Table III. Juvenile Hormone Activity of Oxahomofarnesanes

compd	dose, μg/nymph	% JH act.
I	1.0	68
II	1.0	65
III	1.0	65
methoprene	0.2	75

comparison. Ten insects were used for each concentration, and it was repeated three times. From the experimental data (Table III) it is evident that synthetic oxahomofarnesanes I–III had activity comparable to that of methoprene.

HPLC Separation. Oxahomofarnesanes I–III have been separated by high-performance liquid chromatography using a μ-Bondapak C_{18} column (stainless steel, 300 mm × 3.9 mm (i.d.)) with particle size 10 μm under varying conditions of eluant concentration (methanol–water). Adequate resolution of the above mixtures was observed with methanol–water (4:1), and the retention times have been shown in Table II.

RESULTS AND DISCUSSION

Acetylation of citronellol (1) with acetic anhydride in pyridine at room temperature gave citronellyl acetate (2). This was subjected to oxymercuration–demercuration reaction (Brown and Rei, 1969) to afford 7-methoxycitronellol (3). This on subjection to a O-alkylation reaction with propargyl bromide provided alkynyl ether **5** in more than 80% yield. The next step involved conversion of the terminal acetylene to the corresponding methyl ketone **7**. This was brought about by a mercuric sulfate catalyzed reaction (Thomas et al., 1938) in 80% yield.

Following a similar sequence of reactions, citronellol (1) and dihydrocitronellol (4) were converted to their corresponding oxa ketones **6** and **10**, respectively. Compounds I–III were prepared in a 60:40 *E*:*Z* ratio over 80% yield by a Wittig–Horner reaction between respective methyl ketones and triethyl phosphonoacetate. The isomers were exclusively separated on a silica gel column impregnated with silver nitrate to afford the corresponding *E* and *Z* isomers, which were designated with suffixes a and b, respectively. These were in more than 98% purity as confirmed by its GLC analysis. The structures of the *Z* and *E* isomers of I–III were fully characterized on the basis of their NMR spectra, and the characteristic ^1H NMR signals are shown in Table I. It is evident from the table that the chemical shift values of proton signals at C-2 and C-3 methyls of *Z* isomers fall downfield whereas C-4 fall upfield compared to that of *E* isomers.

ACKNOWLEDGMENT

R.R.I. thanks the Department of Atomic Energy for the award of a research fellowship.

Registry No. (±)-1, 26489-01-0; (±)-2, 67650-82-2; (±)-3, 120444-95-3; (±)-4, 59204-02-3; (±)-5, 120411-85-0; (±)-6, 120411-86-1; (±)-7, 120411-87-2; (±)-8, 120411-88-3; (±)-9, 120411-89-4; (±)-10, 120444-96-4; (±)-Ia, 120411-90-7; (±)-Ib, 120411-93-0; (±)-IIa, 120411-91-8; (±)-IIb, 120411-94-1; (±)-IIIa, 120411-92-9; (±)-IIIb, 120411-95-2.

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Received for review July 25, 1988. Accepted January 17, 1989.

Identification of Hydrolysis Products of the Fungicide Vinclozolin by Spectroscopic and X-ray Crystallographic Methods

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The hydrolysis of vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione], a dicarboximide fungicide, at 20 °C in 0.1 M phosphate buffers of pH 5.0, 7.0, and 9.0 gives three products, identified as 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). The identity of M3 was confirmed by gas chromatography–mass spectrometry. M1 and M2 were isolated from a 0.1 M phosphate buffer of pH 7.0 after incubation at 35 °C for 7 days. After purification, their identities were confirmed by solid-probe mass spectrometry and proton and ^{13}C NMR spectrometry. Furthermore, the identity of M1 was confirmed by unambiguous evidence from X-ray crystallography of its ethyl ester.

Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione] (Figure 1a) was introduced by BASF AG of Germany in 1975 under the code number BAS 35204F. Its fungicidal properties against *Botritis cinerea* were first reported by Pommer and Mangold (1975) and by Hess and Locher (1975). Subsequent research showed that vinclozolin is effective in the control of diseases in grapes, fruits, vegetables, ornamentals, hops, rapeseed, and turfgrass caused by *Botritis spp.*, *Sclerotinia spp.*, and *Monilinia spp.* (Spencer, 1982). Since its introduction vinclozolin has been widely used in Europe for the control of fungal diseases. This fungicide is currently registered in the United States, but not in Canada.

