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Mechanisms of Dicarboximide Ring Opening in Aqueous Media: Procymidone, Vinclozolin and Chlozolinate*

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Abstract: The hydrolysis kinetics of the dicarboximide fungicides procymidone, vinclozolin and chlozolinate in neutral and alkaline solutions of pH 6·0 to 13·7 at 25°C have been determined conjointly by ultraviolet spectrophotometry and by high performance liquid chromatography. Under alkaline conditions, the fungicides undergo attack by the hydroxide ion on a specific carbonyl group and the rate of hydrolysis increases proportionally to the hydroxide ion concentration. Procymidone gives quantitatively and irreversibly 2-(3,5-dichlorophenylcarbamoyl)-1,2-dimethylcyclopropanecarboxylate. The reaction is 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 from chlozolinate, the dicarboximide ring cleavage of the two other fungicides leads, by mechanisms which differ 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. The hydrolysis of vinclozolin and chlozolinate yields, respectively, *N*-(3,5-dichlorophenyl)-2-hydroxy-2-methylbut-3-enanilide and *N*-(3,5-dichlorophenyl)-2-hydroxypropanilide.

1 INTRODUCTION

Procymidone (**1**), *N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide, vinclozolin (**2**), (*R,S*)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione and chlozolinate (**3**), ethyl (±)-3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxo-1,3-oxazolidine-5-carboxylate (Fig. 1) are essentially systemic (**1**) and contact (**2** and **3**) fungicides acting on spores and mycelium which show both preventive and curative activity.[1,2] They are effective against a number of phytopathogenic fungi, especially *Botrytis* and *Sclerotinia* sp.[3–7] *Botrytis cinerea* Pers. ex Fr. is one of the most important fungal diseases in viticulture: its growth causes serious production losses and adversely affects wine quality. Along with iprodione, these fungicides constitute the group of dicarboximides which have been widely employed during the last few years, partly owing to the fact that *Botrytis* spp. display resistance to systemic fungicides containing the benzimidazole nucleus.[8–11] However, their use is decreasing owing to the problems of increasing fungal resistance and to the presence of undesirable residues in the final product. A number of reports have been published on the degradation or the breakdown products of these fungicides, particularly in plants,[2] in soils,[12,13] in wine[4,5] and in tap water.[6]

Because no study of their mechanism of cleavage in aqueous media appears to have been reported in the literature, and in order to understand better their

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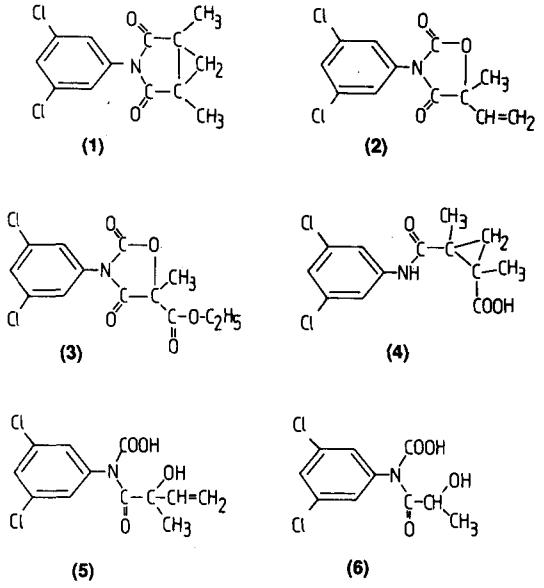

Fig. 1. Procymidone (**1**), vinclozolin (**2**), chlozolinate (**3**), 2-(3,5-dichlorophenylcarbamoyl)-1,2-dimethylcyclopropanecarboxylic acid (**4**), *N*-(3,5-dichlorophenyl)-*N*-(2-hydroxy-2-methylbut-3-enoyl)carbamic acid (**5**) and *N*-(3,5-dichlorophenyl)-*N*-(2-hydroxypropanoyl)carbamic acid (**6**).

behaviour in the environment a kinetic study of their mechanism of hydrolysis has been undertaken.

2 EXPERIMENTAL

2.1 Materials

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.

A Durrum stopped-flow spectrophotometer (Model D-110) coupled to an OS4000 Gould oscilloscope for very fast reactions ($t_{1/2}$ less than 20 s) and a Varian Cary 210 spectrophotometer, both equipped with thermostated cell compartments, were used for spectroscopic measurements. Hydrolysis products of fungicides were analyzed quantitatively and qualitatively by a high performance liquid chromatography (HPLC) system (Gilson Model 305), equipped with an ultraviolet (UV) detector and a reverse-phase column (Hamilton PRP1) that was eluted with mobile phases of acetonitrile + deionized water (70 + 30 by volume) or acetonitrile + phosphoric acid, 5 mM (65 + 35 by volume; pH = 2·72). The flow rate was 1 cm^3 min^{-1} and detection was made at 210 and 245 nm. The substrate was made up as fungicide in aqueous buffer solutions: borate (5 mM, pH = 9·08), phosphate (5 mM, pH = 6·88). The pH measurements were carried out by using a Radiometer PHM 64 pH-meter equipped with a Radiometer GK 2401 B electrode. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker 80 MHz apparatus.

2.2 Substrates

2.2.1 Extraction of procymidone and vinclozolin

Procymidone 500 g kg^{-1} wettable powder ('Sumilex', Sumitomo Chemical Co.) and vinclozolin 500 g kg^{-1} wettable powder ('Ronilan'; BASF AG) were treated respectively with dichloromethane and acetone, refluxed for 24 h and the insoluble material filtered off. The filtrates were washed with saturated aqueous sodium chloride, the organic phases separated, dried and the solvent removed under vacuum. Hexane was added to the remaining oily products and the precipitates formed were recrystallized from hexane to give respectively white crystals of procymidone, mp 166°C (literature,[14] 166°C); NMR (hexadeuteroacetone): δ 7·40 (m, 3H, aromatic), 1·52 (s, 6H, CH_3), 1·30 (m, 2H, CH_2), and white crystals of vinclozolin, mp 108°C (literature,[6] 108°C); NMR (hexadeutero acetone): δ 7·60 (m, 3H, aromatic), 6·05 (m, 1H, vinylic CH), 5·60 (m, 2H, vinylic CH_2), 1·81 (s, 3H, CH_3).

