found to be quite large and remarkably (see below) only 3—5 times smaller than those reported for (IX).

(b) *Bifunctional reagents [including* (IX)]. The bifunctional reagent (IX), a model for trypsin-like enzymes, is the most effective micellar catalyst for activated ester hydrolysis so far reported.[5d] Its high effectiveness has been explained as essentially due to a very rapid acylation of the hydroxamate function followed by its rapid deacylation catalysed by the neighbouring imidazole (anionic at pH > 8) function. The latter was, therefore, denied any relevant direct role in esterolysis. Yet, the rather similar $k_{turnover}$ of (V) and (IX) (see above) could imply for the latter a mechanism different from that suggested which may involve fast acylation of the hydroxamate function followed by very rapid acyl transfer to the neighbouring imidazole which is then deacylated to water at a rate close to that observed for (V). Contrary to this idea, however, is the observation by Brown and his co-workers [8i] that intermolecular acyl transfer from an acylated hydroxamic acid-functionalized surfactant to the imidazole of a histidine-containing surfactant in mixed micelles is thermodynamically quite unfavourable. There is also the possibility of very fast acylation of the hydroxamate function, which is slowly deacylated, and parallel, effective acylation and deacylation of the imidazole ring in an independent cycle which is substantially responsible for the observed turnover rate. Yet, some of the data reported by Kunitake *et al.* seem not to fit this hypothesis also. At any rate, a better definition of the mode of action of the very effective micellar (IX) is highly desirable.

The case of the bifunctional reagent (VI) is more clearly defined.[4b,8g] On one hand, co-operation of the two functions in the acylation stage is excluded by early data from Moss *et al.*[4a] and by the present data. On the other hand, it has been shown that the catalytic cycle involves fast acylation of the imidazole ring, followed by a relatively rapid acyl transfer to the hydroxy-group and its slow deacylation to water. It is here emphasized that this mode of action is closely analogous to that suggested by Hubbard and Kirsch,[19] for the active site of α-chymotrypsin in the esterolysis of phenyl esters: acylation of the enzyme would involve nucleophilic attack by the His-57 imidazole and fast acyl transfer to the Ser-195 hydroxy-function.

EXPERIMENTAL

The *p*-nitrophenyl esters PNPA and PNPH, cetylethyl-(2-hydroxyethyl)methylammonium bromide (I), cetyldimethyl(imidazol-4-ylmethyl)ammonium chloride hydrochloride (IV.HCl), cetyl-(2-hydroxyethyl)(imidazol-4-ylmethyl)methylammonium chloride hydrochloride (VI.HCl) and N^{α}-myristoyl-L-histidine (VII) were synthesized as described.[4b,8f] Cetyltrimethylammonium bromide and α-chymotrypsin (from Worthington Biochemical Corp.) were commercial products crystallized before use. N^{α}-Methylmyristohydroxamic acid was prepared according to Kunitake and his co-workers,[20] m.p. 55—56° (lit.,[20] 55—57°).

Cetyldimethyl-(2-hydroxyiminophenethyl)ammonium Bromide (II).—2-Hydroxyiminophenethyl bromide (1.95 g) prepared according to Korten and Scholl,[21] m.p. 89—91° (lit.,[21] 92°) was dissolved in methanol and added to cetyldimethylamine (1.5 g). After 2 days at room temperature, the solvent was removed and the residue was twice crystallized from ethyl acetate–ether and dried under vacuum over P_2O_5. The product (35%), a mixture of *syn-* and *anti-*isomers, melts at 124—125° (Found: C, 64.4; H, 9.6; N, 5.65. Calc. for $C_{27}H_{46}BrN_2O_2$: C, 64.05; H, 9.7; N, 5.75%), δ ($CDCl_3$) 0.9 (3 H, t), 1.2 (28 H, m), 3.2 (8 H, m), 4.8 (2 H, m), 7.8 (5 H, m), 11.5 and 12.3 (1 H, 2 s, *syn-* and *anti-*isomers).

3-Bromo-1-hydroxyimino-1-phenylpropan-2-one.—A solution of 1-hydroxyimino-1-phenylpropan-2-one (4.0 g) prepared according to Kolb,[22] δ ($CDCl_3$) 2.53 (3 H, s) and 7.3 (5 H, m), in anhydrous ether (40 ml) was added to small amounts (*ca.* 0.1 g) of aluminium chloride and ice-cooled. To this solution, bromine (1.2 g) dissolved in ether (10 ml) was added dropwise with stirring and the resulting mixture was then refluxed for 3 h. After removal of the solvent, the residue was twice crystallized from benzene. The *product* (55%) had m.p. 141—142° (Found: C, 44.95; H, 3.15; N, 5.55. $C_9H_8BrNO_2$ requires C, 44.65; H, 3.30; N, 5.75%), δ ($CDCl_3$) 4.55 (2 H, s) and 7.3 (5 H, m).