Little was known about the degradation of vinclozolin in the environment. Clark (1983) reported that the oxazolidine ring of vinclozolin was opened in ethanolic and

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0021-8561/89/1437-1103$01.50/0 Published 1989 by the American Chemical Society

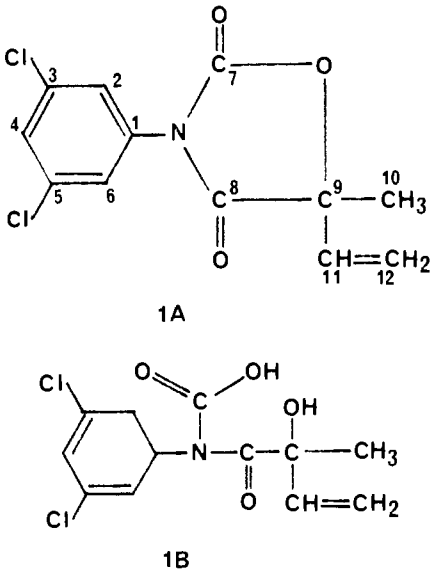

Figure 1. Vinclozolin (A) and *N*-(2-hydroxy-2-methyl-1-oxobuten-3-yl)-3,5-dichlorophenyl-1-carbamic acid (B).

methanolic solutions and water suspension. The reaction products in ethanol and methanol were ethyl- and methyl-*N*-(2-hydroxy-2-methyl-1-oxobuten-3-yl)-3,5-dichlorophenyl carbamate, respectively. Two products were identified from water suspension, *N*-(2-hydroxy-2-methyl-1-oxobuten-3-yl)-3,5-dichlorophenyl-1-carbamic acid (Figure 1B) and its decarboxylation product *N*-3,5-dichlorophenyl-2-hydroxy-2-methylbut-3-enamide (Figure 2a). Clark (1983) synthesized these two products and characterized them by mass spectrometry and proton NMR; the degradation products in water suspension were identified by chromatography with the two synthesized products as reference standards.

The present study describes the degradation of vinclozolin in buffers; three products have been isolated and their identities confirmed by mass spectrometry, proton and ^{13}C NMR spectrometry, and X-ray crystallography.

EXPERIMENTAL SECTION

Preparation of Vinclozolin. Two grams of Ronilan 50 WP, a commercial formulation of vinclozolin, was divided into four aliquots of approximately 500 mg each in 50-mL centrifuge tubes. Each aliquot was extracted with 25 mL of glass-distilled acetone for 1 h in an ultrasonic water bath. After extraction, the acetone suspensions were centrifuged at about 2000 rpm in a bench-top centrifuge for 10 min, and the supernatants were passed through glass fiber filter paper. The combined filtrates were again centrifuged and filtered until the acetone solution was clear. The acetone solution was concentrated to about 50 mL in a flash evaporator at 38 °C, followed by the addition of 450 mL of glass-distilled water. At 4 °C vinclozolin crystals were allowed to precipitate from the aqueous solution, which contained 10% acetone. The precipitates were filtered through glass fiber filter paper and recrystallized from hexane at 4 °C. Crystals of vinclozolin were harvested by filtering through glass fiber filter paper and dried under a gentle stream of nitrogen overnight at room temperature.

The purity of the crystals was acertained by melting point determination, high-pressure liquid chromatography (HPLC), and gas–liquid chromatography (GLC). HPLC analysis was performed with a Varian Model 5000 high-pressure liquid chromatograph equipped with a Hewlett-Packard Model 1040A high-speed spectrophotometric 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% water, isocratic at 1 mL/min; UV detector wavelength, 212 ± 2 nm; UV–visible absorption spectra were measured at up-slope, apex, and down-slope of each chromatographic peak to determine peak purity.