2.2.2 Preparation of 2-(3,5-dichlorophenylcarbamoyl)-1,2-dimethylcyclopropanecarboxylic acid (4)

This was obtained from procymidone according to the method of Belafdal *et al.*[3] for the preparation of the hydantoic acid from iprodione. Procymidone (1 g) was dissolved in aqueous sodium hydroxide (1 M; 100 cm^3) at room temperature, the mixture washed twice with ether, and the aqueous phase recovered. Ice-cold concentrated hydrochloric acid (5 M; 20 cm^3) was added with stirring and the precipitate collected by filtration. The crude product was washed with cold water and recrystallized from light petroleum distillate + diethyl ether (7 + 3 by volume) to give the corresponding acid (**4**) (Fig. 1), mp 185°C, λ_{max} (ethanol) 248 nm, NMR (hexadeuterodimethyl sulfoxide)): δ 10·80 (br, 1H, COOH), 8·50 (br, 1H, NH), 7·40 (m, 3H, aromatic), 1·60 (s, 6H, CH_3), 1·20 (m, 2H, CH_2).

2.2.3 Preparation of N-(3,5-dichlorophenyl)-N-(2-hydroxy-2-methylbut-3-enoyl)carbamic acid (5) and N-(3,5-dichlorophenyl)-N-(2-hydroxypropanoyl)-carbamic acid (6)

These intermediates were obtained respectively from vinclozolin and chlozolinate (supplied by Farmoplant S.A.) by employing the method of Clark.[6] Vinclozolin (1 g) and chlozolinate (0·3 g) in a mixture of acetone and water (4 + 1, by volume; respectively 25 cm^3 and 10 cm^3), containing sodium hydroxide, were stirred in an ice bath (2 h at 0°C), the solutions acidified with concentrated hydrochloric acid (one drop at a time) to pH 1 and the resulting solid precipitates removed and recrystallized twice from chloroform to give the corresponding carbamic acids: (**5**), mp 139°C (literature,[6] 139°C), λ_{max} (ethanol) 245 nm, NMR (hexadeuterodimethyl sulfoxide): δ 10·30 (br, 1H, COOH), 7·50 (m, 3H, aromatic), 6·15 (m, 1H, vinylic CH), 5·50 (m, 2H, vinylic CH_2), 2·42 (s, 1H, OH),

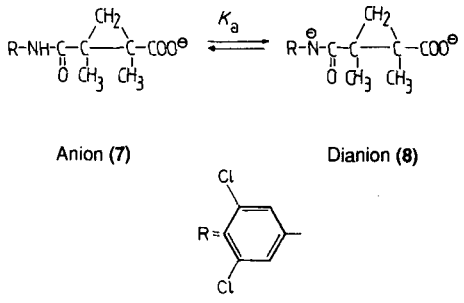

Fig. 2. Ionization of the NH group of the final product of alkaline hydrolysis of procymidone.

1·71 (s, 3H, CH_3); (**6**), mp 169°C, λ_{max} (ethanol) 245 nm, NMR (hexadeuterodimethyl sulfoxide): δ 10·50 (br, 1H, COOH), 7·40 (m, 3H, aromatic), 4·10 (m, 1H, CH), 2·72 (s, 1H, OH), 1·17 (s, 3H, CH_3). The spectral and analytical data were in agreement with literature values.

2.3 Kinetics

The changes in concentration were followed spectrophotometrically by recording the changes in optical density at 248 nm corresponding to the appearance of acid (**4**) and at 242 nm corresponding to the appearance of carbamic acids (**5**, **6**) and anilides. The substrates were made up as concentrated solutions (usually 4·0 mM for procymidone and chlozolinate, and 6·7 mM for vinclozolin) 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). The ionic strength (μ) was maintained at 1·0 M throughout by using potassium chloride. Reactions were followed for at least ten half-lives where possible. Pseudo first-order rate constants (k_{obs}) were calculated from linear plots of log ($D_{00} - D_t$) *versus* time, where D_t and D_{00} represent respectively the absorption at time t and the final absorption, or by the Guggenheim method,[15] when the reaction time is very important.

2.4 pK_a measurement: NH group ionization

The UV spectra of 2-(3,5-dichlorophenylcarbamoyl)-1,2-dimethylcyclopropanecarboxylate (**7**) in sodium hydroxide solutions, obtained *in situ* by alkaline hydrolysis of procymidone, show a bathochromic shift from 248 nm to 275 nm with the increase in concentration of the hydroxide ion. This is consistent with the formation of an anion with a negative charge conjugated with the aromatic nucleus (Fig. 2). As the completely inonized form could not be reached for this substrate, the data obtained from spectroscopic measurements were treated by the method developed by Maroni and Calmon[16] and latter adopted in the revised edition of Albert and Serjeant.[17] The pK_a was obtained from the intercept with the x-axis of the graphs of $1(D_{AH} - D)$ *versus* $1/[OH^-]$ at four wavelengths (245 nm: $y = 4{\cdot}37 + 15{\cdot}62 . x$, $r = 0{\cdot}998$; 248 nm: $y = 5{\cdot}78 + 21{\cdot}81 . x$, $r = 0{\cdot}988$; 251 nm: $y = 7{\cdot}37 + 34{\cdot}28 . x$, $r = 0{\cdot}992$; 254 nm: $y = 90{\cdot}76 + 61{\cdot}42 . x$, $r = 0{\cdot}996$), where D_{AH} and D are respectively the optical densities of the final product of hydrolysis of procymidone (**7**) in a 0·01 M borate solution (pH = 9·05, μ = 1·0 M) and of the mixture of the anion (**7**) and the dianion (**8**) in sodium hydroxide solutions with concentrations ranging from 1 M to 8 M (μ = 1·0 M).

2.5 Thermodynamic parameters of activation

The energy of activation E_a was calculated from the slope of the plots of ($\log k/T$) *versus* ($1/T$). The entropy of activation $\Delta S^{\#}$ was obtained from the equation

$$\mathrm{Ln}\,(k/T) = \mathrm{Ln}\,(K_B/h) - (E_a/(RT)) + (\Delta S^{\#}/R) + 1$$

where K_B and h are the Boltzmann and Planck constants and R the gas constant.[18]

3 RESULTS AND DISCUSSION

3.1 Procymidone

3.1.1 Characterization of the reaction products

The hydrolysis products of procymidone were identified by comparing their UV spectra and high performance liquid chromatograms with those produced by a sample of acid **4**. In aqueous borate solution (pH = 9·08), the UV spectra showed an absorption maximum at 248 nm, and a bathochromic shift of 27 nm with an increase of pH. The retention time (t_R) of the hydrolysis product was 1·90 min; it was identical to that obtained from a sample of the acid, **4**, in the same conditions. These results show that under the experimental conditions employed, in alkaline solutions of pH 8·05 to 13·70, procymidone yields quantitatively and irreversibly the corresponding acid **4**. The study of the hydrolysis of procymidone in a 5 mM borate buffer (pH = 9·05) by HPLC (Fig. 3(a)) revealed a decrease of the concentration of procymidone (t_R = 9·60 min) up to complete disappearance in about 25 h and an increase in inverse ratio of the concentration of the acid **4**. With acetonitrile + phosphoric acid as mobile phase instead of acetonitrile + water, the signal at 1·90 min disappeared and the chromatograms showed a new signal at 3·05 min. The latter corresponded to the protonated form of the carboxylic acid function of **4**, and the signal at 1·90 min to the carboxylate **7**. Finally, there was no trace of 3,5-dichloroaniline by HPLC.

