The Micelle-catalysed Hydrolysis of Benzylpenicillin

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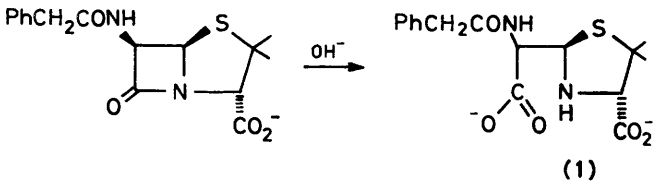
Micelles of cetyltrimethylammonium bromide catalyse the alkaline hydrolysis of benzylpenicillin with a rate enhancement of *ca.* 50-fold. However, the rate of reaction is inhibited by increasing concentrations of hydroxide ion and penicillin anion. A saturation phenomenon is observed with increased concentration of surfactant. Attempts are made to determine the binding- and rate-constants using existing kinetic models. These are not completely satisfactory and a model is proposed which assumes that both hydroxide ion and penicillin have to be bound to the micelle for reaction to occur. Bromide, chloride, acetate, fluoride, and benzylpenicilloate ions all inhibit the micellar catalysis.

NON-FUNCTIONALISED micelles which catalyse reactions provide a simple illustration of the utilisation of the binding energy between a phase or macromolecule and the reactants to lower the free energy of activation.[1-3] Even if the rate constant for a bimolecular reaction within the micelle is the same as that in the bulk solvent a rate enhancement may be observed if the reactants are confined to a smaller volume within the micelle.[1,4] This requires the binding energy of interaction between the reactants and the micelle to compensate for the loss of entropy resulting from the restriction of the reactants within the micelle. Micelles are of particular interest because they can provide different microenvironments for different parts of the reactant molecule. There is a nonpolar, hydrophobic core that can provide binding energy for similar groups on the reactant and a polar, usually charged, outer shell that can interact with the reactant's polar groups.

In general, experimental observations are rationalised in terms of electrostatic and hydrophobic interactions that occur between the substrate, micelle, and other solutes such as salts or nucleophiles.[3] Electrostatic factors do not always account for the different behaviour of structurally similar substances, and, as in the case of enzymic catalysis, differences are attributed to hydrophobic interactions and to the difference in the nature and extent of binding which occurs in the non-covalent intermediate.[3,5] However, based on electrostatic considerations it is possible to predict qualitatively the kinetic effects or reactions in micelles.[3,6,7] For example, hydrophobic substrates and counterions are attracted to the micelle so that a cationic micelle should assist the



(1)

reaction between a neutral molecule and anionic nucleophile while anionic micelles will inhibit such reactions. The present study concerns the micelle-catalysed reaction between two anions, the hydroxide-ion catalysed hydrolysis of the negatively charged benzylpenicillin [reaction (1)]. In the following paper is described the effect of making the penicillin molecule more hydrophobic.[8] The rate of the hydroxide-ion catalysed hydrolysis of benzylpenicillin decreases approximately three-fold in micellar solutions of itself.[9]

EXPERIMENTAL

Cetyltrimethylammonium bromide (Aldrich) was shaken with anhydrous ether, filtered off, and recrystallised from ethanol.

The rates of reaction were determined by following the change in absorbance on a Gilford 240 recording spectrophotometer having the cell compartment controlled at 30 ± 0.05 °C.† The rate constants were calculated using a generalised least-squares program which treated the first-order rate constant and the absorbances at both time zero and infinity as disposable parameters.

RESULTS AND DISCUSSION

Neither solutions of sodium dodecylsulphate or polyoxyethylene lauryl ether catalyse the hydroxide-ion catalysed hydrolysis of benzylpenicillin. The observation that anionic and neutral surfactants do not affect the rate of hydrolysis is not unexpected. Neutral micelles could bind benzylpenicillin but would have no affinity for hydroxide ion. Anionic micelles, simply based on electrostatic consideration, should repel the benzylpenicillin anion as well as the hydroxide ion, and hence have no effect on the observed rate.

