Kinetics and Mechanisms of Hydrolysis of Dicarboximide Fungicides in Micellar Media†

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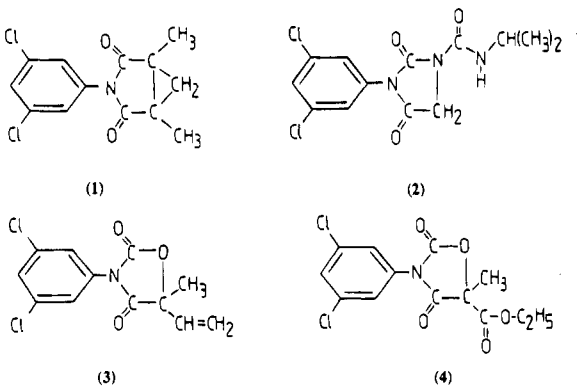
The kinetics of alkaline hydrolysis of the dicarboximide fungicides procymidone, iprodione, vinclozolin, and chlozolinate (**1**–**4**, respectively) were investigated in micellar solutions containing various amounts of either sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), or three nonionic surfactants (two C_{13} alcohols and a copra amine combined with ethoxyl chains) and compared with the kinetics in aqueous media. For all compounds, the rate constants observed are slightly reduced by the SDS micellar media, showing that reactions essentially take place in the aqueous pseudophase. The CTAB micellar media speed up the hydrolysis rates with small quantities of Br^- ions in the medium. As the number of Br^- ions increases, the rate of reactions falls. This is characteristic of an ion exchange (OH^- and Br^-) at the surface of the CTAB micelles. Finally, the presence of nonionic micelles has little influence on the hydrolysis of the fungicides: the reduction in the rate of dicarboximide ring opening is attributed to micelle–substrate association. These results can be explained by means of the pseudophase kinetic model coupled with the mechanisms of hydrolysis of these fungicides in water solution.

Keywords: *Dicarboximide fungicide; surfactant; mechanisms of hydrolysis*

Procymidone (**1**), *N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide, iprodione (**2**), 3-(3,5-dichlorophenyl)-*N*-isopropylimidazolidine-2,4-dione-1-carboximide, vinclozolin (**3**), (*R,S*)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione, and chlozolinate (**4**), ethyl (*R,S*)-3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxo-1,3-oxazolidine-5-carboxylate (Chart 1) constitute the group of dicarboximide fungicides. They are essentially systemic (**1**) and contact (**2**–**4**) fungicides acting on spores and mycelium which show both preventive and curative activity (Ambrus et al., 1991). They are effective against a number of phytopathogenic fungi, especially *Botrytis* and *Sclerotinia* (Clark, 1983; Cabras et al., 1984; Belafdal et al., 1986). *Botrytis cinerea* is one of the most important fungal diseases in viticulture: its growth causes serious production losses and adversely affects wine quality.

The poor solubility of many pesticides in water means that they are often used in micellar solutions. It is thus important to determine the effects of such media on their kinetics of hydrolysis. We report here the results obtained in anionic, cationic, and nonionic surfactants with the four fungicides of varying hydrophobicity in which the mechanisms of hydrolysis in aqueous media were determined in previous studies (Belafdal et al., 1986; Villedieu et al., 1994). Under alkaline aqueous conditions, the fungicides undergo an attack by the hydroxide ion on a specific carbonyl group and the rate of hydrolysis increases proportionally to the hydroxide ion concentration (Scheme 1). Procymidone and iprodione lead quantitatively and irreversibly to 2-[(3,5-dichlorophenyl)carbamoyl]-1,2-dimethylcyclopropanecarboxylate (**5**) and isopropylcarbamoyl-3-(3,5-dichlorophenyl)-5-hydantoate (**6**) (Chart 2). The reactions are not subject to general base catalysis, and experimental data are in agreement with a rate-determining attack by the hydroxide ion. After a rapid hydrolytic loss of the ethoxycarbonyl substituent for chlozolinate, the dicarboximide ring cleavage of the two other fungicides leads by different mechanisms, with respect to the type of base catalysis and the rate-determining step, to the corresponding anilides, producing as intermediates the carbamic acids, which undergo loss of carbon dioxide. These anilides are, respectively, for the hydrolysis of vinclozolin and chlozolinate, 3,5-dichlorophenyl-2-hydroxy-2-methylbut-3-enanilide (**7**) and 3,5-dichlorophenyl-2-hydroxypropanilide (**8**).