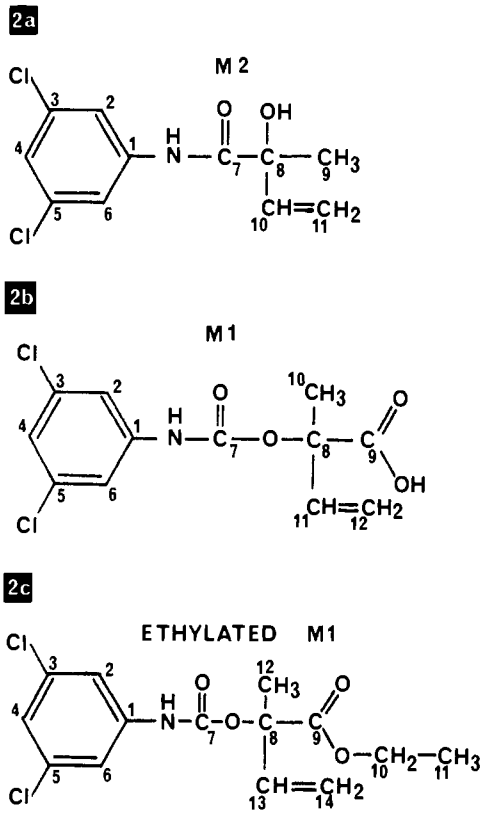

Figure 2. 3′,5′-Dichloro-2-hydroxy-2-methylbut-3-enanilide (a); 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid (b); and 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methylbut-3-enoate (c).

GLC analysis was performed with a Hewlett-Packard Model 5890 gas–liquid chromatograph equipped with a ^{63}Ni electron capture detector. The operating parameters were as follows: column, DB-17 fused silica capillary column, 30 m × 0.253 mm (i.d.) from J & W Scientific, Inc.; column head pressure, 140 kPa with a total flow of 40 mL of helium/min; column temperature, programmed from 70 to 225 °C at 20 °C/min with the final temperature held for 15 min; injector temperature, 225 °C; detector temperature, 300 °C; makeup gas, 5% methane in argon at 20 mL/min; injection, splitless.

Hydrolysis of Vinclozolin. A stock solution of vinclozolin at 1000 μg/mL was prepared in glass-distilled methanol. Aliquots of 1 mL of the stock solution were thoroughly mixed with the 0.1 M phosphate buffers of pH 5.0, 7.0, and 9.0 in 100-mL volumetric flasks to give concentrations of 10 μg of vinclozolin/mL. These three solutions were incubated at 20 °C in a water bath. The volumetric flasks were wrapped in aluminum foil to shield them from light. After incubation at 20 °C for 7 and 30 days, an aliquot of 20 μL of each incubated solution was injected directly into a high-pressure liquid chromatograph for the determination of vinclozolin and any degradation products formed in the aqueous solutions.

Isolation and Identification of Degradation Products. A 500-mg portion of crystalline vinclozolin was added to 1 L of 0.1 M phosphate buffer of pH 7.0 and the resultant mixture heated to about 60 °C until dissolved. The buffered vinclozolin solution was incubated at 35 °C in a water bath for 7 days. At the end of incubation, the amount of vinclozolin remaining in the buffered solution was determined by HPLC. When degradation of more than 90% of the added vinclozolin was indicated, the degradation products were isolated from the buffered solution.

The buffered solution was removed from the water bath, allowed to cool to room temperature, and then extracted three times with 250 mL of glass-distilled dichloromethane. The combined extracts were dried with anhydrous Na_2SO_4, and the degradation product so isolated was designated as M2.

After isolation of M2, the aqueous solution was acidified to about pH 1.0 with concentrated HCl. The acidified solution was extracted three times with 250 mL of glass-distilled dichloro-

methane. The combined extracts were dried with acidified Na_2SO_4, and the degradation product so isolated was designated as M1. The acidified anhydrous Na_2SO_4 was prepared by washing anhydrous Na_2SO_4 with glass-distilled acetone saturated with concentrated H_2SO_4 and then drying at 120 °C in an oven overnight. The dichloromethane solution containing M1 was concentrated to dryness in a flash evaporator at 38 °C, and M1 was recrystallized twice from chloroform. The crystals were harvested by filtering through glass fiber filter paper, dried under a gentle stream of nitrogen for 2 h, and then stored in a desiccator before the melting point was determined and the structure identified.