Initial experiments in the presence of cetyltrimethylammonium bromide (CTABr) showed that the pseudo-first-order rate constant for hydrolysis increases rapidly with surfactant concentration once above the critical micelle concentration (c.m.c.) of the surfactant. The c.m.c. of CTABr was determined by the dye method [10,11] using bromophenol as the indicator and at 30 °C with 0.05M-NaOH was found to be 5×10^{-4}M. C.m.c. values have been shown to be fairly insensitive to small changes in sodium hydroxide concentration.[10-12] Increasing the surfactant concentration eventually leads to a slow decrease in the observed rate. Figure 1 shows

† °C = K − 273.15.

the change in observed rate with increasing surfactant concentration and the data are given in Table 1. This general shape of surfactant–rate profile has been found

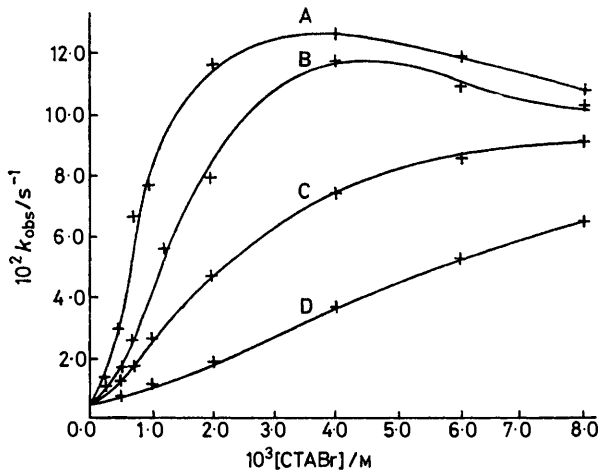


FIGURE 1 Observed pseudo-first-order rate constants for the hydrolysis of benzylpenicillin as a function of cetyltrimethylammonium bromide (CTABr) concentration and varying benzylpenicillin concentrations, at 30 °C. [Hydroxide ion] 0.05 M; A = 2×10^{-4}, B = 5×10^{-4}, C = 1×10^{-3}, and D = 2×10^{-3}M-benzylpenicillin. The lines are calculated, see text

TABLE 1

Pseudo-first-order rate constants ($s^{-1} \times 10^2$) for the hydroxide-ion catalysed hydrolysis of benzylpenicillin as a function of substrate and cetyltrimethylammonium bromide (CTABr) concentration at 30 °C with 0.05M-hydroxide ion

	[Benzylpenicillin]/M			
10^4[CTABr]/M	2×10^{-4}	5×10^{-4}	1×10^{-3}	2×10^{-3}
0.0	0.68	0.624	0.641	
2.5	1.36	0.93	0.73	0.69
5.0	2.94	1.64	1.15	0.87
7.5	6.64	2.49	1.67	0.97
10.0	7.71	4.26	2.06	
20.0	11.66	7.83	4.74	1.81
40.0	12.44	11.67	7.34	3.08
60.0	11.87	10.77	8.49	5.26
80.0	10.79	10.20	8.97	6.39

for many bimolecular reactions catalysed by cationic micelles.[5,13,14]

The observed pseudo-first-order rate constant, $k_{obs.}$, is not independent of penicillin concentration, unlike previous examples such as the base-catalysed hydrolysis of *p*-nitrophenyl esters.[15] Figure 1 shows the surfactant–rate profile for the hydroxide-ion catalysed reaction at different benzylpenicillin concentrations. The binding constant between the micelle and substrate is unlikely to change significantly with concentration yet the lower the concentration of benzylpenicillin the faster the rate increases and the greater the maximal rate obtained, the rate maximum shifting to a lower surfactant concentration. This observation could be explained if both hydroxide ion and benzylpenicillin compete for the same type of sites in the micelle and if both reactants are required to bind to the micelle. The competition between benzylpenicillin and hydroxide ion was also shown by carrying out the hydrolysis at constant hydroxide ion and surfactant concentration while increasing the substrate concentration (Table 2 and Figure 2). Unfortunately, using spectrophotometric methods it was not possible to reduce the benzylpenicillin concentration below 10^{-4}M.