Chart 1. Procymidone (1), Iprodione (2), Vinclozolin (3), and Chlozolinate (4)



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† This paper formed part of a thesis by J.C.V.

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0021-8561/95/1443-1948$09.00/0

Scheme 1. Mechanism of Dicarboximide Ring Opening in Aqueous Medium: R = 3,5-Dichlorophenyl (X = C for Compounds 1 and 2; X = O for Compounds 3 and 4; Y = C for Compounds 1, 3, and 4; Y = N for Compound 2)

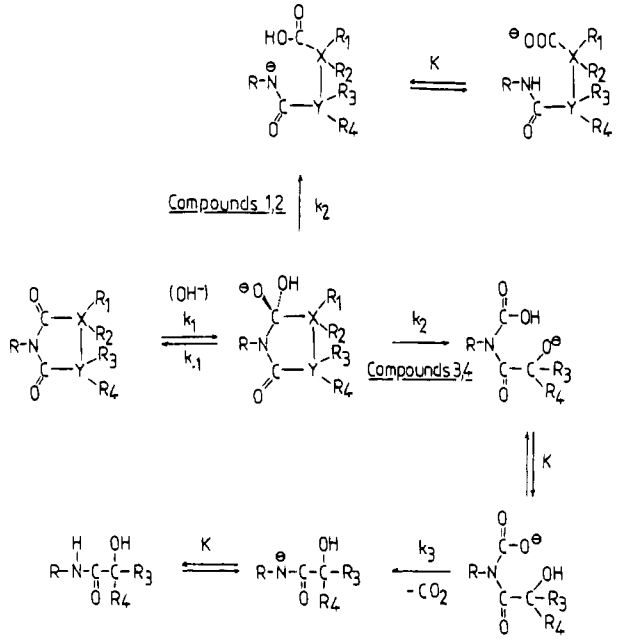
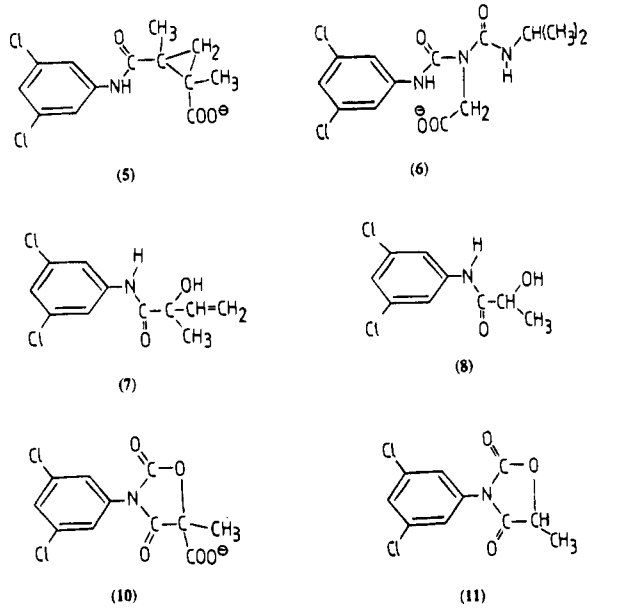


Chart 2. Structures of Products of Degradation of Dicarboximide Fungicides



EXPERIMENTAL PROCEDURES

Chemicals. The dicarboximide fungicides were obtained by methods previously described (Belafdal et al., 1986; Villedieu et al., 1994).

Sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB) used as anionic and cationic surfactants were of analytical reagent grade. The three nonionic surfactants were kindly supplied by A. Chamel and J. Coret. There are two C_{13} alcohols each combined with an ethoxyl chain (AA6OE and AA10OE) and a copra amine combined with two ethoxyl chains (AmC10OE). The used products were characterized by their average number of ethoxylation indicated by numerals. Buffer components were from analytical grade material, and the aqueous solutions were prepared by using deionized water, which was then distilled twice over potassium permanganate and sodium hydroxide. The ionic strength (μ) was maintained at 1.0 M throughout using potassium chloride.