3.1.2 pH and buffer effects

The study of the hydrolysis of procymidone by UV spectrophotometry showed an increase with time of the optical density at 248 nm up to a maximum, leading to an observed rate constant. The dicarboximide ring opening reaction of procymidone was first-order with

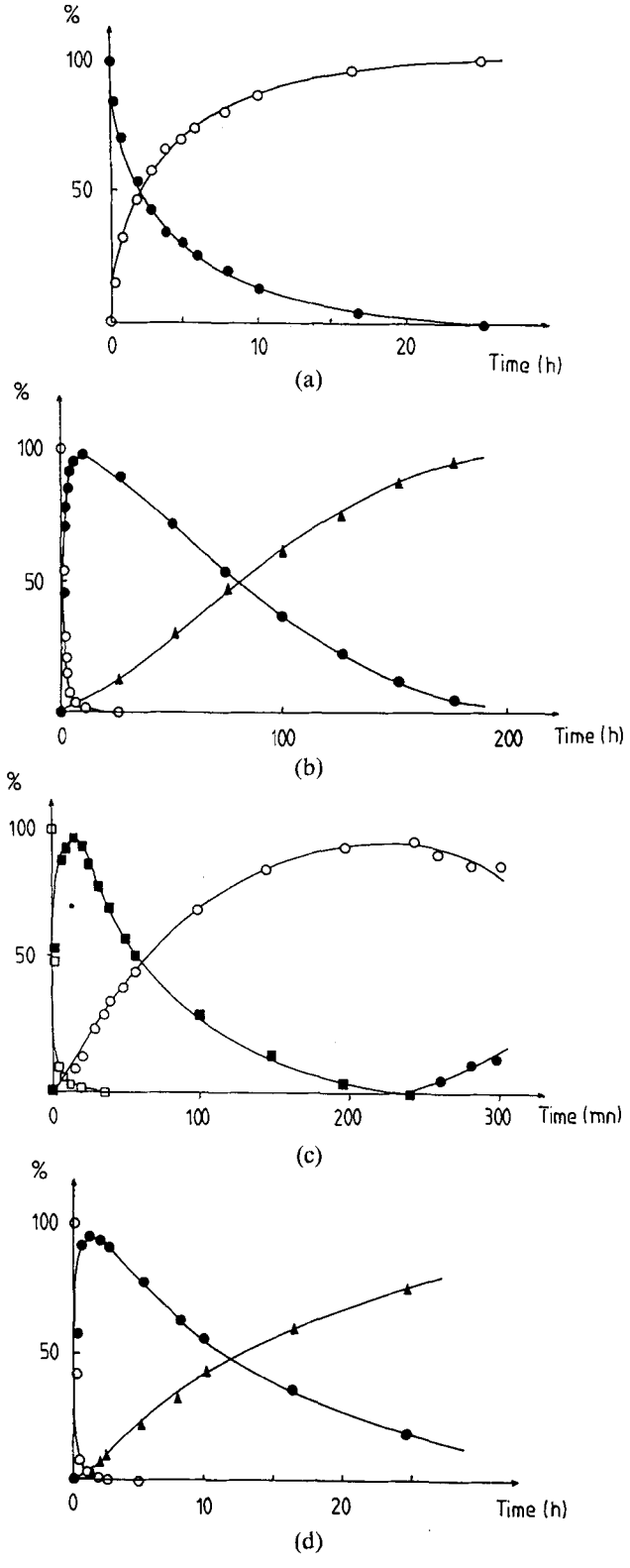

Fig. 3. Percentage-time profiles for hydrolysis of: (a) Procymidone in 5 mM borate (pH 9·05); (●) procymidone (**1**); (○) acid (**4**). (b) Vinclozolin in 5 mM borate (pH 9·05); (○) vinclozolin (**2**); (●) carbamic acid (**5**); (▲) anilide (**11**). (c) Chlozolinate in 5 mM phosphate (pH 6·88); (□) chlozolinate (**3**); (■) carboxylate (**15**); (○) oxazolidine-2,4-dione (**16**); (●) carbamic acid (**6**). (d) Chlozolinate in 5 mM borate (pH 9·05); (○) oxazolidine-2,4-dione (**16**); (●) carbamic acid (**6**); (▲) anilide (**17**).

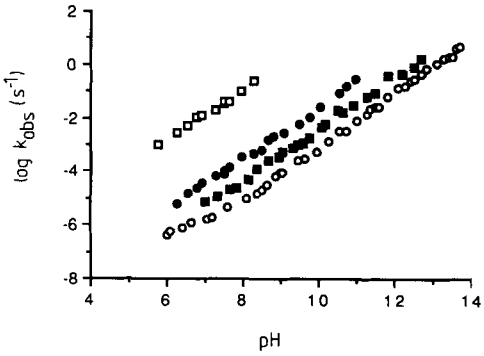

Fig. 4. Plots of log k_{obs} (s^{-1}) against pH for the hydrolysis of procymidone (○, k^{p}_{obs}), vinclozolin (■, k^{v}_{obs}) and chlozolinate (□, $k^{c}_{1\,obs}$; ●, $k^{c}_{3\,obs}$) in water at 25°C (μ = 1·0 M, KCl).

respect to the substrate. The pseudo first-order rate constants k^{p}_{obs} were measured at 25°C in sodium hydroxide solutions for pH values greater than 11·50 and in buffer solutions (disodium hydrogen phosphate, sodium borate, potassium dihydrogen phosphate) for pH values between 6·07 and 11·50. The graph of the variation of logarithm of the rate constants k^{p}_{obs} as a function of pH (Fig. 4) had a slope close to unity ($\log k^{p}_{obs} = -13{\cdot}26 + 1{\cdot}02$ pH; $r = 0{\cdot}999$) for pH values ranging from 8·05 to 13·70 and this slope decreased for pH values smaller than 8·05. According to the results of Cabras *et al.*[4] on the hydrolysis of procymidone in wine (at pH 3 and 4), a horizontal plateau occurs at acidic pH. The present results show that, under alkaline conditions, the rate of hydrolysis increases proportionally with the hydroxide ion concentration. The half-lives of the reaction at 25°C were 19 h at pH = 8·12 and 21 min at pH = 10·08. At higher pH values, procymidone became very unstable ($t_{1/2}$ = 1 s at pH = 12·80).