Now if inhibition of the reaction occurs by increasing

TABLE 2

Observed pseudo-first-order rate constants for the hydrolysis of benzylpenicillin as a function of penicillin concentration at 30 °C

10^4 [Benzylpenicillin]/M	$k_{obs.}/s^{-1}$ [a]	$10^2 k_{obs.}/s^{-1}$ [b]
0.5	0.152	3.17
1.0	0.144	3.15
2.0	0.119	2.73
3.0	0.100	2.43
5.0	0.078	2.01
7.5	0.050	
15.0	0.036	
20.0	0.028	
40.0	0.015	

[a] 2×10^{-3}M-Cetyltrimethylammonium bromide and 0.10M-sodium hydroxide. [b] 4×10^{-3}M-Cetyltrimethylammonium bromide and 5×10^{-3}M-sodium hydroxide.

the benzylpenicillin concentration then increasing the hydroxide ion concentration may also inhibit the rate of the micellar-catalysed reaction while the rate in the bulk aqueous phase increases. Experimentally the observed pseudo-first-order rate constant does not increase linearly with increasing hydroxide ion concentration at constant surfactant concentration (Figure 3). It appears therefore, that at high hydroxide ion concentration, the hydroxide ion excludes the penicillin molecule from the surface of the micelle.

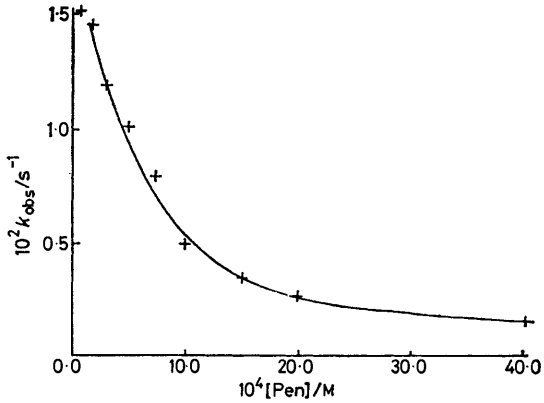


FIGURE 2 Observed pseudo-first-order rate constants for the hydrolysis of benzylpenicillin as a function of benzylpenicillin concentration at 30 °C with 2×10^{-3}M-cetyltrimethylammonium bromide and 0.1M-sodium hydroxide

The kinetic evidence implies that there must be some binding between the benzylpenicillin anion and the micelles of CTABr. However, no direct evidence of substrate binding to the micelle was found. Solubility

changes have been used to evaluate binding constants of water-insoluble compounds with detergent molecules.[16] Unfortunately the benzylpenicillin anion is readily water soluble and micelles have no significant effect on its

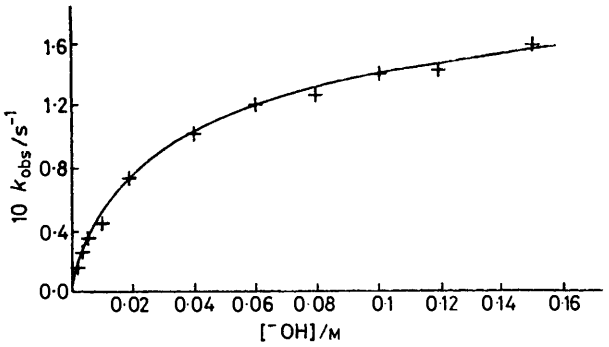
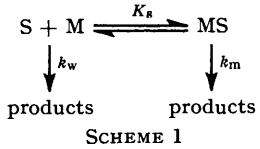


FIGURE 3 Observed pseudo-first-order rate constants for the hydrolysis of benzylpenicillin as a function of hydroxide ion concentration at 30 °C with 2×10^{-4}M-benzylpenicillin and 6×10^{-3}M-cetyltrimethylammonium bromide

solubility. U.v. measurements have also been used to determine binding constants[17] but spectroscopic measurement did not show a change in absorbance in the presence of surfactant. Therefore, the values of the binding constants were evaluated only from kinetic data and are subject to any approximation that is made in deriving the kinetic equations.

The rate increase observed for many reactions upon the addition of detergents above the c.m.c. has been explained on the basis of Scheme 1, attributed to Menger



SCHEME 1

and Portnoy.[5] The substrate, S, associates with the micelle, M, to form a substrate–micelle complex. The substrate and substrate–micelle complex break down with rate constants k_w and k_m, referring to bulk and micellar phase respectively. Although several kinetic equations based on this general Scheme have been developed,[4,13,18,19] the most successful appears to be that of Romsted[20] who suggested expression (1), which takes into account the effects of ions present in solution as well as substrate binding to the micelle. The major assumption in deriving equation (1) is that the total number of

$$k_{obs.} = \frac{k_m \beta S K_s (C_D - \text{c.m.c.})}{[K_s(C_D - \text{c.m.c.}) + 1][I_t + X_t K_i]} + \frac{k_w}{[K_s(C_D - \text{c.m.c.}) + 1]} \quad (1)$$

counterions bound to a micelle is constant which allows evaluation of the rate constant associated with the substrate–micelle complex. The exchange constant K_i refers to equilibrium (2) where I is the reactive, and X the unreactive counterion, and subscripts m and w define the micelle and bulk phases, respectively. The

$$I_m + X_w \rightleftharpoons I_w + X_m \quad (2)$$

constant β is the degree of binding of the counterions to the Stern layer (Romsted chose 0.8), S is the molar density of the micellar phase, and C_D is the surfactant concentration.