Apparatus and Kinetic Methods. The time course of the hydrolysis reaction was followed spectrophotometrically by recording the changes in optical density corresponding to the appearance of products. This was recorded at fixed wavelengths of 248 nm for acids **5** and **6** and 242 nm for anilides **7** and **8** in a Cary 210 UV spectrophotometer equipped with a thermostated sample holder (±0.1 °C). The substrates were made up as concentrated solutions (usually 4.0 mM for compounds **1**, **2**, and **4**, and 6.7 mM for compound **3**) in ethanol, and the reaction was initiated by addition of 30 μL of one of these solutions to a 1 cm path length cell containing the alkaline or neutral reaction solution (3 cm^3). Reactions were followed for at least 10 half-lives when possible. The dicarboximide ring opening reaction of the fungicides is first order with respect to the substrate. The observed rate constants (k_{obs}) were calculated from linear plots of $\log(D_\infty - D_t)$ vs time, where D_t and D_∞ represent, respectively, the absorption at time t and the final absorption. The rate constants were obtained by at least-squares fit with a correlation coefficient of 0.99 or higher.

The study of the hydrolysis of procymidone and iprodione by UV spectrophotometry shows an increase with time of the optical density at 248 nm up to a maximum, leading to observed rate constants k^{p}_{obs} and k^{i}_{obs}, respectively. For vinclozolin, the maximum optical density is obtained at 242 nm, leading to the observed rate constant k^{v}_{obs}. The observed step of the hydrolysis of vinclozolin corresponds to the dicarboximide ring opening leading to *N*-(3,5-dichlorophenyl)-*N*-(2-hydroxy-2-methylbut-3-enoyl)carbamic acid (**9**). The decarboxylation of this carbamic acid cannot be observed in UV spectrophotometry. Hydrolysis of chlozolinate in aqueous phosphate solution evolves according to three steps with different half-lives in UV spectrophotometry: the first step consists of an increase of the optical density at 237 nm up to a maximum corresponding to the hydrolysis of the ethyl ester of the ethoxycarbonyl group leading to 3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxooxazolidine-5-carboxylate (**10**) (k^{c}_{1obs}); the second step consists of a decrease of the optical density, finding expression in the decarboxylation of **10** leading to 3-(3,5-dichlorophenyl)-5-methyloxazolidine-2,4-dione (**11**) (k^{c}_{2obs}); finally, the last step consists of an increase of the optical density at 242 nm of large amplitude up to a maximum corresponding to the dicarboximide ring opening of the dione **11** (k^{c}_{3obs}). Except for k^{c}_{2obs}, which is independent of pH in the range examined, the two other observed rate constants of hydrolysis increase proportionally to the hydroxide ion concentration.

The pH measurements were carried out by using a Radiometer PHM 64 pH meter equipped with a Radiometer GK 2401 B electrode. The critical micellar concentrations (cmc) were determined from surface tension (obtained by means of a Prolabo K8 apparatus) vs concentration plots. In water at 25 °C in the presence of sodium phosphate (10^{-2} M) and borate (10^{-2} M), the average cmc values of SDS, CTAB, AA6OE, AA10OE, and AmC10OE are 5.5×10^{-4}, 2.8×10^{-4}, 7.8×10^{-5}, 9.6×10^{-5} and 6.9×10^{-5} M, respectively.

RESULTS AND DISCUSSION

For compounds **1**–**4**, the changes in k_{obs} at 25 °C as a function of micellized surfactant concentration [D_n] are plotted in Figure 1. [D_n] is generally assumed to be given by [D_n] = [D_T] − cmc, [D_T] being the total concentration of surfactant in the medium and the concentration of monomeric surfactant given by the cmc.

The results can be explained by means of the pseudophase kinetic model proposed by Menger and Portnoy (1967) and developed by Bunton (1979), Romsted (1982) and Vera and Rodenas (1986a,b). In developing a quantitative model for the kinetic results, we make certain key assumptions: (i) that reaction occurs independently in the micellar and aqueous pseudophases and that rate constants can be estimated for these reactions; and (ii) that the distribution of counterions between aqueous and micellar pseudophases is governed by an ion-exchange equilibrium (Romsted, 1982).

