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Kinetics of Hydrolysis of the Dicarboximide Fungicide Vinclozolin

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The hydrolysis of the dicarboximide fungicide vinclozolin was studied in several aqueous buffers of pH 4.5–8.3 at 13–35 °C. The reaction was base-catalyzed and the rate proportional to pH. At 35 °C the pseudo-first-order rate constants ranged from 1.30×10^{-3} to 1.11 h^{-1}, and the second-order rate constants, from 4.10×10^{6} to 5.56×10^{5} M^{-1} h^{-1}. The energy of activation for the hydrolysis of vinclozolin at pH 7.0 was calculated from the Arrhenius plot to be 97.2 kJ mol^{-1}. On the basis of kinetics, a degradation pathway was proposed. On hydrolysis the 2,4-oxazolidinedione ring opens to yield 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid (M1) and 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2) independently. The conversion to M1 is reversible, which leads to the formation of vinclozolin by recyclization. At basic pH the forward reaction to yield M1 is favored whereas at acidic pH the reverse reaction to yield vinclozolin is favored. 3,5-Dichloroaniline (M3) was detected as a minor degradation product.

Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione] (Figure 1) is a protectant fungicide marketed by BASF AG. It is effective for controlling fungal diseases caused by *Botrytis* spp., *Sclerotinia* spp., and *Monilinia* spp. in grapes, fruits, vegetables, ornamentals, hops, rapeseed, and turfgrass (Spencer, 1982). Since its introduction, vinclozolin has been widely used in Europe for the control of fungal diseases. Vinclozolin is currently registered in the United States, but not in Canada.

Vinclozolin is unstable in methanolic and ethanolic solutions and water suspension (Clark, 1983). Three hydrolytic degradation products of vinclozolin have been isolated and identified by us, namely 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid (M1), 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2) (Figure 1), and 3,5-dichloroaniline (M3) (unpublished data). Vinclozolin was more susceptible to hydrolysis at basic than acidic pH (Melkebeke et al., 1986). Melkebeke et al. (1986) studied the chemical hydrolysis of vinclozolin in the range pH 3.0–11.0 and reported the half-lives and rate constants for the disappearance of the parent compound. However, they did not study the mechanism of degradation. Considering the structures of the two degradation products, the butenoic acid and the enanilide, it is highly unlikely that the enanilide is formed via the butenoic acid as an intermediate because conversion from the butenoic acid to the enanilide would require complicated rearrangement of the molecule.

A comprehensive study is required to investigate the disappearance of vinclozolin and the production of degradation products, so that the mechanism of hydrolytic degradation can be better understood. The present study describes the kinetics of hydrolysis of vinclozolin in several aqueous buffers in the range pH 4.5–8.3 and in the temperature range 13–35 °C, and on the basis of these data, a degradation pathway is proposed.

EXPERIMENTAL SECTION

Preparation of Vinclozolin and Its Hydrolytic Degradation Products. Vinclozolin, 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid, and 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide were obtained from BASF Aktiengesellschaft, and their purities were, respectively, 99%, 98%, and 96%. 3,5-Dichloroaniline (98%) was obtained from the Laboratory Services Division of Agriculture Canada in Ottawa.

Preparation of Aqueous Buffers. Buffered solutions of 0.01 M were prepared with sterilized deionized water by adjusting the pH with the following chemicals: NaH_2PO_4/H_3PO_4 and NaOAc/HOAc for pH 4.5;

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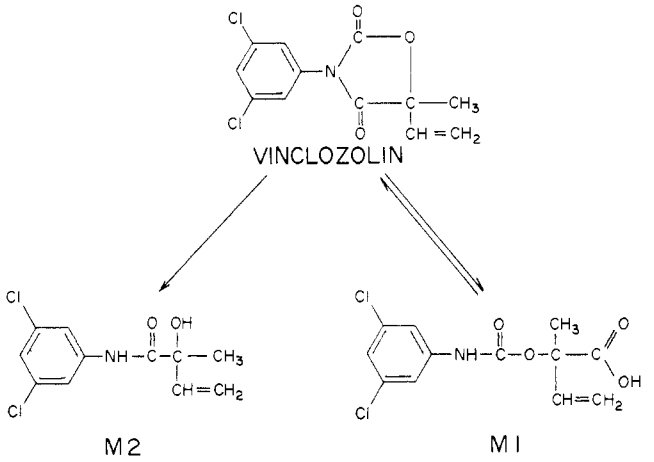

Figure 1. Proposed degradation pathway for the hydrolysis of vinclozolin leading reversibly to the formation of M1 and M2.

NaH_2PO_4/Na_2HPO_4 and NaOAc/HOAc for pH 5.5; NaH_2PO_4/Na_2HPO_4 and NaOAc/HOAc for pH 6.5; Na_2HPO_4/NaH_2PO_4 and $Na_2B_4O_7$/HCl for pH 7.0; $Na_2B_4O_7$/HCl for pH 7.3; Na_2HPO_4/NaH_2PO_4 for pH 7.5; Na_2HPO_4/NaH_2PO_4 for pH 8.0; $Na_2B_4O_7$/HCl for pH 8.3 (Dawson et al., 1969).

Incubation of Vinclozolin in Aqueous Buffers. Aliquots of 1 mL of a stock solution of vinclozolin at 1000 μg/mL in methanol were thoroughly mixed with the aqueous buffers. The final concentration of vinclozolin in the buffered solutions was 10 μg/mL. The controls were similarly prepared with methanol alone to acertain that no UV response was produced during incubation without the addition of vinclozolin. Aliquots of approximately 2 mL of the buffered solutions were transferred to 2-mL brown ampules that were then sealed under nitrogen. All sealed ampules were incubated at 35 °C in a water bath in darkness; those of pH 7.0 were also incubated in darkness at 13, 20, and 26 °C in environmental chambers.