A chromatographic column (30 cm × 2.5 cm (i.d.)) was prepared by packing from the bottom with a glass wool plug, 2 cm of anhydrous Na_2SO_4, 8 cm of Florisil deactivated with 2% water, 2 cm of anhydrous Na_2SO_4, and another glass wool plug. The packed column was prewashed with 25 mL of 40% dichloromethane in hexane. The dichloromethane solution containing M2 was concentrated to about 8 mL in a flash evaporator at 38 °C, followed by the addition of 12 mL of glass-distilled hexane. The resultant solution was transferred quantitatively to the prewashed column and then eluted three times with 100, 50, and 25 mL of 40% dichloromethane in hexane. The third eluate was concentrated just to dryness in a flash evaporator at 38 °C; the residues were dissolved in acetone and analyzed by GLC. When the presence of degradation product M2 was indicated, the column was eluted with 125 mL of 10% acetone in dichloromethane. The eluate containing degradation product M2 was concentrated just to dryness in a flash evaporator at 38 °C, and the M2 was recrystallized twice from 5% benzene in hexane. The crystals were harvested by filtering through glass fiber filter paper, dried under a gentle stream of nitrogen for 2 h, and then stored in a desiccator before determination of the melting point and identification of the structure.

Preparation of Ethylated M1. Diazoethane was prepared from its precursor, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine. A 300-mg sample of the precursor in 5 mL of glass-distilled ether reacted at room temperature with 2 mL of 5 N NaOH solution in a gas bubbler. One of the two side arms of the gas bubbler was connected to a gentle flow of nitrogen and the other arm connected with Tygon tubing to a Pasteur pipet for dispensing diazoethane. *Since diazoethane is highly toxic, mutagenic, carcinogenic, and potentially explosive, all reactions involving diazoethane were carried out in an efficient fume hood and behind a safety shield.*

A 100-mg portion of M1 was dissolved in 5 mL of glass-distilled dichloromethane in a graduated, glass-stoppered reaction tube and the resultant mixture cooled to about 4 °C in an ice–water bath. Freshly generated diazoethane was gently purged with nitrogen into the dichloromethane solution until a pale yellow color appeared, indicating the presence of excess diazoethane. The reaction mixture was evaporated just to dryness under a gentle stream of nitrogen in a heating block at 40 °C. The ethylated product was recrystallized twice from 5% benzene in hexane. The crystals were harvested by filtering through glass fiber filter paper, dried under a gentle stream of nitrogen for 2 h, and then stored in a desiccator before determination of the melting point and identification of the structure.

The purity of M1 and its ethylated product and M2 was acertained as described for vinclozolin except that the HPLC mobile solvent system consisted of 72% methanol and 28% 0.05 M phosphate buffer of pH 3.3 instead of 72% methanol and 28% water and a Hewlett-Packard Model 5970 mass selective detector was used in the GLC analysis instead of an electron capture detector. The detector temperature was 250 °C, and the scanning mass range was from 40 to 450 amu.

Identification of Degradation Products. The structures of degradation products M1 and M2 were elucidated by analyzing data obtained from solid-probe mass spectrometry, proton and ^{13}C NMR spectrometry, and X-ray crystallography of the ethylated product of M1.

A Kratos Model MS 50 mass spectrometer was used to obtain mass spectra for vinclozolin, M1 and its ethylated product, and M2. The temperature of the solid probe was 120 °C. Proton and ^{13}C NMR spectra of vinclozolin, M1 and its ethylated product, and M2 were obtained, respectively, on a Bruker Model WP-400 spectrometer operating at 400 MHz and a Varian Model XL-300 spectrometer operating at 75 MHz. A 5-mm broad-band probe was used in both spectrometers. Solutions of each compound were prepared in dichloromethane-*d* or chloroform-*d*, to which a small amount of tetramethylsilane (TMS) was added for reference. A crystal of the ethylated product of M1 was selected for crystal structure determination at 22 °C.