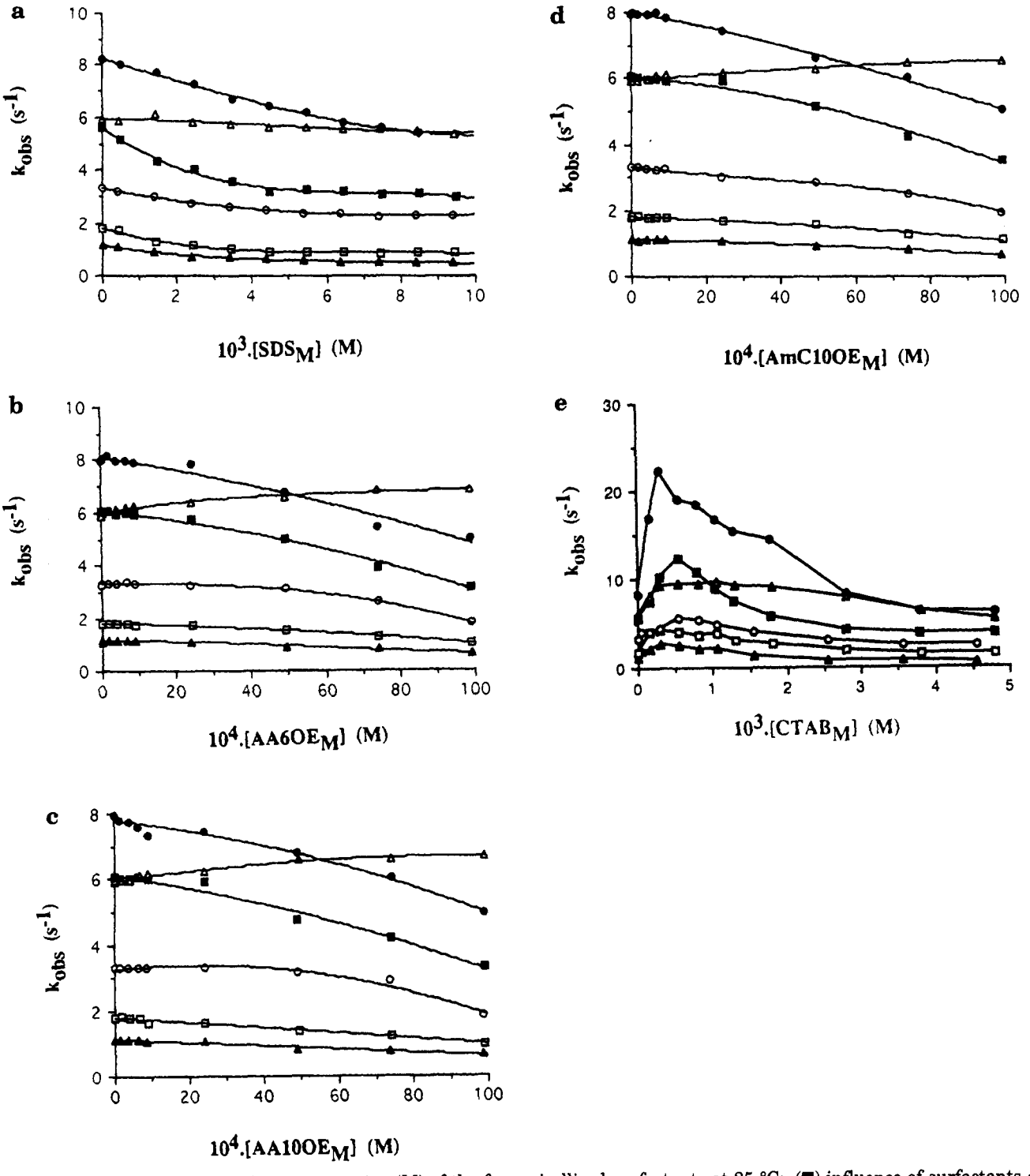
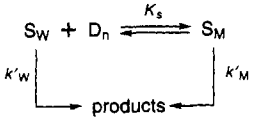