Incubation of M1 in Aqueous Buffers. Aliquots of 1 mL of the stock solution of M1 at 1000 μg/mL in methanol were thoroughly mixed with the aqueous buffers of pH 4.5 and 8.0. The final concentration was 10 μg/mL, and the controls were similarly prepared with methanol alone. Aliquots of approximately 2 mL of the buffered solutions were transferred to 2-mL brown ampules that were then sealed under nitrogen. The sealed ampules were incubated at 35 °C in a water bath in darkness.

A separate incubation experiment at pH 8.0 was similar. After incubation at 35 °C for 24 h, half of the samples were acidified with 0.01 M H_3PO_4 to pH 4.5 and then incubated at 35 °C in a water bath in darkness.

Analysis by High-Pressure Liquid Chromatography (HPLC). Concentrations of vinclozolin and its degradation products M1, M2, and M3 present after various intervals of incubation were determined by HPLC with a Varian Model 5000 high-pressure liquid chromatograph equipped with a Hewlett-Packard Model 1040A high-speed spectrophotometeric detector. The operating parameters were as follows: column, Varian Micro Pak MCH-10, 30 cm × 4 mm (i.d.); mobile solvent system, 72% methanol and 28% 0.05 M phosphate buffer of pH 3.3, isocratic at 1 mL/min; UV detector wavelength, 212 ± 2 nm. Aliquots of 20 μL of the incubated solution was injected directly into the high-pressure liquid chromatograph for determination of vinclozolin and its degradation products. Under the chromatographic conditions described, the absolute retention times were 5.04, 6.43, 7.25, and 8.86 min for M1, M3, M2, and vinclozolin, respectively.

Table I. Kinetic Data of Hydrolysis of Vinclozolin at 35 °C in Buffered Solutions

pH	buffer	no. of readings	correln coeff	k_{obsd},[a] × 10^{-3} h^{-1}	k_{OH^-},[b] × 10^5 M^{-1} h^{-1}	half-life, h
4.5	phosphate	11	0.9996	1.31	41.4	529
4.5	acetate	11	0.9991	1.28	40.5	541
5.5	phosphate	10	0.9999	12.4	39.2	56.0
5.5	acetate	10	0.9993	12.1	38.3	57.5
6.5	phosphate	8	0.9980	46.4	14.6	15.0
6.5	acetate	8	0.9971	44.3	14.0	15.6
7.0	phosphate	10	0.9995	107	10.7	6.45
7.0	borate	10	0.9995	107	10.7	6.45
7.3	borate	9	0.9981	210	10.5	3.31
7.5	phosphate	8	0.9987	248	7.84	2.80
8.0	phosphate	9	0.9973	599	5.99	1.16
8.3	borate	8	0.9963	1109	5.56	0.62

[a] k_{obsd} = pseudo-first-order rate constant. [b] k_{OH^-} = second-order rate constant.

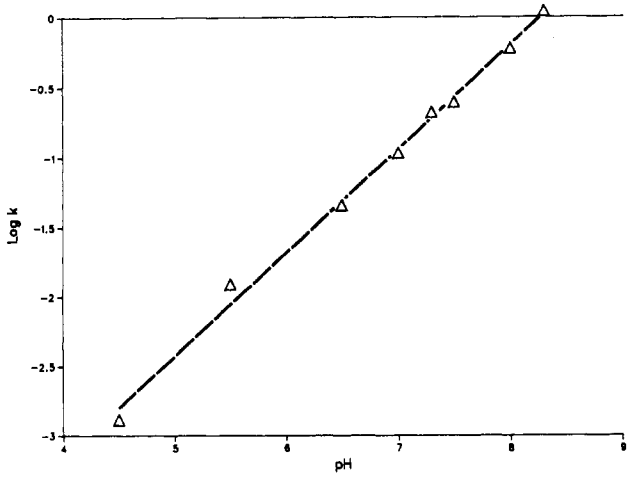

Figure 2. Profile of log rate constant vs pH for the hydrolysis of vinclozolin at 35 °C.

Quantification of analytes was based on an external standard. Detector response was calibrated for each analysis with reference standards of vinclozolin, M1, M2, and 3,5-dichloroaniline. Calculation was based on average peak areas of these external standards, which were injected before and after each sample.

RESULTS AND DISCUSSION

Disappearance of Vinclozolin. All data for the hydrolysis of vinclozolin at 35 °C and pH 4.5–8.3 followed simple pseudo-first-order kinetics. Table I summarizes these data. Buffer catalysis was not observed. At pH 4.5, 5.5, 6.5, and 7.0 the observed rates at each pH were similar between the two buffers. The disappearance of vinclozolin at 35 °C was very fast at basic pH but much slower at acidic pH. At pH 8.3 the half-life was 0.62 h whereas at pH 4.5 it was approximately 530 h. A linear relationship was indicated between the logarithm of the observed rate and the pH from 4.5 to 8.3 (Figure 2). By a least-squares method a linear regression was calculated as follows: log (obsd rate) = 0.7450pH − 6.1562 (n = 12, r = 0.9967*, significant at p = 0.05). It was evident that the rate of disappearance of vinclozolin was dependent on hydroxide ion concentration. The second-order rate constants (k_{OH^-}) were calculated from $k_{obsd}/[OH^-]$ and are given in Table I.