The hydrolysis of procymidone is not subject to a general base catalysis since its rate is independent of the concentration of basic species of buffer at constant pH (Table 1).

3.1.3 Temperature and solvent deuterium isotope effects

The influence of temperature on the rate of hydrolysis of procymidone has been examined in buffers (borate, phosphate) and sodium hydroxide solutions for pH values ranging from 9·08 to 11·50. The values of the bimolecular rate constants k_{OH} measured for three different temperatures are shown in Table 2. The activation entropy of the reaction is calculated from these data to be $-81\,(\pm 24)$ J mol^{-1} K^{-1}. The value of the solvent deuterium isotope effect in 5 mM sodium hydroxide and deuteroxide solutions is 0·82 at 25°C.

3.1.4 Ionization of the NH group of the acid 4

The product of alkaline hydrolysis of procymidone has two acidic sites. The pK_a value of the carboxylic group lies between 3 and 4 for 2-aryl and 2-alkyl hydantoic acids.[19] To estimate the pK_a of the amide group which

TABLE 1
Observed Rate Constants of the Hydrolysis of Procymidone and Vinclozolin in Buffers at 25°C (μ = 1·0 M, KCl)

Buffer	*pH*						
Borate	9·05	$[B_t]^a$ (M)	0·05	0·04	0·03	0·02	0·01
		$10^5 . k^p_{obs}$ (s^{-1})	9·6	9·3	9·5	9·4	9·2
		$10^4 . k^v_{obs}$ (s^{-1})	5·6	6·4	6·5	5·7	5·4
Carbonate	9·55	$[B_t]^a$ (M)	0·25	0·20	0·15	0·10	
		$10^4 . k^p_{obs}$ (s^{-1})	4·7	5·2	4·8	4·6	
		$10^3 . k^v_{obs}$ (s^{-1})	2·6	3·1	2·6	2·0	
Phosphate	11·00	$[B_t]^a$ (M)	0·025	0·020	0·015	0·010	0·005
		$10^3 . k^p_{obs}$ (s^{-1})	7·9	7·8	7·9	6·7	7·1
		$10^3 . k^v_{obs}$ (s^{-1})	8·5	7·2	5·9	6·0	6·1

[a] $[B_t]$ = Total buffer concentration: $[B_t] = [BH^+] + [B]$.

TABLE 2
Temperature Effect on Rates of Hydrolysis of Procymidone, Vinclozolin and Chlozolinate in Water

Fungicide	*Rate constants* k_{OH} ($dm^3\ mol^{-1}\ s^{-1}$); k_{obs} (s^{-1})				E_a ($kJ\ mol^{-1}$)
Procymidone	T (°C)	25	35	45	
	k^p_{OH}	5·0	9·1	18·2	47·2
Vinclozolin	T (°C)	25	35	45	
	k^v_{OH}	33·9	94·2	223·4	68·8
Chlozolinate	T (°C)	15	25	35	
	$k^c_{1\,OH} . 10^{-4}$	5·4	7·2	10·2	21·5
	$k^c_{2\,obs} . 10^{-4}$	3·2	6·4	17·0	60·5
	$k^c_{3\,OH}$	123·0	160·3	215·2	18·5

leaves the dicarboximide ring during the hydrolysis of procymidone, the second ionization constant for the acid **4** was determined. The pK_a value of the NH group carrying the 3,5-dichlorophenyl substituent is 14·75 at 25°C.

3.1.5 Mechanism of hydrolysis

The hydrolysis of procymidone is first-order with respect to OH^-, and this together with the absence of general catalysis suggests the reaction mechanism shown in Fig. 5. Owing to the fact that the molecule of procymidone is symmetrical, the hydroxide ion can attack either carbonyl group of the dicarboximide ring with the same probability. Moreover, the anion **10** produced by the decomposition of the tetrahedral intermediate **9** is stabilized by resonance of the negative charge carried by the nitrogen atom with the aromatic nucleus. It is the bathochromic shift with the increase in concentration of hydroxide ion that suggests the ionization of the acid **4**. The rate equation is

$$k_{obs} = (k_1 k_2/(k_{-1} + k_2)) . [OH^-] = k_{OH}[OH^-]$$

and is in good agreement with the experimental results.

The rate-determining step can be either the formation (k_1) of the tetrahedral intermediate, **9**, or its decomposition (k_2), respectively, leading to the equations

$$k_{obs} = k_1[OH^-]$$

and

$$k_{obs} = (k_1 k_2/k_{-1}) . [OH^-].$$

However, the activation entropy of $-81\ (\pm 24)$ J mol^{-1} K^{-1} and the value of 0·82 for the solvent isotope effect favour the addition of the hydroxide ion as the rate-determining step. These values are close to those measured for reactions showing a rate-determining attack of the hydroxide ion on a carbonyl group. For the basic hydrolysis of esters,[20] the entropy of activation lies between -41 and -147 J mol^{-1} K^{-1}. Our value is also comparable to those obtained from the hydantoin ring-opening of 3-aryl hydantoins,[21] which lie between -41 and -83 J mol^{-1} K^{-1}, and from the alkaline hydrolysis of iprodione[3] (-77 J mol^{-1} K^{-1}).