The Romsted equation has been shown to describe most features of micellar catalysis in a semi-quantitative way and it was hoped that this equation would allow the evaluation of rate and equilibrium constants in the alkaline hydrolysis of penicillins in the presence of micelles.

Accepting, for the moment, that the equation derived by Romsted gives a reasonable description of the micelle-catalysed reaction, then using equation (1) it should be possible to evaluate relative if not absolute binding constants between the substrate and micelle. The solid line shown on the surfactant–rate profile (Figure 4),

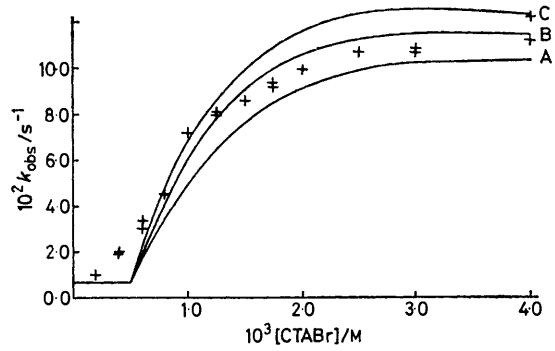


FIGURE 4 Observed pseudo-first-order rate constants (+) for the hydrolysis of benzylpenicillin as a function of cetyltrimethylammonium bromide concentration at 30 °C with 2×10^{-4}M-benzylpenicillin and 0.05M-sodium hydroxide. The lines are theoretical using equation (2) (see text): A, K_m 250 l mol^{-1}; B, K_m 300 l mol^{-1}; C, K_m 350 l mol^{-1}

has been generated by setting the rate constant in the micelle equal to that in the aqueous phase ($k_m = k_w$). For bimolecular reactions that have been shown to occur predominantly in the Stern layer the rate constant in the micelle phase is no faster than that in the aqueous phase. The binding constant between micelle and substrate, K_s, and the exchange constant between the active and unreactive anions, K_i, may be determined by treating K_s and K_i as disposable parameters. Again there is no direct method for measuring the hydroxide-ion concentration in the Stern layer, hence the need for treating K_i as a disposable parameter.[21] At this stage it was necessary to assume that hydroxide ion and/or bromide ion does not exclude the benzylpenicillin anion from the micelle. Using this procedure the binding constant, K_s, is estimated to be 3×10^2 l mol^{-1}, while the ion-exchange constant of bromide ion relative to hydroxide ion is 25 l mol^{-1}. Figure 4 plots the effect of changing the value of K_s, and shows the sensitivity of equation (1) to the value of K_s. It should also be remembered that it is

assumed that the total number of counterions bound to a micelle is constant and in this case β was assigned the value of 0.8. Therefore, the results actually generate values of βK_s.

It appears that, at this time, it is not possible to account quantitatively for the rate of anion–anion reactions occurring on or in the micellar phase. Equations developed by Berezin [19] and Romsted [20] have been based on the reaction rate *versus* surfactant concentration profile being independent of one of the reactants. This is not true for the bimolecular alkaline hydrolysis of benzylpenicillin in the presence of CTABr micelles.

The mechanism of alkaline hydrolysis of benzylpenicillin involves the rate-limiting formation of a tetrahedral intermediate.[22] It is possible that the environment within the micelle where reaction occurs may affect the energetics of bond making and breaking. The most likely place for the reaction between anions to occur is in the double layer which surrounds the micelle core, referred to as the Stern layer.[23]

^{1}H N.m.r. evidence suggests that molecules with polar characteristics, such as benzene and nitrobenzene, are solubilised on the micelle surface of cationic micelles while cyclohexane molecules are located within the micelle core.[24] The benzylpenicillin anion will probably be located in the Stern layer. There is evidence to suggest that water penetration does occur into the micelle core,[24,25] which may in turn create regions within the micelle core capable of solvating the anionic reactants. If the reactant molecules are located in the micelle core it makes interpretation of the salt effect difficult to explain.