Figure 1. Plot of k_{obs} (s^{-1}) vs the concentration (M) of the four micellized surfactants at 25 °C: (■) influence of surfactants on compound **1** in phosphate (10^{-2} M, pH 11.02), $k^{P}_{obs} \times 10^3$; (▲) influence of surfactants on compound **2** in borate (10^{-2} M, pH 9.08), $k^{i}_{obs} \times 10^3$; (●), influence of surfactants on compound **3** in phosphate (10^{-2} M, pH 10.50), $k^{v}_{obs} \times 10^3$; (□, △) influence of surfactants on the first and second steps of hydrolysis of compound **4** in phosphate (10^{-2} M, pH 7.07), $k^{c}_{1obs} \times 10^2$ and $k^{c}_{2obs} \times 10^4$; (○) influence of surfactants on the third step of hydrolysis of compound **4** in borate (10^{-2} M, pH 11.02), $k^{c}_{3obs} \times 10^3$. (a) $k_{obs} = f$[SDS_M]; (b) $k_{obs} = f$[$AA6OE_M$]; (c) $k_{obs} = f$[$AA10OE_M$]; (d) $k_{obs} = f$[$AmC10OE_M$]; (e) $k_{obs} = f$[$CTAB_M$].

The pseudophase model for bimolecular reactions of substrates in aqueous micelles is shown in the scheme



where k'_W and k'_M, the first-order rate constants in the aqueous and micellar pseudophases, are given by (Bunton et al., 1980)

$$k'_W = k_W[OH^-_W] \quad (1)$$

$$k'_M = k_M m_{OH} = k_M[OH^-_M]/[D_n] \quad (2)$$

The binding constant K_S is given by $K_S = [S_M]/[S_W][D_n]$, where S_W and S_M represent, respectively, the

substrate in aqueous and in micellar pseudophase. In eq 2 k_M is written in terms of the mole ratio, m_{OH}, of micellar bound OH^- to micellar head groups. The concentrations $[OH^-_W]$ and $[OH^-_M]$ are molarities expressed in terms of total solution volume, so that $[OH^-_T]$, the total concentration of OH^-, is given by

$$[OH^-_T] = [OH^-_W] + m_{OH}[D_n]$$

The rate law is expressed as

$$v = k_{obs}[S_T] = k[OH^-_T][S_T] = k'_W[S_W] + k'_M[S_M]$$

with $[S_T] = [S_W] + [S_M]$. The first-order rate constant k_{obs} is given by

$$k_{obs} = \frac{k'_W + k'_M K_S[D_n]}{1 + K_S[D_n]} \quad (3)$$

For a pseudo-first-order reaction in which OH^- acts as nucleophile, the relation 3 can be expressed by

$$k_{obs} = \frac{k_W[OH^-_W] + k_M K_S m_{OH}[D_n]}{1 + K_S[D_n]} \quad (4)$$

$$k_{obs} = \frac{k_W[OH^-_W] + k_M K_S[OH^-_M]}{1 + K_S[D_n]} \quad (5)$$

Equations 4 and 5 take into account the distribution of both the substrate and the hydroxide ion between the aqueous and micellar pseudophase (Bunton et al., 1979). It is convenient to write eq 4 in terms of $[OH^-_T]$, giving

$$k_{obs} = \frac{k_W[OH^-_T] + (k_M K_S - k_W)m_{OH}[D_n]}{1 + K_S[D_n]} \quad (6)$$

Moreover, when the OH^- and Br^- ions compete for access to the charged micellar surface, Romsted (1984) assumes that the interaction of two counterions with an ionic micelle is governed by an ion-exchange equilibrium. This approach explains qualitatively a number of micellar effects upon reaction rates and equilibria and leads to equations that describe the competition between counterions for an ionic micelle. This approach is illustrated by relation 7, for micellar binding of OH^- to the cationic micelle having Br^- as counterion.

$$OH^-_M + Br^-_W \overset{K_{Br}^{OH}}{\rightleftharpoons} OH^-_W + Br^-_M \quad (7)$$

The ion-exchange constant K_{Br}^{OH} is given by

$$K_{Br}^{OH} = \frac{[OH^-_W][Br^-_M]}{[OH^-_M][Br^-_W]} \quad (8)$$

Considering that the β ratio, which corresponds to the ratio of ionic headgroups of the micelle neutralized by counterions, is constant ($\beta = 0.8$ for a halogenated counterion; Romsted, 1982), m_{OH} is expressed vs K_{Br}^{OH} from

$$m^2_{OH} + m_{OH}\left[\frac{[OH^-_T] + K_{Br}^{OH}[Br^-_T]}{(K_{Br}^{OH} - 1)[D_n]} - \beta\right] - \frac{\beta[OH^-_T]}{(K_{Br}^{OH} - 1)[D_n]} = 0 \quad (9)$$

The experimental results can be fitted to eq 3 or 6, and k'_W, k'_M, and K_S are determined by simulation technique. Table 1 shows the values of k'_W, k'_M, K_S, and $[S_M]/[S_T]$ for the four fungicides.