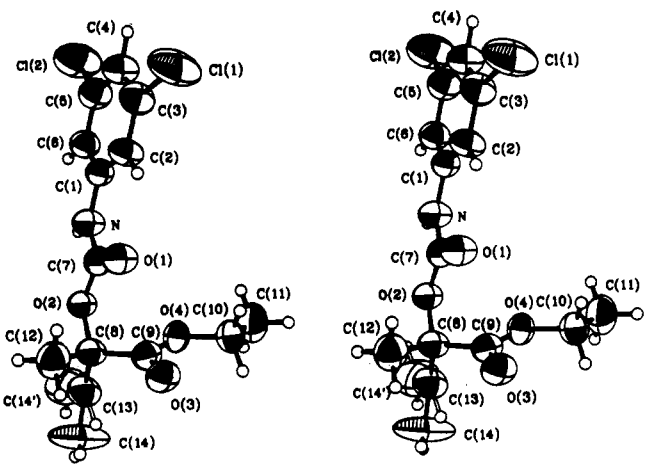

Figure 3. Stereoview of the molecule of the M1 ethyl ester, with 50% probability ellipsoids for the non-hydrogen atoms.

X-ray Crystallographic Analysis of Ethyl 2-[[(3,5-Dichlorophenyl)carbamoyl]oxy]-2-methylbut-3-enoate. A crystal bounded by the 10 faces (followed by their distances (mm) from a common origin) [{100} 0.14, {010} 0.14, {011} 0.19, ±(212) 0.19] was mounted in a general orientation. Unit-cell parameters were refined by least-squares on 2 sin (θ/λ) values for 25 reflections (θ = 22–30°) measured on a diffractometer with Cu Kα radiation ($\lambda(K\alpha_1)$ = 1.540 56 Å). Crystal data at 295 K: $C_{14}H_{15}Cl_2NO_4$; fw = 332.18; monoclinic; a = 10.994 (2), b = 12.488 (2), c = 12.760 (2) Å; β = 112.20 (1)°; V = 1622.0 (5) Å^3; Z = 4; D_x = 1.360 mg m^{-3}; $F(000)$ = 688; μ(Cu Kα) = 38 cm^{-1}; space group $P2_1/c$ (C_{2h}^5–No. 14).

Intensities were measured with nickel-filtered Cu Kα radiation on an Enraf-Nonius CAD$_4$-F diffractometer. An ω-2θ scan at 1.5–10° min^{-1} over a range of (1.00 + 0.14 tan θ)° in ω (extended by 25% on both sides for background measurement) was employed. Data were measured to 2θ = 150°. The intensities of three check reflections, measured every 4000 s throughout the data collection, showed a 4% decay (correction applied). After data reduction, an absorption correction was applied with the Gaussian integration method (Coppens et al., 1965; Busing and Levy, 1967), transmission factors ranging from 0.336 to 0.486 for 224 integration points. Of the 3305 independent reflections measured, 1363 (41%) had $I \geq 2\sigma(I)$, where $\sigma^2(I) = S + 4(B_1 + B_2) + (0.06S)^2$, with S = scan count and B_1 and B_2 = background counts.

The structure was determined by direct methods and refined by full-matrix least-squares techniques. The vinyl group proved to be disordered, with two (50% occupancy) sites for C(14), related by 180° rotation about C(8)–C(13) (Figure 3). In the final stages, the non-hydrogen atoms were refined with anisotropic thermal parameters; hydrogen atoms were fixed in idealized positions, except for H(N), which was refined. Scattering factors for all atoms and anomalous scattering corrections for Cl were taken from the *International Table for X-Ray Crystallography* (1974). The weighting scheme $w = 1/\sigma^2(F)$, where $\sigma^2(F)$ is derived from the previously defined $\sigma^2(I)$, gave uniform average values of w ($|V_o| - |F_c|)^2$ over ranges of both $|F_o|$ and sin (θ/λ) and was employed in the final stages of refinement of 203 variables using 1363 reflections with $I \geq 2\sigma(I)$. Convergence was reached at R = 0.066 and R_w = 0.067 for the 1363 reflections (R = 0.154 for all 3305 data). On the final cycle of refinement the mean and maximum shifts were 0.13 and 0.68σ, respectively, and a final difference map showed maximum fluctuations of ±0.3 e Å^{-3}. The mean error in an observation of unit weight was 1.12. Final positional parameters are given in Table I.