Melkebeke et al. (1986) studied the kinetics of the chemical hydrolysis of iprodione, vinclozolin, and metalaxyl. They reported that the disappearance of vinclozolin at 60 °C from pH 3.0 to 11.0 was pseudo-first-order and that vinclozolin was much more persistent in acidic than

Table II. Kinetic Data of Hydrolysis of Vinclozolin in 0.01 M Phosphate Buffer of pH 7.0 at 13, 20, 26, and 35 °C

temp, °C	k_{obsd}, $\times 10^{-3}$ h^{-1}	half-life, h	no. of readings	correln coeff
13	4.94	140	10	0.9999
20	26.8	25.9	9	0.9999
26	35.1	19.7	8	0.9999
35	107	6.45	10	0.9995

in alkali pH, results in general agreement with those reported here. However, the pseudo-first-order rate constants and half-lives reported by them are not in agreement with ours. For example, they reported a pseudo-first-order rate of 1.42×10^{-5} s^{-1}, i.e., 51.1×10^{-3} h^{-1}, for the hydrolysis of vinclozolin at 25 °C and pH 7.0, and the calculated half-life of 13.4 h, which differs from the rate of 35.1×10^{-3} h^{-1} and the half-life of 19.7 h for 26 °C and pH 7.0 as determined in our study (Table II). This discrepancy may be attributed to the fact that Melkebeke et al. (1986) actually determined the pseudo-first-order rates at 60 °C for pH 3.0, 5.0, 7.0, 9.0, and 11.0 but their results are given at standard conditions of 25 °C (1 atm). They stated that their data had been converted to standard conditions, but their methods were not described.

In order to study the influence of temperature on the rate constant, the Arrhenius plot for the hydrolysis of vinclozolin at pH 7.0 was established with kinetic data generated at 13, 20, 26, and 35 °C (Table II). The linear regression of the Arrhenius plot of log rate vs $1/T$ (Figure 3) was as follows: log rate = $-5074[1/T] + 15.5$ ($n = 4$, $r = 0.9698$*, significant at $p = 0.05$). The energy of activation and the frequency factor A for the hydrolysis of vinclozolin at pH 7.0 were calculated to be 97.2 kJ mol^{-1} and 3.47×10^{15} h^{-1}, respectively.

By comparison with other protectant fungicides used extensively for crop protection, vinclozolin appears to be more resistant to hydrolysis. According to the study of Wolfe et al. (1976), the pseudo-first-order rate constants of hydrolysis at 28 °C and about pH 7.0 are 0.234 h^{-1} for captan (pH 7.07), 0.277 h^{-1} for captafol (pH 7.17), and 0.504 h^{-1} for folpet (pH 7.14). By comparison, the value for vinclozolin at 28 °C and pH 7.0 is calculated to be 0.048 h^{-1}, which is almost 1 order of magnitude lower than those of captan, captafol, and folpet. The effects of pH on the hydrolysis of vinclozolin are different from those on the hydrolysis of captan. The rate constant was pH dependent from pH 4.5 to 8.3 for vinclozolin, and a linear relationship existed between log rate constant and pH (Figure 2). By comparison the rate constant for the hydrolysis of captan is independent of pH over the range of pH 2–6 and the average pseudo-first-order rate constant at 28 °C is 6.48×10^{-2} h^{-1}. Above pH 7.0, the rate constant is pH dependent and the mean and standard deviation of second-order rate constants for the hydrolysis of captan at 28 °C in the range pH 7.07–8.25 are 2.05×10^{6} and 0.14×10^{6} M^{-1} h^{-1} (Wolfe et al., 1976).

In the range pH 5.5–8.0, which is typical of natural water, vinclozlin underwent hydrolysis readily. At 35 °C the half-lives were about 56 h at pH 5.5 and 1.16 h at pH 8.0 (Table I). At room temperature (20 °C) and neutral pH the half-life was about 26 h (Table II). These findings suggest that Ronilan 50 WP, a commercial formulation of vinclozolin, may be unstable in water especially in slightly basic water. Indeed, Ronilan 50 WP has been shown to be unstable in water by Clark (1983). When 150 mg of Ronilan 50 WP was stirred in 100 mL of tap water (pH 8) at room temperature, 98% of vinclozolin was recovered from the suspension after 1 h but only 7% after 23 days. On the basis of data of Clark (1983), the half-life of Ronilan

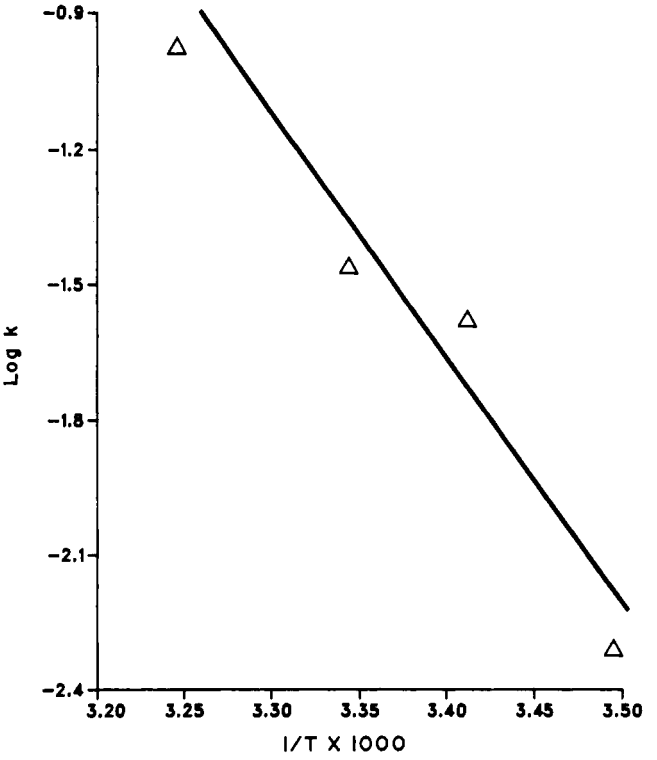

Figure 3. Arrhenius plot of log k vs $1/T \times 1000$ for the hydrolysis of vinclozolin at pH 7.0.