The ratio $k_{obs}(H_2O)/k_{obs}(D_2O) = k_{OH}/k_{OD}$ indicates

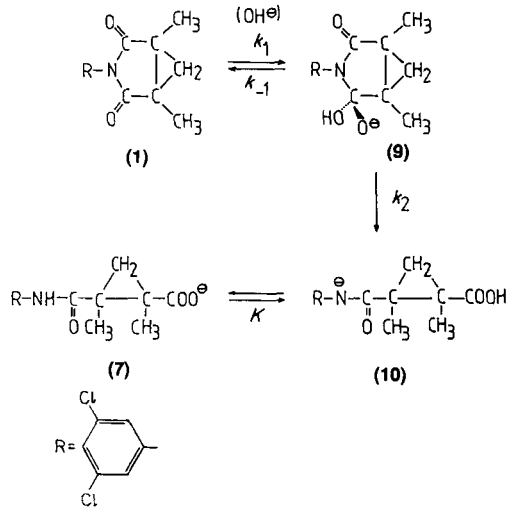

Fig. 5. Mechanism of alkaline hydrolysis of procymidone.

that the reaction is accelerated in heavy water. This has been observed for the hydantoin ring-opening reaction of 3-aryl hydantoins[21] or iprodione.[3] This could be explained by a greater rate of attack by OD^- than by OH^- owing to the greater nucleophilicity of OD^- compared to OH^-.[22] In addition, the value of 14·75 for the pK_a of the leaving group is in good agreement with the proposed reaction mechanism. Thus, according to Bender,[23] the rate-determining step for the basic hydrolysis mechanism of amides or esters is the addition of the hydroxide ion when the pK_a value of the leaving group is less than or equal to that of water (pK_a = 15·70), as has been observed for 3-aryl hydantoins.[21]

3.2 Vinclozolin

3.2.1 Characterization of the reaction products

The hydrolysis products were identified by HPLC and UV spectrophotometry. The HPLC study of the hydrolysis of vinclozolin (t_R = 10·79 min) in aqueous borate solution (5 mM, pH = 9·08) (Fig. 3(b)) revealed a decrease of the concentration of the fungicide up to complete disappearance in about 20 h and an increase in inverse ratio of the concentration of a compound with a retention time of 1·45 min, identical to that obtained from a sample of carbamic acid **5**. Moreover, with the HPLC system, the UV spectrum of a compound appearing on the chromatogram at a particular time could be determined. The UV spectrum of the compound with the retention time of 1·45 min displayed λ_{max} at 245 nm identical to that obtained from a sample of carbamic acid **5** in aqueous borate solution. With acetonitrile + phosphoric acid as mobile phase instead of acetonitrile + water, a new signal at 3·92 min took the place of the signal at 1·45 min: it corresponded to the protonated form of the carboxylic acid function of **5**. HPLC results showed that, after 10 h, the concentration of **5** began to decrease while a new product (t_R = 6·31 min) appeared, increasing in inverse ratio to **5**, corresponding probably to 3,5-dichlorophenyl-2-hydroxy-2-methylbut-3-enanilide (**11**), the final product of the hydrolysis of vinclozolin under the above conditions.

3.2.2 pH and buffer effects

The study of the hydrolysis of vinclozolin by UV spectrophotometry showed an increase with time of the optical density at 242 nm up to a maximum, leading to an observed rate constant. The dicarboximide ring-opening reaction was first-order with respect to the substrate. The pseudo first-order rate constants k^v_{obs} were measured at 25°C in sodium hydroxide solutions for pH values higher than 11·50 and in buffer solutions (as in Section 3.1.2) for pH values between 6·85 and 11·50. The graph of the variation of the logarithm of the rate constants k^v_{obs} as a function of pH (Fig. 4) had a slope close to unity ($\log k^v_{obs} = -12{\cdot}27 + 0{\cdot}98$ pH; r = 0·999) for pH values ranging from 7·95 to 13·10 and this slope decreased at pH values smaller than 7·95. The results showed that, under alkaline conditions, the rate of hydrolysis increased proportionally to the hydroxide ion concentration. The half-lives of the reaction at 25°C were 3·5 h at pH = 8·32 and 5 min at pH = 9·82. At higher pH values, vinclozolin became very unstable ($t_{1/2}$ = 2 s at pH = 12·08).

The hydrolysis of vinclozolin is not subject to a general base catalysis since k^v_{obs} is independent of concentration of basic species of buffer at constant pH (Table 1).

3.2.3 Temperature and solvent deuterium isotope effects

The influence of temperature on the rate of hydrolysis of vinclozolin has been examined in buffers (phosphate and borate) and sodium hydroxide solutions for pH values ranging from 9·08 to 11·50. The values of the bimolecular rate constant k_{OH} measured for three different temperatures are shown in Table 2. The activation entropy of the reaction was calculated from these data to be +7 (±2) J mol^{-1} K^{-1}. The value of the solvent deuterium isotope effect in 5 mM sodium hydroxide and deuteroxide solutions was 0·94 at 25°C.

3.2.4 Mechanism of hydrolysis

Comparison of the hydrolysis rate constants obtained on the one hand by HPLC and on the other hand by UV spectrophotometry (Table 3) allowed the identification of the reaction corresponding to the rate constant measured spectrophotometrically. Thus the hydrolysis of vinclozolin corresponds to the dicarboximide ring opening leading to the carbamic acid **5**. The decarboxylation of this carbamic acid is not observed by UV spectrophotometry, according to certain results in the literature.[21,24] Johnson and Morisson[25] have studied the kinetics of decarboxylation of the *N*-aryl carbamates and have found general acid catalysis.

The hydrolysis of vinclozolin is first-order with respect to OH^-, and this, together with the absence of general

TABLE 3
Comparison of UV and HPLC Observed Rate Constants of Hydrolysis of Vinclozolin, Chlozolinate and Their Derivatives

Compounds	k_{obs} *UV* 10^4 (s^{-1})	k_{obs} *HPLC* 10^4 (s^{-1})
Vinclozolin[a]	5·60	4·93
Chlozolinate[b]	91	57
Carboxylate (**15**)[b]	5·20	4·10
Oxazolidine-2,4-dione (**16**)[a]	28	23

[a] In 5 mM borate (pH = 9·05).
[b] In 5 mM phosphate (pH = 6·88) at 25°C.

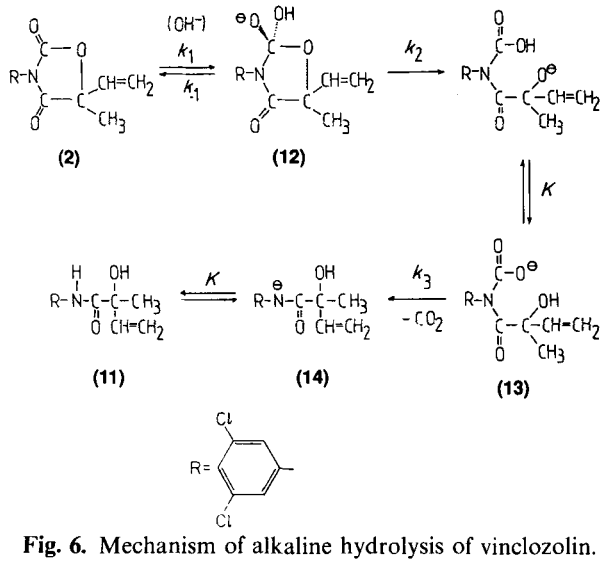

Fig. 6. Mechanism of alkaline hydrolysis of vinclozolin.

catalysis, suggests the reaction mechanism shown in Fig. 6. The hydroxide ion attacks the carbonyl group near the oxygen heteroatom, probably owing to the fact that it has greater electrophilic character and less steric constraint than the other carbonyl group near the methyl and vinyl groups. The carbamate **13**, produced by decomposition of the tetrahedral intermediate **12**, corresponds, in the pH range studied, to the ionized form of the carbamic acid **5**. The rate equation is identical to that of the hydrolysis of procymidone and is in good agreement with the experimental results.