RESULTS AND DISCUSSION

Hydrolysis of Vinclozolin. With use of 99% pure vinclozolin, its hydrolysis was carried out at 35 °C in

Table I. Final Positional (Fractional × 10^4, H × 10^3) and Isotropic Thermal Parameters ($U \times 10^3$, Å^2) with Estimated Standard Deviations in Parentheses

atom	x	y	z	U_{eq}/U_{iso}
Cl(1)	10854 (2)	6959 (2)	3560 (2)	128
Cl(2)	11478 (2)	2779 (2)	4510 (2)	135
O(1)	6069 (3)	6032 (3)	2086 (4)	70
O(2)	4804 (3)	4581 (2)	2029 (3)	58
O(3)	3355 (3)	7113 (3)	1836 (3)	72
O(4)	4331 (4)	6062 (3)	3343 (4)	65
N	6956 (5)	4392 (4)	2738 (5)	62
C(1)	8314 (5)	4597 (4)	3123 (5)	57
C(2)	8824 (5)	5597 (4)	3098 (5)	67
C(3)	10197 (6)	5699 (5)	3548 (6)	74
C(4)	11020 (6)	4854 (6)	3960 (6)	81
C(5)	10462 (6)	3876 (5)	3958 (5)	76
C(6)	9113 (6)	3726 (4)	3547 (5)	68
C(7)	5964 (5)	5095 (4)	2261 (5)	58
C(8)	3630 (5)	5248 (4)	1543 (5)	59
C(9)	3775 (6)	6248 (5)	2239 (7)	61
C(10)	4557 (6)	6974 (5)	4101 (6)	77
C(11)	4988 (7)	6581 (5)	5256 (7)	98
C(12)	3347 (6)	5479 (5)	313 (6)	83
C(13)	2546 (7)	4594 (6)	1681 (7)	82
C(14)[a]	1341 (13)	4745 (14)	1218 (18)	156
C(14')[a]	2546 (18)	3719 (18)	1910 (20)	153
H(N)	673 (4)	383 (3)	285 (4)	37 (15)

[a] Occupancy factor 0.5.

phosphate buffers of pH 5.0, 7.0, and 9.0. Regardless of pH, approximately 90% of vinclozolin disappeared after 7 days and two new peaks (M1 and M2) were detected by HPLC. One of them (M1) had its retention time changed from 3.48 to 5.04 min when 0.05 M phosphate buffer of pH 3.3 was used instead of water for the mobile solvent system. The longer retention time obtained with acidified water in reversed-phase HPLC suggests a carboxylic function in the unknown M1.

After incubation for 30 days at pH 5.0, 7.0, and 9.0, 3,5-dichloroaniline was detected as a minor degradation product. Its identity was confirmed by GC–MS with an authentic reference standard obtained from the Laboratory Services Division of Agriculture Canada in Ottawa.

Isolation and Identification of Degradation Products. The melting point, HPLC, and GC–MS analyses of M1 and M2 isolated from the hydrolysis reaction mixture indicated >98% purity.

Vinclozolin consists of a 3,5-dichlorophenyl and a 2,4-oxazolidinedione moiety. It was hypothesized that on hydrolysis 3,5-dichloroaniline could be produced and the oxazolidinedione ring may open. In fact, 3,5-dichloroaniline was identified and confirmed as a minor product, and two major products, M1 and M2, were isolated, which might have resulted from the cleavage of the 2,4-oxazolidinedione ring. According to the review by Clark-Lewis (1958) it is generally believed, without proof, that the hydrolysis of 3-alkyl-2,4-oxazolidinedione will lead to amides, whereas the hydrolysis of 3-phenyl-2,4-oxazolidinedione yields phenylurethanes. For vinclozolin it is hypothesized that upon hydrolysis the 2,4-oxazolidinedione ring opens to yield 2-[[(3,5-dichlorophenyl)carbamoyl]-oxy]-2-methyl-3-butenoic acid (Figure 2b) and 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide (Figure 2a). The chromatographic behavior of M1 indicated a carboxylic group. Therefore, we felt that M1 could be the butenoic acid (Figure 2b) and M2 the enanilide (Figure 2a).