was approximately 6 days, which is considerably longer than that of the methanol solution of vinclozolin used in our study. The longer half-life of Ronilan is probably attributable to its lower solubility in water than the methanol solution of vinclozolin. To ensure the efficacy of Ronilan 50 WP, it is important to prepare the spray mix fresh with water that is neutral or slightly acidic or the formulation may be buffered to prevent spray tank hydrolysis. With water that is slightly basic, e.g. pH 8, to prepare the spray mix would result in loss of efficacy from hydrolysis of the vinclozolin.

Conversion Products of Vinclozolin. When vinclozolin hydrolyzed in aqueous buffers at 35 °C in the range pH 4.5–8.0, the parent compound was gradually converted to 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid, 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide, and 3,5-dichloroaniline. Similar conversions were observed at each pH regardless of the type of buffer used, indicating that buffer catalysis did not occur in the hydrolysis of vinclozolin.

Within the range pH 4.5–8.0 the major conversion product was M1, of which the concentrations increased steadily with time. The highest concentrations of M1 (in percent of the total of vinclozolin + M1 + M2 + M3) reached 70–85% with the exception of pH 4.5 (Tables III–VII). At pH 4.5 the highest concentration of M1 reached approximately 18% of the total (Table III). After reaching the highest levels, the concentrations of M1 decreased gradually with time. Another major conversion product was M2. The concentrations of M2 increased steadily with time and were at their highest when the hydrolysis studies were ended. M3 was detected only after at least 21 days (504 h) of incubation. It was detected at pH 4.5, 5.5, 6.5, and 7.0, but not 8.0 where the analysis was terminated after 4.3 h.

Vinclozolin hydrolyzed to yield M1, M2, and M3 (Tables III–VII). The concentrations of the parent compound and the conversion products varied depending on pH and incubation time. However, the sum of concentrations of

Table III. Hydrolysis of Vinclozolin in 0.01 M Phosphate Buffer of pH 4.5 at 35 °C

time, h	concentration, μM (%)[a]				
	vinclozolin	M1[b]	M2[b]	M3[b]	total[b]
0	32.4 (100)	ND[d]	ND	ND	32.4 (100)[c]
26.8	30.6 (85.2)	4.79 (13.3)	0.46 (1.5)	ND	35.9 (110)
48.5	29.8 (82.3)	5.18 (14.3)	1.20 (3.4)	ND	36.2 (112)
72.6	28.8 (79.8)	5.41 (15.0)	1.85 (5.2)	ND	36.1 (112)
121.5	27.0 (72.4)	7.06 (18.9)	3.32 (8.7)	ND	37.3 (115)
170.3	25.3 (68.8)	7.43 (20.2)	4.02 (11.0)	ND	36.8 (114)
264.0	22.4 (60.7)	6.73 (18.3)	7.76 (21.0)	ND	36.9 (114)
387.3	19.0 (51.8)	5.15 (14.1)	12.5 (34.1)	ND	36.7 (113)
672.3	12.6 (34.4)	4.88 (13.3)	17.6 (48.1)	1.49 (4.2)	36.6 (113)
1008	8.73 (23.3)	3.04 (8.1)	24.2 (64.7)	1.49 (3.9)	37.4 (115)
1536	4.25 (11.2)	1.25 (3.3)	30.3 (79.5)	2.36 (6.0)	38.1 (117)

[a] Vinclozolin, M1, M2, or M3 as percent of total of vinclozolin + M1 + M2 + M3. [b] M1 = 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid, M2 = 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide, M3 = 3,5-dichloroaniline, and total = vinclozolin + M1 + M2 + M3. [c] Vinclozolin + M1 + M2 + M3 at time t expressed as percent of vinclozolin + M1 + M2 + M3 at time 0. [d] ND = not detected.

Table IV. Hydrolysis of Vinclozolin in 0.01 M Phosphate Buffer of pH 5.5 at 35 °C

time, h	concentration, μM (%)[a]				
	vinclozolin	M1[b]	M2[b]	M3[b]	total[b]
0	33.4 (100)	ND[d]	ND	ND	33.4 (100)[c]
23.3	25.9 (71.3)	9.11 (25.1)	1.31 (3.6)	ND	36.3 (109)
28.4	24.4 (67.6)	10.4 (28.8)	1.31 (3.6)	ND	36.1 (108)
32.0	22.3 (63.5)	11.4 (32.5)	1.39 (4.0)	ND	35.1 (105)
47.0	18.9 (52.8)	15.0 (41.9)	1.93 (5.3)	ND	35.8 (107)
71.1	14.3 (41.4)	17.6 (51.0)	2.55 (7.6)	ND	34.5 (103)
96.3	10.2 (29.2)	21.2 (60.7)	3.47 (10.1)	ND	34.9 (104)
119.7	7.65 (21.2)	24.2 (67.0)	4.29 (11.8)	ND	36.1 (108)
167.3	4.28 (11.8)	24.9 (68.6)	7.10 (19.6)	ND	36.3 (109)
387.3	0.28 (0.8)	24.8 (70.0)	10.3 (29.2)	ND	35.4 (106)
1537	ND	13.6 (36.4)	21.7 (58.0)	2.11 (5.6)	37.4 (112)

[a] Vinclozolin, M1, M2, or M3 as percent of total of vinclozolin + M1 + M2 + M3. [b] M1 = 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid, M2 = 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide, M3 = 3,5-dichloroaniline, and total = vinclozolin + M1 + M2 + M3. [c] Vinclozolin + M1 + M2 + M3 at time t expressed as percent of vinclozolin + M1 + M2 + M3 at time 0. [d] ND = not detected.