The value of the activation entropy of +7 (±2) J mol^{-1} K^{-1} and the value of 0·94 for the solvent isotope effect are characteristic of reactions showing a rate-determining decomposition of tetrahedral intermediate ($k_{obs} = (k_1k_2/k_{-1}).[OH^-]$). The positive activation entropy results from a negative entropy for the attack of hydroxide ion on the carbonyl group (loss of degrees of freedom of OH^- in the transition state) and from a positive entropy higher in absolute value for the decomposition of the tetrahedral intermediate **12** owing to the dicarboximide ring opening. The ratio $k_{obs}(H_2O)/k_{obs}(D_2O) = k_{OH}/k_{OD}$ does not allow the rate-determining step to be known with certainty, because, in the case of the formation of the tetrahedral intermediate as in the case of its decomposition, the solvent isotope effect could be near unity. UV spectra of **11** in sodium hydroxide solutions, obtained *in situ* by alkaline hydrolysis of vinclozolin, show a bathochromic shift of 25 nm with increase in concentration of the hydroxide ion. This is consistent with the formation of the anion **14** with a negative charge conjugated with the aromatic nucleus.

The mechanism proposed for the alkaline hydrolysis of vinclozolin is in accordance with certain results in the literature. Alkaline hydrolysis of 2-oxazolidones, molecules having a structural similarity to the dicarboximide ring of vinclozolin, has been studied by Branstad.[26] 2-Oxazolidones are degraded in alkaline media to β-aminoalcohols by a comparable reaction mechanism to that of the hydrolysis of alkyl carbamates. In the hydrolysis of 3-phenyl-2-oxazolidones and 3-(4-chlorophenyl)-2-oxazolidones, Branstad observed the formation of carbamic acids that led by decarboxylation to β-aminoalcohols. Sumida *et al.*[27,28] studied the degradation of dichlozoline (3-(3,5-dichlorophenyl)-5,5-dimethyl-1,3-oxazolidone-2,4-dione), an analogue of vinclozolin, by plants, soil and light. Their studies were hindered by the spontaneous degradation of dichlozoline in aqueous solution at neutral pH. The products were identified as the carbamic acid and its decarboxylation product. These two products were also found in soil degradation studies of dichlozoline, but, in soil, the product of decarboxylation was further degraded to 3,5-dichloroaniline, a chlorinated aromatic amine that could be toxic to living organisms. Finally, Clark [6] has proved the presence of the carbamic acid, **5**, and of the anilide, **11**, in the hydrolysis of vinclozolin at pH = 8 (in tap water), and that the carbamic acid is less stable than its decarboxylation product and consequently leads to the corresponding anilide.

3.3 Chlozolinate

3.3.1 Characterization of the reaction products

The study of HPLC of the hydrolysis of chlozolinate (t_R = 8·44 min) in aqueous phosphate solution (5 mM,

pH = 6·88) (Fig. 3(c)), in order to observe the first steps, which were too rapid to be accessible at pH = 9·08, revealed the presence of a product (t_R = 2·10 min, λ_{max} at 237 nm) whose concentration increases proportionally to the decrease of the concentration of chlozolinate. That signal does not appear with acetonitrile + phosphoric acid as mobile phase: it is replaced by a new signal at 4·09 min, showing that the product responsible for that signal is probably an acid. These results led to the hypothesis that this product was 3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxo-1,3-oxazolidine-5-carboxylate (**15**). This molecule was then hydrolysed and a new product appeared on the chromatograms with a retention time of 9·51 min; its UV spectrum was very similar to that of vinclozolin. Finally, after 200 min, this last product began to decrease, while a new product appeared with a concentration in inverse ratio, a retention time of 1·86 min, a λ_{max} at 245 nm, and identical HPLC and UV data to those obtained from a sample of carbamic acid **6** in the same conditions. With acetonitrile + phosphoric acid as mobile phase, a new signal at 3·46 min appeared instead of that at 1·86 min, corresponding to the protonated form of the acid. These results showed that the product with a retention time of 9·51 min was 3-(3,5-dichlorophenyl)-5-methyloxazolidine-2,4-dione (**16**).

An HPLC study in aqueous borate solution (Fig. 3(d)) showed that chlozolinate did not appear on the chromatograms, owing to the fact that the ethoxycarbonyl group is extremely labile in that pH range. On the other hand, chromatograms revealed the presence of the dione **16**, whose concentration decreased up to complete disappearance in about 2 h, and the appearance of the carbamic acid, **6**, which increased in inverse ratio. Finally, with the decrease of the concentration of **6**, the final product of the hydrolysis of chlozolinate became visible and was probably 3,5-dichlorophenyl-2-hydroxypropionanilide (**17**) (t_R = 6·75 min, λ_{max} at 242 nm).

The similarity of the percentage-time profiles of hydrolysis of the product with a retention time of 9·51 min and vinclozolin (Figs 3(d) and 3(b)) indicate, from the structural similarity of the two molecules, that the former is the oxazolidine-2,4-dione, **16**.

3.3.2 *pH-rate profiles*

Hydrolysis of chlozolinate in aqueous phosphate solution (pH = 7·21) evolved according to three steps with different lives in UV spectrophotometry: the first step consisted of an increase of the optical density at 237 nm up to a maximum (15 min); the second, of a decrease of the optical density leading to a UV spectrum very similar to that of vinclozolin (90 min); and finally, an increase in the optical density at 242 nm of large amplitude up to a maximum (about 40 h). Three observed rate constants were measured at 25°C in buffer solutions (phosphate, borate and carbonate) for pH values between 6·25 and 11·03: $k^c_{1\,obs}$, $k^c_{2\,obs}$ and $k^c_{3\,obs}$. $k^c_{2\,obs}$ was independent of pH in the range examined while the other two observed rate constants of hydrolysis increased proportionally to the hydroxide ion concentration (Fig. 4): the graphs of the variation of the logarithm of the rate constants $k^c_{1\,obs}$ and $k^c_{3\,obs}$ as a function of pH had slopes close to unity ($\log k^c_{1\,obs} = -7{\cdot}97 + 0{\cdot}93$ pH, $r_1 = 0{\cdot}998$; $\log k^c_{3\,obs} = -11{\cdot}18 + 0{\cdot}94$ pH, $r_3 = 0{\cdot}998$). The mean value of $k^c_{2\,obs}$ over the pH range 6·57 to 7·65 was 5.10^{-4} s^{-1}. The half-lives of the reaction at 25°C were, for $k^c_{1\,obs}$ and $k^c_{3\,obs}$, respectively, 2 min and 6 h at pH = 6·78, and 4 s and 16 min at pH = 8·28.