Catalysis by micelles of the hydroxide-ion catalysed hydrolysis of substrates appears to be qualitatively understood on the basis of a concentration effect of reactant on, or around, the micelle surface and need not necessarily involve a difference in the free energies of activation in the micelle and bulk phases. That is not to say that the cationic micelles could not and do not cause electrostatic stabilisation of the transition state. The cationic micelle surface can act as an electrostatic sink for the anionic intermediate leading to its stabilisation but a rate enhancement requires preferential stabilisation of this intermediate compared with the reactant. But the small rate enhancement, *ca.* 46-fold, is equally well explained by considering that the increased concentration of reactants at the micelle surface leads to a higher observed rate. Incorporation of the reactants into a limited volume decreases the entropy loss that is associated with bringing reactants together in the transition state, leading to an increase in the pseudo-first-order rate constants in the presence of surfactant micelles.[1,26,27]

The Inhibition of Micelle Catalysis by Electrolytes.— Figure 5 shows the effect of increasing anion concentration on the rate of alkaline hydrolysis of benzylpenicillin at constant surfactant and hydroxide ion concentration. All the anions studied bring about a substantial inhibition of the CTABr-catalysed reaction. The changes in electrolyte concentration used have little effect upon the rate of alkaline hydrolysis in aqueous solution.[28] The salt effect can be considered to be due to a competitive binding of the anions present with the micelle. Initially the only unreactive anion present is bromide ion present as the micelle counterion and the ratio of unreactive anion to hydroxide ion is small and a large rate enhancement is observed. However, increasing the unreactive anion concentration increases the

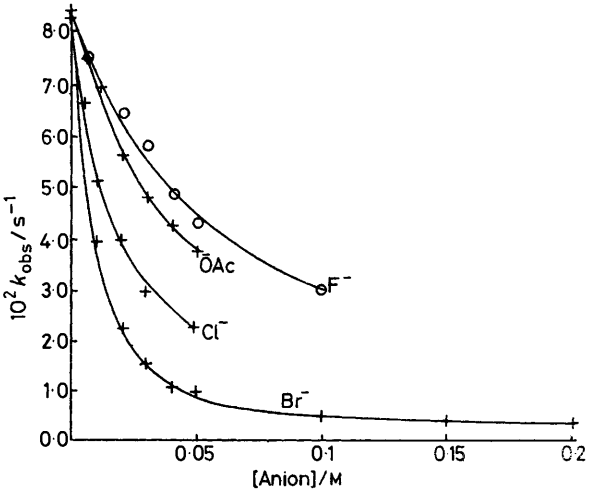


FIGURE 5 Observed pseudo-first-order rate constants for the cetyltrimethylammonium bromide-(4×10^{-3}M)-catalysed hydrolysis of benzylpenicillin (2×10^{-4}M) with 0.025M-hydroxide ion at 30 °C as a function of salt concentration. The lines are calculated, see text

ratio and decreases the amount of bound hydroxide ion, leading to a reduction in the observed rate.

The addition of unreactive anions to solutions of cationic detergents, in general, decreases the c.m.c.,[29,30] increases the micellar size,[30] and affects the amount of residual change in the micelle surface.[31,32] It is possible that these factors do lead to inhibition but the most likely reason is the replacement of reactive hydroxide ion by the non-reactive anion.

If the assumption is made that the inhibition is competitive but only between the added anion and hydroxide ion then the equation derived by Romsted can be applied to the kinetic data. From the previous section the value of the association constant, K_s, between benzylpenicillin and micelle has been estimated to be 300 l mol^{-1} and the exchange constant, K_i, for bromide relative to hydroxide ion was 25 l mol^{-1}. Using these values for K_s and K_i and the concentration of bromide added one can use the Romsted equation to calculate the expected results as a fraction of anion concentration. The calculated results are compared with the experimental results in Table 3. The results of this calculation are important because not only can two independent sets of experiments be explained reasonably well, *i.e.* (a) the effect of increasing surfactant concentration at constant reactant concentration and (b) the effect of increasing the non-reactive counterion concentration on