Table V. Hydrolysis of Vinclozolin in 0.01 M Phosphate Buffer of pH 6.5 at 35 °C

time, h	concentration, μM (%)[a]				
	vinclozolin	M1[b]	M2[b]	M3[b]	total[b]
0	39.6 (100)	ND[d]	ND	ND	39.6 (100)[c]
1.0	37.9 (96.2)	1.45 (3.8)	ND	ND	39.4 (99.5)
2.5	34.7 (91.1)	3.40 (8.9)	ND	ND	38.1 (96.2)
4.0	29.6 (80.2)	7.29 (18.8)	ND	ND	36.9 (93.2)
9.5	22.6 (61.1)	11.8 (31.9)	2.63 (7.0)	ND	37.0 (93.4)
24.0	13.2 (33.7)	22.8 (58.2)	3.24 (8.1)	ND	39.2 (99.0)
29.0	10.1 (25.8)	24.4 (62.4)	4.59 (11.8)	ND	39.1 (98.7)
48.0	4.07 (10.9)	28.1 (75.3)	5.10 (13.8)	ND	37.3 (94.2)
192.7	ND	29.7 (79.6)	7.64 (20.4)	ND	37.3 (94.2)
338.7	ND	27.2 (76.0)	8.61 (24.0)	ND	35.8 (90.4)
505.8	ND	25.9 (68.9)	10.3 (27.4)	1.43 (3.7)	37.6 (94.9)
1203	ND	21.2 (55.2)	14.3 (37.2)	2.86 (7.6)	38.4 (97.0)

[a] Vinclozolin, M1, M2, or M3 as percent of total of vinclozolin + M1 + M2 + M3. [b] M1 = 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid, M2 = 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide, M3 = 3,5-dichloroaniline, and total = vinclozolin + M1 + M2 + M3. [c] Vinclozolin + M1 + M2 + M3 at time t expressed as percent of vinclozolin + M1 + M2 + M3 at time 0. [d] ND = not detected.

vinclozolin + M1 + M2 + M3 remained relatively unchanged throughout the hydrolysis study. The fact that the conversion products M1, M2, and M3 were relatively stable in aqueous media suggests that residues may be present in wine produced from vinclozolin-treated grapes. The possibility of 3,5-dichloroaniline being produced has importance for public health researchers because 3,5-dichloroaniline is a chlorinated aromatic amine that could be toxic to living organisms. Cabras et al. (1984) studied the degradation of vinclozolin in wine at pH 3.0 and 4.0, and 3,5-dichloroaniline was not detected. In our study of the hydrolysis of vinclozolin at pH 4.5 and 35 °C, M3 was detected after 28 days (672 h) (Table III). In the study of Cabras et al. (1984) the starting concentration of vinclozolin was about 10 μM, which was approximately 30% of the concentration (32.4 μM) used in our hydrolysis study. Taking into consideration that M3 accounted for only about 6% of the total of vinclozolin and hydrolysis products after 28 days, the concentration of M3 that might have occurred in the wine would probably have been below the limit of detection of the analytical method used by Cabras et al. (1984). In the same study Cabras et al. (1984) reported that the disappearance of vinclozolin was pseudo-first-order. The rate constants at 30° C were 0.17×10^6 s^{-1} at pH 3.0 and 0.22×10^6 s^{-1} at pH 4.0; the half-lives were 48.5 days at pH 3.0 and 36.7 days at pH 4.0. Comparing their reported rate constants and half-lives, it is evident that the reported rate constants are several orders of magnitude too high for the reported half-lives. It appears highly likely that there was a typographical error in the reported rate constants. according to their reported half-lives, the rate constants should be 0.17×10^{-6} s^{-1} at

Table VI. Hydrolysis of Vinclozolin in 0.01 M Phosphate Buffer of pH 7.0 at 35 °C

time, h	concentration, μM (%)[a]				
	vinclozolin	M1[b]	M2[b]	M3[b]	total[b]
0	38.2 (100)	ND[d]	ND	ND	38.2 (100)[c]
1.0	33.7 (89.6)	3.30 (8.8)	0.62 (1.6)	ND	37.6 (98.4)
2.0	29.3 (79.6)	6.01 (16.3)	1.47 (4.1)	ND	36.8 (96.3)
3.0	25.5 (69.1)	9.31 (25.2)	2.05 (5.7)	ND	36.9 (96.6)
4.0	23.5 (62.3)	11.5 (30.5)	2.66 (7.2)	ND	37.7 (98.7)
5.0	21.5 (57.6)	12.8 (34.3)	2.97 (8.1)	ND	37.3 (97.6)
6.0	19.4 (51.2)	14.9 (39.3)	3.55 (9.5)	ND	37.9 (99.2)
9.0	13.8 (36.3)	19.8 (52.1)	4.40 (11.6)	ND	38.0 (99.5)
10.3	12.4 (32.6)	20.7 (54.5)	4.94 (12.9)	ND	38.0 (99.5)
24.0	2.81 (7.4)	28.5 (75.0)	6.68 (17.6)	ND	38.0 (99.5)
48.0	ND	29.8 (79.0)	7.92 (21.0)	ND	37.7 (98.7)
72.0	ND	30.0 (78.5)	8.19 (21.5)	ND	38.2 (100)
96.0	ND	30.0 (78.5)	8.19 (21.5)	ND	38.2 (100)
168.0	ND	29.5 (77.4)	8.57 (22.6)	ND	38.1 (99.7)
240.0	ND	29.2 (75.5)	9.46 (24.5)	ND	38.7 (101)
360.0	ND	28.1 (73.9)	9.88 (26.1)	ND	38.0 (99.5)
504.0	ND	26.6 (71.9)	10.4 (28.1)	ND	37.0 (96.9)
696.0	ND	25.2 (67.4)	12.2 (32.6)	ND	37.4 (97.9)
1108	ND	23.8 (62.3)	14.4 (37.7)	ND	38.2 (100)
1370	ND	20.4 (51.4)	16.6 (41.8)	2.67 (6.8)	39.7 (104)
1683	ND	18.6 (47.4)	17.6 (44.9)	2.98 (7.7)	39.2 (103)
2381	ND	15.1 (38.0)	21.0 (52.9)	3.60 (9.1)	39.7 (104)