3.3.3 *Buffer catalysis*

The first and the last observed steps of the hydrolysis of chlozolinate are subject to general acid-base catalysis since $k^c_{1\,obs}$ and $k^c_{3\,obs}$ increase linearly with total concentration of buffer at constant pH. The observed rate constant is

$$k_{obs} = k_o + k_H a_H + k_{OH} a_{OH} + k_{BH}[BH^+] + k_B[B]$$

where BH^+ and B are the acid and base components of buffer; k_H, k_{OH}, k_{BH} and k_B, the catalytic constants of different acids and bases of the media. The term k_o corresponds to the catalysis of the reaction by water. At constant pH, the previous relation becomes

$$k_{obs} = k'_o + k_{BH}[BH^+] + k_B[B].$$

If a reaction is subject to a general acid-base catalysis, the observed rate constant in buffer solution at constant pH is

$$k_{obs} = k'_o + k'_r[buffer]_{total}$$

where $k'_r[buffer]_{total} = k_{BH}[BH^+] + k_B[B]$.

The observed buffer constant k'_r still can be written as

$$k'_r = (k_B - k_{BH})[B]/[buffer]_{total} + k_{BH}.$$

The observed buffer constants were plotted against the proportion of the buffer in the free base form (Fig. 7). Straight lines were obtained and the intercept was zero at 0% free base and positive, equal to k_B, at 100% free base for the two buffers examined (acetate and borate). Thus we conclude that general base catalysis of the hydrolysis

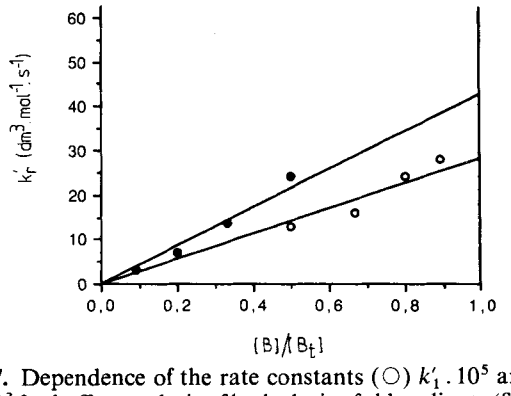

Fig. 7. Dependence of the rate constants (○) $k'_1 . 10^5$ and (●) $k'_3 . 10^3$ for buffer catalysis of hydrolysis of chlozolinate (first and third steps) respectively on the composition of acetate and borate buffers at 25°C (μ = 1·0 M, KCl). The I intercepts are k_B.

of the ethyl ester group and of the dicarboximide ring opening occurs in the mechanism of hydrolysis of chlozolinate.

3.3.4 Temperature and solvent deuterium isotope effects
The influence of the temperature on the rate of hydrolysis of chlozolinate was examined in buffers (acetate, phosphate and borate). The values of the bimolecular rate constants k^c_{1OH} and k^c_{3OH} and the pseudo first-order rate constant k^c_{2obs} (pH-independent reaction) measured for three temperatures are shown in Table 2. The activation entropies of the first, second and third observed steps of the reaction were calculated from these data to be respectively -88 (± 26), -110 (± 33) and -149 (± 44) J mol^{-1} K^{-1}.

From experiments in 5 mM sodium hydroxide and deuteroxide solutions, solvent deuterium isotope effects were measured at 15°C (except for the first step because this step was too rapid to be measured in sodium hydroxide solutions): the values were, respectively, 0·85 and 1·95 for the second and third observed steps.

3.3.5 Mechanism of hydrolysis
Comparison of the hydrolysis rate constants obtained on the one hand by HPLC and on the other hand by UV spectrophotometry (Table 3) permitted the identification of the reactions corresponding to the rate constants of the successive reactions determined spectrophotometrically. The three observed steps of the hydrolysis of chlozolinate are respectively the hydrolysis of the ethyl ester of the ethoxycarbonyl group, the decarboxylation of the resulting carboxylate (**15**) and the dicarboximide ring opening of the oxazolidine-2,4-dione (**16**).

The hydrolysis of the ethyl ester is dependent on the concentration of the base components of the buffer and this, with a negative activation entropy comparable to that of the basic hydrolysis of esters,[20,29] suggests a true general base catalysis. General base-catalyzed ester hydrolysis may proceed through one of the kinetically indistinguishable transition states (Fig. 8). Structure C1 involves removal of a proton from the attacking water molecule by the general base and structure C3, the addition of a proton to the leaving alcohol molecule. Structure C2 involves proton transfer to the carbonyl oxygen atom to aid hydroxide addition to the ester. In the case of esters with a good leaving group relative to the attacking group, the rate-determining step reflects principally the nucleophilic attack on the carbonyl group and is sensitive to the nucleophilic reactivity of the attacking reagent, while with a relatively poor leaving group the transition state also reflects the expulsion of this group from the tetrahedral intermediate. In the latter case, nucleophilic reactivity, as reflected in the rate of nucleophilic attack, becomes of secondary importance, while proton transfer, to aid expulsion of the leaving group and prevent expulsion of the attacking group, becomes an important part of the reaction. The mechanism proposed in Fig. 9(a) seems to be the more plausible.

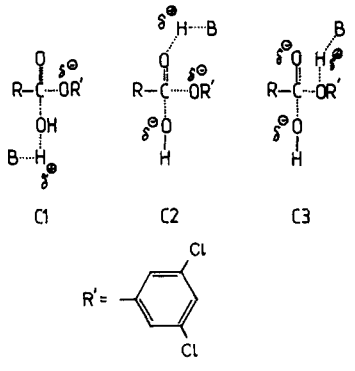

Fig. 8. Transition states for the general base catalysis of ester hydrolysis.