TABLE 3

Pseudo-first-order rate constants for the hydroxide-ion catalysed hydrolysis of benzylpenicillin as a function of bromide ion concentration at 30 °C with 0.025M-sodium hydroxide and 4×10^{-3}M-cetyltrimethylammonium bromide

$[Br^-]$/M	$10^2 k_{obs.}/s^{-1}$	$10^2 k_{calc.}/s^{-1}$
0.0	8.50	
0.01	3.91	3.45
0.02	2.20	1.88
0.03	1.47	1.35
0.04	1.02	1.10
0.05	0.758	0.93
0.10	0.45	0.58
0.15	0.436	0.45
0.20	0.409	0.38
0.25	0.343	0.35

the observed rate at constant surfactant and reactant concentration, but also because the values of K_s and K_i obtained in experiment (a) and the initial assumption that $k_m = k_w$ appear reasonable. It was therefore decided to use Romsted's equation to determine the relative exchange constants for the series of anions studied. An iterative procedure was adopted to evaluate K_i, using K_i as a disposable parameter, until a reasonable fit with the experimental data was obtained. The calculated values for the ion-exchange constant K_i and the relative values K_{X^-}/K_{F^-} are shown in Table 4.

TABLE 4

Relationship between-anion, the ion-exchange constant K_i and the ion-exchange ratio K_{X^-}/K_{F^-}

		K_{X^-}/K_{F^-}		
Anion	K_i/l mol^{-1}	*a*	*b*	*c*
Bromide	25.0 ± 5.0	11.3	14.5	18.5
Chloride	7.4 ± 0.8	3.3	7.6	5.9
Acetate	3.1 ± 0.8	1.3		
Fluoride	2.5 ± 0.6	1.0	1.0	1.0

[a] Hydroxide-ion catalysed hydrolysis of benzylpenicillin in the presence of CTABr. [b] Addition of cyanide ion to n-dodecyl-3-carbamoylpyridinium bromide: J. Baumrucker, M. Calzadilla, M. Centeno, G. Lehrmann, M. Urdaneta, P. Lindquist, D. Dunham, M. Price, B. Sears, and G. C. Cordes, *J. Am. Chem. Soc.*, 1972, **94**, 8164. [c] Base-catalysed hydrolysis of *p*-nitrophenyl hexanoate.[32]

The exchange constant $K_i = 25 \pm 5.0$ l mol^{-1} for bromide ion relative to hydroxide ion is also supported by the hydroxide-ion catalysed hydrolysis of *p*-nitrophenyl acetate in aqueous cetyltrimethylammonium bromide where the results were rationalised in terms of bromide ion being bound 40 ± 10 times more strongly than hydroxide ion to the micelle.[33] Incidentally, for this ester hydrolysis a value of 6.5 l mol^{-1} s^{-1} was calculated for the second-order rate constant in the micelle phase compared with a value of 10.9 l mol^{-1} s^{-1} in aqueous sodium hydroxide solution. This supports the suggestion of Romsted and others that rate constants for bimolecular reactions are generally slower in the presence of micelles than in aqueous solution. Also the binding constant of chloride ion to CTABr has been estimated to be 7 l mol^{-1} from the hydrolysis of 2,6-dinitrophenyl phosphate in the presence of CTABr,[34] in reasonable agreement with the value of 7.4 ± 0.8 l mol^{-1} determined in this study.

From Table 4 and other results it appears that the relative degree of anion inhibition is quite general; the larger the anion the lower is the charge density possessed by the anion and the larger the inhibition. The inhibition by bromide ion is of interest because initially the micelle-catalysed reaction was studied in solutions of high ionic strength, the ionic strength being adjusted by the addition of KBr, and no catalysis was observed. It was not until the ionic strength control was removed that any effect by CTABr was observed. Presumably, micelles of cetyltrimethylammonium hydroxide would result in a large rate enhancement. Possibly a little surprising is the rather low K_i value of 3.1 ± 0.8 l mol^{-1} for acetate ion which is a more hydrophobic anion than the other anions and yet it binds less tightly to the micelle. However, the hydrolysis reaction is inhibited by the addition of the hydrolysis product, benzylpenicilloate, which contains two carboxylate groups (Table 5). The exchange constant is estimated