[a] Vinclozolin, M1, M2, or M3 as percent of total of vinclozolin + M1 + M2 + M3. [b] M1 = 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid, M2 = 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide, M3 = 3,5-dichloroaniline, and total = vinclozolin + M1 + M2 + M3. [c] Vinclozolin + M1 + M2 + M3 at time t expressed as percent of vinclozolin + M1 + M2 + M3 at time 0. [d] ND = not detected.

Table VII. Hydrolysis of Vinclozolin in 0.01 M Phosphate Buffer of pH 8.0 at 35 °C

time, h	concentration, μM (%)[a]				
	vinclozolin	M1[b]	M2[b]	M3[b]	total[b]
0	41.1 (100)	ND[d]	ND	ND	41.1 (100)[c]
0.233	33.6 (87.5)	4.75 (12.5)	ND	ND	38.4 (93.3)
0.467	28.1 (74.1)	9.80 (25.9)	ND	ND	37.9 (92.2)
0.717	24.9 (63.5)	11.6 (29.6)	2.66 (6.9)	ND	39.2 (95.3)
0.967	20.6 (53.4)	14.9 (38.6)	3.05 (8.0)	ND	38.6 (93.8)
1.117	17.6 (45.4)	17.7 (45.6)	3.51 (9.0)	ND	38.8 (94.4)
1.450	14.8 (37.8)	20.0 (51.0)	4.44 (11.2)	ND	39.2 (95.5)
2.767	7.12 (18.1)	27.0 (68.5)	5.25 (13.4)	ND	39.4 (95.9)
3.783	4.14 (10.3)	29.5 (73.6)	6.45 (16.1)	ND	40.1 (97.5)
4.300	ND	31.5 (80.8)	7.53 (19.2)	ND	39.0 (95.0)

[a] Vinclozolin, M1, M2, or M3 as percent of total of vinclozolin + M1 + M2 + M3. [b] M1 = 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid, M2 = 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide, M3 = 3,5-dichloroaniline, and total = vinclozolin + M1 + M2 + M3. [c] Vinclozolin + M1 + M2 + M3 at time t expressed as percent of vinclozolin + M1 + M2 + M3 at time 0. [d] ND = not detected.

pH 3.0 and 0.22×10^{-6} s^{-1} at pH 4.0. These findings are in general agreement with our kinetic data for hydrolysis of vinclozolin in aqueous buffers (Table I), indicating that the degradation of vinclozolin in wine was mainly due to hydrolysis.

M2 was the only degradation product detected by Cabras et al. (1984) in their study of wine. They speculated that M2 was derived from vinclozolin by hydrolytic opening of the 2,4-oxazolidine ring. In a subsequent study by the same group (Pirisi et al., 1986), the identity of M2 was confirmed by elemental analysis and proton NMR. They hypothesized that M2 was derived from the intermediate, *N*-(2-hydroxy-2-methyl-1-oxobuten-3-yl)-3,5-dichlorophenyl-1-carbamic acid. However, Cabras et al. (1984) did not detect any carbamic acid in the reaction mixture during the course of their study. A careful analysis of their HPLC method shows that their mobile solvent system consisted of 55% of acetonitrile and 45% water with a flow rate of 1 mL/min. With their mobile solvent system for reversed-phase HPLC, the acid would be coeluted with the solvent front and not be detected as a degradation product.

Reaction of M1 in Aqueous Buffers. Conversion of vinclozolin to the butenoic acid (M1) and the enanilide (M2) occurred simultaneously at 35 °C for pH 4.5, 5.5, and 7.0 (Tables III, IV, and VI). At pH 6.5 and 8.0 there was a time lapse between the occurrence of M1 and M2. M1 appeared sooner than M2. When vinclozolin hydrolyzed at pH 4.5, 5.5, 6.5, and 7.0, the concentrations of M1 increased steadily to a maximum and decreased thereafter whereas the concentrations of M2 continued to increase steadily (Tables III–VI). At pH 8.0 the concentrations of both M1 and M2 increased steadily during hydrolysis (Table VII). Considering the structures of M1 and M2 it seems unlikely that the formation of M2 is via M1 as the intermediate because such conversion would require complicated rearrangement of the molecule. Therefore, it was hypothesized that vinclozolin was converted independently to both M1 and M2 and that the conversion from vinclozolin to M1 was reversible and pH dependent. Results of the studies on chemical conversion of M1 in aqueous buffers of pH 4.5 and 8.0 confirmed this hypothesis (Tables VIII and IX). At pH 4.5, M1 was rapidly converted to vinclozolin and its concentration increased steadily to a maximum after about 3 days (71 h) and decreased gradually thereafter. M2 was not detected until almost 2 days (46 h) after the appearance of vinclozolin. At the end of this study with M1, i.e., after about 70 days (1680 h), the concentration of M2 accounted for approximately 80% of the total (Table VIII). The conversion of M1 at 35 °C and pH 4.5 appeared to be the reversal of the hydrolysis of vinclozolin under identical conditions (Tables III and VIII).