Cetyldimethyl-(3-hydroxyimino-3-phenyl-2-oxopropyl)-ammonium Chloride (III).—Cetyldimethylamine (1.07 g) dissolved in ether (20 ml) was added to an ethereal (20 ml) solution of 3-bromo-1-hydroxyimino-1-phenylpropanone (0.48 g) during 4 h under stirring. After 1 day at room temperature, the precipitated waxy material was separated by centrifugation, washed with ether, and dissolved in methanol. The resulting solution was treated with dry Na_2CO_3 (2 g) and rapidly filtered. After removal of the solvent the residue was washed several times with light petroleum and then dissolved in methanol saturated with HCl. The solvent was evaporated and the residue was crystallized from dichloromethane–ether and from moderately warm water. The product (63%), dried under vacuum under P_2O_5, m.p. 142—144°, is probably contaminated by small amounts of inorganic salts (Found: C, 66.55; H, 9.8; N, 5.55; Cl, 7.65. Calc. for $C_{27}H_{47}ClN_2O_2$: C, 69.3; H, 10.1; N, 6.0; Cl, 7.55%), δ ($CDCl_3$) 0.9 (3 H, t), 1.2 (28 H, m), 3.4br (8 H, m), 5.5 (2 H, s), and 7.33 (5 H, m).

NN-Dimethyl-[2-(imidazol-4-ylcarbamoyl)ethyl]amine.—Imidazole-4-carbonyl chloride hydrochloride (0.83 g), prepared according to Lau and Gutsche,[23] was added in small portions over a period of 15 min to a solution of *NN*-dimethyl-(2-aminoethyl)amine (0.88 g) and triethylamine (1.6 g) in chloroform. The solution was then refluxed for 6 h, the solvent removed, and the oily residue dissolved in methanol. This solution was treated with Na_2CO_3 for 5 min, filtered, and the solvent and triethylamine were evaporated. The resulting viscous oil was used without further purification, δ (CD_3OD) 2.3 (6 H, s), 2.5 (2 H, t), 3.5 (2 H, m), and 7.4 (2 H, s).

Cetyldimethyl-[2-(imidazol-4-ylcarbamoyl)ethyl]ammonium Chloride Hydrochloride (V.HCl).—A solution of cetyl bromide (1.98 g) and *NN*-dimethyl-[2-(imidazol-4-ylcarbamoyl)ethyl]amine (1.1 g) in absolute ethanol was refluxed for 8 h. The resulting solution was then treated with HCl in methanol to convert the free imidazole to its hydrochloride. The solvent was removed and the residue was purified by chromatography on silica gel, using methanol as eluant. The product (15%), needles, had m.p. 186—189°; it is probably contaminated by small amounts of inorganic salts (Found: C, 58.75; H, 9.6; N, 11.20; Cl, 15.4. Calc. for $C_{24}H_{48}Cl_2N_4O$: C, 60.1; H, 10.1; N, 11.7; Cl, 14.8%), δ (CD_3OD) 0.9 (3 H, t), 1.27 (28 H, m), 3.2 (6 H, m), 3.7 (6 H, m), 8.23 (1 H, s), and 9.07 (1 H, s).

p$K_a$app. *Measurements*.—The degree of dissociation of surfactants (II), (III), and (VIII) was determined for solutions of co-micelles with [CTABr] : [D^f] (7—9) : 1 in Tris and borate buffers, by measuring the absorption at 300 nm, using the extinction coefficients of the acidic and anionic forms, and following literature methods.[5a,8f] The p$K_a$app. values are the pH values of half-neutralization.

Kinetic Measurements.—The general procedure has been described.[4b] The appearance of *p*-nitrophenol was monitored at 410 nm using a Gilford 2400 or Varian Cary 219, or, occasionally, a Durrum stopped-flow spectrophotomer. Stock solutions of α-chymotrypsin were prepared by dissolving the enzyme (*ca.* 2×10^{-4}M) in 1×10^{-3}N-HCl. The enzyme concentration was determined by literature methods [24] and from the ' burst ' size in the kinetic runs performed under conditions of excess ester. Under conditions [ester] ⩽ [enzyme], the apparent second-order rate constants were determined as described by Bender *et al.*[25]

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