For all compounds, the presence of SDS micelles has little influence. The shape of curves of Figure 1a and the value of k'_W (compared to k_{obs} in H_2O) show that the reactions essentially take place in aqueous pseudophase. The reduction in k_{obs} is attributed to micelle–substrate association. For compounds **1** and **2**, such associations inhibit the dicarboximide ring opening reaction between the substrate and OH^- because of the electrostatic repulsion between the hydroxide ions and the negatively charged micelles ($R-OSO_3^-$). Compound **3** seems to have a particular behavior owing to its weak hydrophobicity. Indeed, it is 220 times more soluble in water than procymidone, 75 times more soluble than iprodione, and 30 times more soluble than chlozolinate. The inhibition of dicarboximide ring opening of compound **3** by SDS micelles is, respectively, 1.5 and 1.2 times weaker than inhibition of the hydrolysis of compounds **1** and **2**. In accordance with its poor hydrophobic character, little or no vinclozolin enters the micelle and consequently remains vulnerable to an attack by hydroxide ions that involve a weak change in the observed rate constant. The ethyl ester of chlozolinate is relatively well protected by SDS micelles against hydroxide ions. On the other hand, the rate of decarboxylation of the carboxylate **10**, resulting in the hydrolysis of the ethyl ester group, weakly changes between aqueous and micellar media. Indeed, the acid **10** under its ionized form enters with difficulty the anionic micelle, owing to the electrostatic repulsion with ionic heads of the negatively charged micelles. The carboxylate remains in the aqueous pseudophase, and consequently, the observed rate constant weakly changes in the presence of surfactants. Finally, the dicarboximide ring opening of the dione **11** is inhibited in the presence of SDS.

Parts b–d of Figure 1 show for compounds **1–3** a progressive fall in reaction rate in the presence of nonionic micelles, indicating a significant association between the substrates and the micelles hindering the dicarboximide ring opening reaction. This association is logically more weak concerning vinclozolin owing to its poor hydrophobic character. The hydrolysis of chlozolinate reveals a peculiar behavior. The loss of the ethyl ester group and the dicarboximide ring opening of the dione **11** are inhibited in the presence of nonionic micelles. On the other hand, the decarboxylation of the carboxylate **10** is weakly accelerated. Contrary to anionic micelles, the ionic heads of which are opposed to the carboxylate–micelle association, the nonionic micelles offer no resistance to the substrate incorporation. The effect of this incorporation inside the nonionic micelles is to decrease the solvation of the carboxylate and to make the dispersion of the negative charges easy. In aqueous media, the decarboxylation step implements a cyclic mechanism involving a molecule of water as showed in Chart 3a (Villedieu et al., 1994). In micellar media, this mechanism cannot occur because of the carboxylate–micelle association. There is every reason

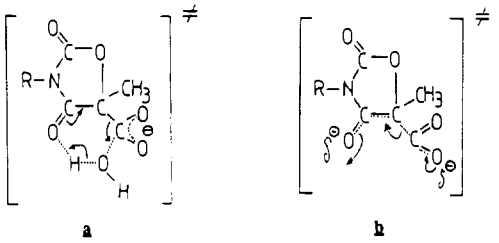
Table 1. Values of k'_W, k'_M, K_S, and $[S_M]/[S_T]$[a] for Compounds 1–4 in the Presence of Different Surfactants

substrate	H_2O 10^3k_{obs}[b] (s^{-1})	SDS $10^3k'_W$ (s^{-1})	SDS $10^{-2}K_S$ (M^{-1})	SDS $[S_M]/[S_T]$	CTAB $10^3k'_M$ (s^{-1})	CTAB $10^{-2}K_S$ (M^{-1})	CTAB $[S_M]/[S_T]$
1	5.62	2.98	1.69	0.62	0.44	1.10	0.34
2	1.15	0.49	1.89	0.64	0.06	1.13	0.34
3	8.20	5.32	0.70	0.40	3.15	0.41	0.16
4	18.00	0.86	2.10	0.66	4.30	1.28	0.38