Table VIII. Chemical Conversion of M1 in 0.01 M Phosphate Buffer of pH 4.5 at 35 °C

	concentration, μM (%)[a]				
time, h	M1[b]	vinclozolin	M2[b]	M3[b]	total[b]
0	33.0 (100)	ND[d]	ND	ND	33.0 (100)[c]
2	30.8 (90.6)	3.19 (9.4)	ND	ND	34.0 (103)
3	28.9 (87.3)	4.18 (12.7)	ND	ND	33.1 (100)
22	15.4 (47.4)	17.1 (52.6)	ND	ND	32.5 (98.5)
46	9.70 (29.9)	21.6 (66.7)	1.12 (3.4)	ND	32.4 (98.2)
71	7.66 (23.7)	22.7 (70.3)	1.89 (6.0)	ND	32.3 (97.7)
144	7.36 (22.2)	20.8 (62.7)	5.02 (15.1)	ND	33.2 (101)
240	6.60 (19.5)	19.0 (56.2)	8.19 (24.3)	ND	33.8 (102)
313	5.84 (17.4)	17.3 (52.0)	10.2 (30.6)	ND	33.3 (101)
480	5.54 (16.6)	15.9 (47.7)	11.9 (35.7)	ND	33.3 (101)
672	4.95 (14.6)	14.1 (41.7)	14.7 (43.7)	ND	33.8 (102)
840	4.29 (12.3)	12.5 (35.9)	17.4 (50.0)	0.62 (1.8)	34.8 (105)
1008	2.84 (8.0)	7.72 (21.8)	23.3 (65.8)	1.49 (4.4)	35.4 (107)
1176	1.91 (5.3)	7.19 (20.1)	25.0 (69.8)	1.74 (4.8)	35.8 (109)
1488	1.68 (4.6)	4.84 (13.3)	27.5 (75.5)	2.36 (6.6)	36.4 (110)
1680	1.45 (4.0)	2.98 (8.2)	29.6 (81.1)	2.48 (6.7)	36.5 (110)

[a] Vinclozolin, M1, M2, or M3 as percent of total of vinclozolin + M1 + M2 + M3. [b] M1 = 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid, M2 = 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide, M3 = 3,5-dichloroaniline, and total = vinclozolin + M1 + M2 + M3. [c] Vinclozolin + M1 + M2 + M3 at time t expressed as percent of vinclozolin + M1 + M2 + M3 at time 0. [d] ND = not detected.

Table IX. Chemical Conversion of M1 in 0.01 M Phosphate Buffer of pH 8.0 at 35 °C

	concentration, μM (%)[a]				
time, h	M1[b]	vinclozolin	M2[b]	M3[b]	total[b]
0	33.0 (100)	ND[d]	ND	ND	33.0 (100)[c]
2	32.0 (100)	ND	ND	ND	32.0 (97.0)
3	32.4 (100)	ND	ND	ND	32.4 (98.3)
22	32.3 (100)	ND	ND	ND	32.3 (97.9)
46	32.5 (100)	ND	ND	ND	32.5 (98.6)
71	32.0 (100)	ND	ND	ND	32.0 (96.9)
144	32.3 (100)	ND	ND	ND	32.3 (98.0)
240	32.2 (100)	ND	ND	ND	32.2 (97.5)
313	31.5 (100)	ND	ND	ND	31.5 (95.3)
480	30.1 (93.5)	ND	2.08 (6.5)	ND	32.2 (97.5)
672	29.2 (91.0)	ND	2.90 (9.0)	ND	32.1 (97.3)
840	27.4 (83.3)	ND	4.90 (14.9)	0.62 (1.8)	32.9 (99.8)
1008	25.7 (75.8)	ND	6.72 (19.8)	1.49 (4.4)	33.9 (103)
1176	25.2 (72.4)	ND	7.64 (22.0)	1.99 (5.6)	34.8 (106)
1488	24.2 (70.8)	ND	7.80 (22.8)	2.24 (6.4)	34.2 (104)
1680	23.5 (67.7)	ND	8.42 (24.3)	2.80 (8.0)	34.7 (105)

[a] Vinclozolin, M1, M2, or M3 as percent of total of vinclozolin + M1 + M2 + M3. [b] M1 = 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid, M2 = 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide, M3 = 3,5-dichloroaniline, and total = vinclozolin + M1 + M2 + M3. [c] Vinclozolin + M1 + M2 + M3 at time t expressed as percent of vinclozolin + M1 + M2 + M3 at time 0. [d] ND = not detected.

The disappearance of M1 was pseudo-first-order for the first 46 h. The calculated pseudo-first-order rate constant was 2.69×10^{-2} h^{-1}, and the second-order rate constant was 8.51×10^{7} M^{-1} h^{-1}. During this period, M1 was mainly converted to vinclozolin and the rate constant was about 20× the rate constant (1.30×10^{-3} h^{-1}) for the hydrolysis of vinclozolin under identical temperature and pH. After the first 46 h the disappearance of M1 appeared to deviate from pseudo-first-order, presumably because more than 60% of the M1 was converted to vinclozolin after 46 h, and the reverse conversion of vinclozolin to M1 was then significant. The time lapse of more than 22 h between the appearance of vinclozolin and M2 clearly demonstrated that M2 was not converted from M1 but from vinclozolin.