TABLE 5

Inhibition of the hydroxide-ion (2.5×10^{-2}M) catalysed hydrolysis of benzylpenicillin (2×10^{-4}M) in the presence of CTABr (4×10^{-3}M) by the addition of benzylpencilloate

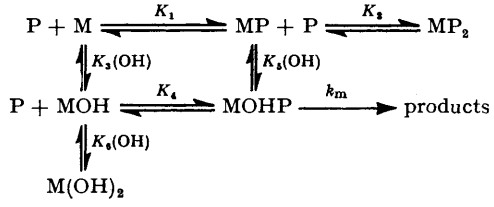
10^4 [Benzylpenicilloate]/M	10^2 $k_{obs.}/s^{-1}$
0.0	8.91
1.0	8.24
2.0	7.80
3.0	6.44
4.0	6.26
5.0	5.51
10.0	3.25
15.0	2.17
20.0	1.30

to be *ca.* 250 l mol^{-1} and thus benzylpenicilloate appears to bind no more tightly to the micelle than does benzylpenicillin itself. In benzylpenicilloate there are two carboxylate anions yet the inhibition which results from increasing its concentration is similar to that caused by increasing the benzylpenicillin concentration. Therefore carboxylate anions are rather ineffective anions in terms of binding with the CTABr micelle relative to simple inorganic anions.

A cautionary note should be added here. Although it has been inferred that the added anion prevents the incorporation of hydroxide ion into the cationic micelle, it is possible that the added anion significantly affects the interaction between the micelle and benzylpenicillin. No independent measurement of the binding constant for benzylpenicillin to CTABr was obtained; hence, limiting the calculation to include only the exchange constant K_i between added anion and hydroxide ion is an over-simplification of the system.

Although Romsted's equation (1) has been used to evaluate exchange constants for non-reacting anions relative to hydroxide ion and to explain the inhibition by these anions it cannot explain either the inhibition by

hydroxide ion or by penicillin. For a bimolecular reaction catalysis is due to the concentration of *both* reactants in the micellar phase. The hydrophilic ion concentration in the micelle is estimated by considering an apparent ion-exchange process between reactive and unreactive counterions in the Stern layer [reaction (2)]. However, Romsted's treatment of a bimolecular reaction between an anion and a neutral molecule assumes that binding of the two reactants is independent of each other. This does not allow for competition between *reactants* for micelle binding when both reactants are anions. Attempts were therefore made to quantitatively describe this situation and Scheme 2 is postulated to



SCHEME 2

explain the experimental results. P, M, and OH refer to the penicillin anion, micelle, and hydroxide ion respectively, while MOH, $M(OH)_2$, MP, MP_2, and MOHP refer to binary or ternary complexes. Only MOHP, the ternary complex between micelle and the two reactant molecules, leads to product. No account is taken of the micelle counterion hence the situation is even more complicated than that shown in Scheme 2, because bromide ion will exchange or replace hydroxide ion and probably the benzylpenicillin anion. Scheme 2 does not include product interaction with the micelle, *i.e.* the equilibrium that controls the dissociation of the product–micelle complex. In Scheme 2, $K_1K_5 = K_3K_4$ and if it is assumed that binding of one reactant molecule has no effect on the binding of a second molecule of the *same* reactant, then $K_1 = K_2$, $K_3 = K_6$, and $K_4 = K_5$.

The overall rate (v) can be represented by equation (2). From Scheme 2, the concentration of MOHP is

$$v = k_m[\text{MOHP}] + k_{OH}[P][OH] \qquad (2)$$

given by equation (3), and substitution in equation (2) gives (4). Limiting cases of equation (4) can explain the

$$[\text{MOHP}] = \frac{([P]_{TOT} - [P]_{aq.})[OH]}{[OH] + \frac{1}{K_2} + \frac{K_1[P]_{aq.}}{K_2}} \qquad (3)$$

$$\text{Rate} = \frac{k_m([P]_{TOT} - [P]_{aq.})[OH]}{\frac{1}{K_2} + \frac{K_1[P]_{aq.} + [OH]}{K_2}} + k_{OH}[P]_{aq.}[OH]_{aq.} \qquad (4)$$

kinetic behaviour with respect to hydroxide ion and penicillin concentrations but Scheme 2 does not take into account competitive binding between the two reactant anions and the surfactant counterions. Hence, the above equation for the rate cannot account for the decrease in observed first-order rate constant with increasing surfactant concentration and is of limited value.

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