substrate	H_2O 10^3k_{obs}[b] (s^{-1})	AA6OE $10^{-2}K_S$ (M^{-1})	AA6OE $[S_M]/[S_T]$	AA10OE $10^{-2}K_S$ (M^{-1})	AA10OE $[S_M]/[S_T]$	Amc10OE $10^{-2}K_S$ (M^{-1})	Amc10OE $[S_M]/[S_T]$
1	6.05	1.18	0.54	1.03	0.50	0.94	0.48
2	1.12	0.92	0.48	0.91	0.47	0.90	0.47
3	7.95	0.80	0.44	0.72	0.42	0.68	0.40
4	18.05	0.92	0.48	0.93	0.48	0.79	0.48

[a] $[S_M]$ corresponds to the maximum of micellized surfactant concentration in the plot $k_{obs} = f([D_n])$. [b] k_{obs} is the rate constant in H_2O medium.

Chart 3. Decarboxylation of the Carboxylate (a) in Aqueous Media and (b) in Micellar Media (R = 3,5-Dichlorophenyl)



a b

to suppose that a spontaneous decarboxylation takes place in the presence of micelles (Chart 3b). The shapes of the curves of degradation of the fungicides are very similar for the three nonionic micelles. There is no significant difference for the K_S and $[S_M]/[S_T]$ values between the surfactants.

The curves of Figure 1e are characteristic of an ion exchange between Br^- and OH^- at the surface of the CTAB micelles. With small quantities of Br^- ions in the medium, relative concentrations of fungicide and hydroxide ion in the Stern layer of micelles of CTAB rapidly increase with the surfactant concentration, leading to an acceleration of the reaction of hydrolysis. This effect of linking explains the rising part of the curve. Moreover, the micellar pseudophase constitutes a favorable medium because of its weak polarity (Bunton, 1976), which contributes to the stabilization of the activated complex with delocalized charge and a reduction of solvation of OH^- when this last leaves the aqueous pseudophase for the micellar pseudophase (Broxton and Chung, 1986). When the fungicide is totally included in the micellar pseudophase, an increase in the concentration of CTAB leads, on the one hand, to a decrease of local concentration of reactants by dilution effect and, on the other hand, to an increase of the concentration of nonreactive counterions Br^- that displace hydroxide ions bound to micelles. In effect, counterions Br^- more easily join with cetyltrimethylammonium cations than OH^- (Rodenas and Vera, 1985), so as the number of Br^- ions increases, competition between Br^- and OH^- for access to the positive charges on the micelle is in favor of Br^- and the rate of reaction falls. The reaction takes place essentially in the aqueous pseudophase (level part of the curves). The calculated exchange constant $K_{Br}^{OH} = 0.050$ is indicative of a weak association between OH^- and the micelles. The value is close to that found by Bunton (1979). The K_S and $[S_M]/[S_T]$ values obtained are in good agreement with the poor hydrophobic character of compound **3** as compared to that of other compounds.

In conclusion, these results show that it is possible to increase or reduce the rate of degradation of substrates as fungicides in micellar media by changing the type of surfactant and varying its concentration. Electrostatic interactions are responsible for repulsion and attraction phenomena between substrates, reactants, and micelles and, consequently, for inhibitory and catalyst effects. The substrates dissolved in micellar solutions are distributed in the aqueous and micellar pseudophases: there is compartmentalization of reactants or products. This compartmentalization can be used positively to locally change concentrations, to hinder the approach of an unwanted reactant, or to favor separations of charges. The stabilization of reactants, intermediates, or products leads to modifications of rate and mechanism. These modifications depend on reaction molecularity: monomolecular reactions have their rates changed by medium effect, while bimolecular reaction rates change by medium and concentration effects.

ACKNOWLEDGMENT

We thank André Chamel and Joël Coret of Centre d'Etudes Nucléaires de Grenoble for the gift of the three nonionic surfactants (AA6OE, AA10OE, and AmC10OE).

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Received for review July 14, 1994. Accepted May 10, 1995.[⊗]

JF9403898

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1995.