At pH 8.0, M1 was very stable in the aqueous buffer and the conversion product, M2, was first detected after 20 days (480 h) (Table IX). The other conversion product M3 first appeared after 35 days (840 h). The concentrations of both M2 and M3 increased steadily throughout the study, and vinclozolin was never detected in the aqueous buffer. After 70 days (1680 h) the concentrations of M1, M2, and M3 accounted for approximately 68, 24, and 8% of the total, respectively. These results clearly showed that M1 was stabilized by the basic medium and that what little vinclozolin may have formed would be converted back to M1 because of the high rate constant for the hydrolysis of vinclozolin at 35 °C and pH 8.0 (0.599 h^{-1}) (Table I). Although vinclozolin was not detected, it was conceivable that very minute amounts were produced and then partly converted to M2. The concentration of M2 increased slowly to the limit of detection by HPLC, so that it was first detected in the aqueous buffer after 20 days (480 h).

The effect of pH on the conversion of M1 to vinclozolin by recyclization was further demonstrated in a separate experiment. After preincubation at 35 °C and pH 8.0 for 24 h, half of the samples were acidified to pH 4.5. Conversion of M1 to vinclozolin occurred immediately after the acidification, and the concentrations of M1, vinclozolin, M2, and M3 became similar to those detected in the earlier studies conducted at 35 °C and pH 4.5 with a 24-h delay.

On the basis of results of the incubation studies with both vinclozolin and M1, a degradation pathway was proposed for the hydrolysis of vinclozolin leading to the formation of M1 and M2 (Figure 1). On hydrolysis the 2,4-oxazolidine ring opens to yield both the butenoic acid (M1) and the enanilide (M2). The conversion to the butenoic acid is reversible, which leads to the formation of vinclozolin by recyclization. At basic pH (8.0) the forward reaction leading to the formation of the butenoic acid was favored. At acidic pH (4.5) the reverse reaction, i.e., recyclization to form vinclozolin, is favored.

Registry No. M1, 119209-27-7; M2, 83792-61-4; M3, 626-43-7; vinclozolin, 50471-44-8.

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Persistence of the Fungicide Vinclozolin on Pea Leaves under Laboratory Conditions

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When pea leaflets were treated with Ronilan 50 WP or an acetone solution of vinclozolin under laboratory conditions, the fungicide persisted 21–46 days. Its persistence was higher with Ronilan 50 WP, a commercial formulation of vinclozolin, than with an acetone solution. However, most of the Ronilan deposits were easily dislodged by rinsing with water, indicating that Ronilan was susceptible to weathering. The dissipation of vinclozolin on leaves was linear, and the calculated half-life was 33.1 days for Ronilan and 13.4 days for the acetone solution. Translocation of vinclozolin was not detected in pea plants after its application to one of the leaflets. None of the hydrolytic degradation products, namely 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid (M1), 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2), and 3,5-dichloroaniline, were detected in the treated plants. However, vinclozolin, M1, and M2 were detected in leaves of pea and bean grown in nutrient solutions containing either vinclozolin or its degradation product M1.

Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione] is marketed by BASF AG as a protectant fungicide under the trade name Ronilan. It is effective in the control of diseases caused by *Botrytis* spp., *Sclerotinia* spp., and *Monilinia* spp. in grapes, fruits, vegetables, ornamentals, hops, rapeseed, and turfgrass (Spencer, 1982). This fungicide is widely used in Europe for controlling fungal diseases; and it is registered in the United States, but not in Canada.

Vinclozolin is known to undergo hydrolysis. Three degradation products, namely 2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (M1), 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2), and 3,5-dichloroaniline (M3), have been isolated from hydrolysis and identified by us (Figure 1) (unpublished data); and the kinetics of hydrolysis at various pHs and temperatures have been determined (Melkebeke et al., 1986; Szeto et al., 1989). According to Clark (1983) both M1 and M2 were noninhibitory in vitro against mycelial growth of *Botrytis cinerea*, indicating that they have no antifungal activity. M3 is a chlorinated aromatic amine that may be toxic to higher animals, and its possible formation is important to environmental toxicologists. Therefore, it is important to know whether these degradation products are formed in plants after application of vinclozolin.

This paper reports the findings of our studies under laboratory conditions on the persistence of vinclozolin and Ronilan 50 WP in garden pea, *Pisum sativum* L., and on translocation in garden pea and red kidney bean, *Phaseolus vulgaris* L., grown in nutrient solutions containing either vinclozolin or M1.

EXPERIMENTAL SECTION

Preparation of Vinclozolin and Its Hydrolytic Degradation Products. Vinclozolin, 2-[[(3,5-dichlorophenyl)carbamoyl]]oxy-2-methyl-3-butenoic acid, and 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide were obtained from BASF Aktiengessellschaft, and their purities were respectively, 99%, 98%, and 96%. 3,5-Dichloroaniline (98%) was obtained from the Laboratory Services Division of Agriculture Canada in Ottawa. Ronilan 50 WP was provided by BASF Canada Inc.

Growing Plants. Garden pea, *P. sativum* (cv. Improved Laxton's Progress), were seeded in square pots (10 cm × 10 cm × 9 cm) and grown in a greenhouse. Twelve days after seeding, the plants were used to study the persistence of vinclozolin and Ronilan 50 WP. For the

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