The highly negative activation entropy and the solvent isotope effect of less than one for the decarboxylation step may be interpreted by a cyclic mechanism involving a molecule of water. Figure 9(b) can account for the solvent isotope effect value only if the normal effect corresponding to the transfer of a proton to the oxygen is compensated for by the inverse isotope effect that can be expected from the nucleophilic attack of incipient OH^- on the carboxylate group, OD^- being more nucleophilic than OH^-. The entropy of activation is consistent with the loss of degrees of freedom of the molecule of water in the transition state. The rate-determining step is the loss of carbonate and the mechanism includes a keto-enol equilibrium. The decarboxylation of **15** is pH-independent, owing to the carboxylic acid group being completely ionized in the pH range examined, and the development of the reaction does not require general acid catalysis. The hydrolytic loss of the ethoxycarbonyl substituent of chlozolinate leads to an oxazolidine-2,4-dione (**16**) that differs from vinclozolin only by the presence in the latter of a vinyl group at C_5. In spite of their structural similarity, the two molecules have different hydrolytic behaviour. In contrast to vinclozolin, basic hydrolysis of **16** is subject to true general base catalysis and shows a highly negative activation entropy and a characteristic value of 1·95 for the solvent isotope effect. The mechanism in Fig. 9(c) has a proton transfer from H_2O to the base (B) as the rate-determining step leading to the tetrahedral intermediate **18**. The ionized form (**19**) of the carbamic acid **6** is formed by decomposition of that intermediate. Finally, the decarboxylation of the carbamate, **19**, leads to the anilide **17**.

UV spectra of **17** in sodium hydroxide solutions, obtained *in situ* by alkaline hydrolysis of chlozolinate, show a bathochromic shift of 27 nm with increase in concentration of the hydroxide ion. This is consistent with the formation of the anion **20**, with a negative charge conjugated with the aromatic nucleus.

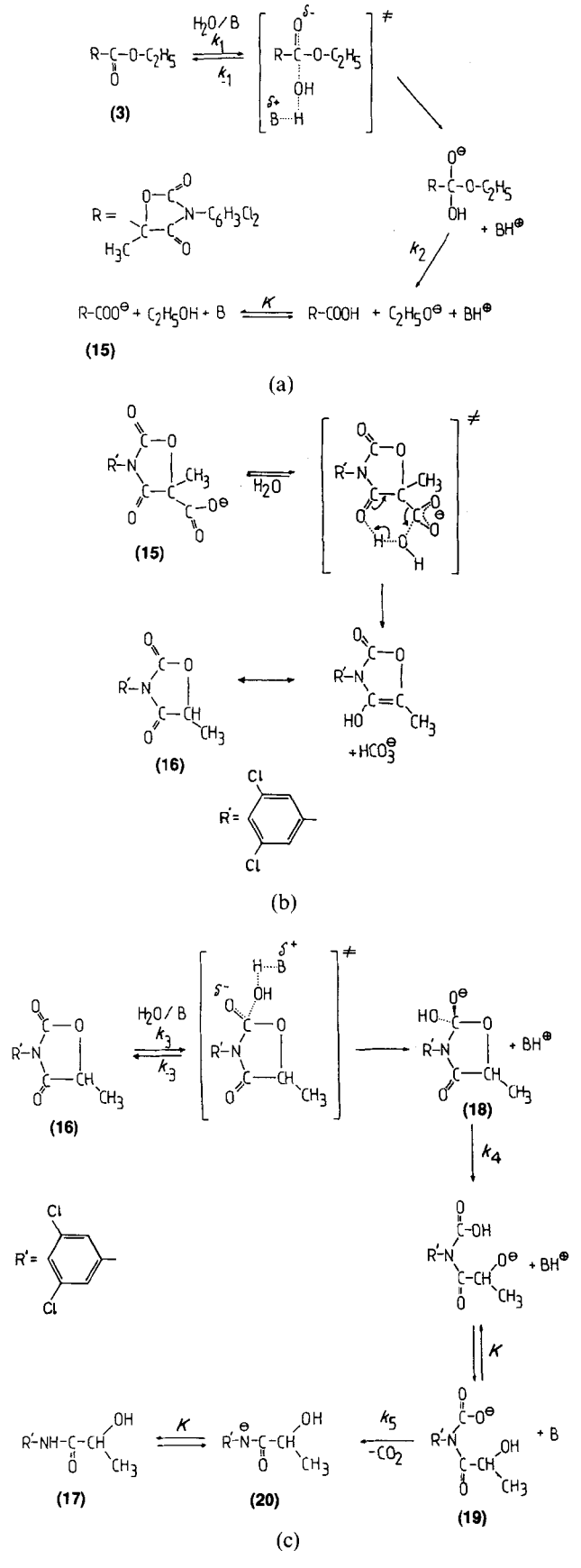

Fig. 9. Mechanism of alkaline hydrolysis of chlozolinate. (a) Hydrolysis of the ethyl ester; (b) decarboxylation of the carboxylate **15**; (c) hydrolysis of the oxazolidine-2,4-dione **16**.

4 CONCLUSIONS

In aqueous alkaline media, procymidone is converted quantitatively and irreversibly to 2-(3,5-dichlorophenylcarbamoyl)-1,2-dimethylcyclopropanecarboxylate. The hydrolysis of procymidone is not subject to general base catalysis and the rate-determining step is the formation of the tetrahedral intermediate. At lower pH values close to that of natural media, the two compounds are in equilibrium.

Chlozolinate, after a rapid hydrolytic loss of the ethoxycarbonyl substituent, and vinclozolin undergo attack by the hydroxide ion on the same carbonyl group to produce, according to different mechanisms, the carbamic acids (**5**, **6**) and after decarboxylation, the corresponding anilides (**11**, **17**). The kinetic data showed a significant difference between the observed rate constants of the basic hydrolysis of the dione **16** and of vinclozolin: $k^{c}_{3\,obs}/k^{v}_{obs} = 5{\cdot}3$. That ratio can be explained by the general base catalysis for the hydrolysis of **16**, where the rate-determining step is the formation of the tetrahedral intermediate. On the other hand, for the hydrolysis of vinclozolin, the rate-determining step seems to be the decomposition of the tetrahedral intermediate, owing to the fact that the electronic and steric effects of the vinyl and methyl groups at C_5 cause an increase in the difficulty of leaving group expulsion. In the case of the dione **16**, the separation of the leaving group can be also assisted by the conjugated acid (BH^+); this is not possible for vinclozolin owing to the steric constraint of the vinyl and methyl groups. In aqueous alkaline media, the two fungicides are converted quantitatively and irreversibly to the anilides and thus the equilibrium between the open and closed forms of the dicarboximide ring is totally displaced towards the open form. In the pH range examined, there was no trace of 3,5-dichlorophenyl signifying that the amide linkage is stable in alkaline media.

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