Identification of M2. Solid-probe mass spectral data of M2 with the major ions (amu) and their relative intensity are as follows: 259 (29.0%), 217 (17.2%), 187 (25.9%), 161 (13.8%), 71 (100%). The ratios of the isotopes indicated the presence of two chlorines, and the major ions were consistent with the enanilide (Figure 2a). Ions at 259 amu represented the molecular ion. Ions at 217 amu indicated the combined loss of CH_3 (15 amu) and $CH{=}CH_2$ (27 amu) for a total of 42 amu. Ions at 187 amu indicated the ion fragment of $C_6H_3Cl_2N{=}C{=}O$, which was commonly found in mass spectra of (dichlorophenyl)urea herbicides such as linuron, diuron, and neburon (Hites, 1985). Ions at 161 amu indicated that 3,5-dichloroaniline ion fragment resulting from a single proton transfer coupled with elimination of a neutral particle (Schlunegger, 1980). The dichloroaniline ion fragment was observed in mass spectra of many dichlorophenylanilides such as *N*-(3,4-dichlorophenyl)-2′-methyl-2′,3′-dihydroxypropionamide, *N*-(3,4-dichlorophenyl)-2′-methylvaleramide, and *N*-(3,4-dichlorophenyl)-2′-methyl-3′-hydroxyvaleramide (Safe and Hutzinger, 1973). Ions at 71 amu indicated the ion fragment of $C(CH_3)(CH{=}CH_2)OH$.

The proton NMR spectrum of M2 was consistent with the enanilide (Figure 2a) as indicated by the following chemical shifts (ppm): 1.58, singlet, 3 H (aliphatic CH_3); 2.48, singlet (OH); 5.25–5.48, doublet of doublets (vinylic CH_2); 6.13-6.25, doublet of doublets (vinylic CH); 7.10, triplet, 1 H (aromatic CH at the para position); 8.60, singlet (NH). The peaks of OH and NH were very broad and appeared at varying positions depending upon solute concentrations and solvent conditions, which may be attributed to the occurrence of both intra- and intermolecular hydrogen bonding. Similarly, the ^{13}C NMR spectrum of M2 was also consistent with the enanilide as indicated by the following chemical shifts (ppm): 26.2 (C_9); 81.1 (C_8); 118.2 (C_{11}); 146.5 (C_{10}); 139.8 (C_1); 128.2 (C_2 and C_6); 146.3 (C_3 and C_5); 123.0 (C_4); 178.5 (C_7) (Figure 2a).

On the basis of spectral data from MS, ^1H NMR, and ^{13}C NMR, the degradation product M2 isolated from hydrolysis of vinclozolin was identified as 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanilide. Our findings are in agreement with Clark (1983) who identified the enanilide present in the water suspension of Ronilan.

Identification of M1. Solid-probe mass spectral data with the major ions (amu) and their relative intensity are as follows: 303 (1.4%), 285 (3.4%), 187 (100%), 161 (50.7%), 99 (16.8%), 71 (59.6%). The ratios of the isotopes indicated the presence of two chlorines, and the major ions were consistent with the butenoic acid (Figure 2b). Ions at 303 amu represented the molecular ion. Ions at 285 amu indicated recyclization to yield vinclozolin by eliminating water (18 amu). Recyclization of M1 to yield vinclozolin was also demonstrated simply by heating M1 at 120 °C for 5 min. The ion fragment at 187 amu indicated $C_6H_3Cl_2N{=}C{=}O$ commonly found in mass spectra of (dichlorophenyl)urea herbicides (Hite, 1985). The mass spectrum of the enanilide M2 (Figure 2a) also showed this ion fragment of 187 amu. Ions at 161 amu indicated the 3,5-dichloroaniline ion fragment resulting from a single proton transfer coupled with elimination of a neutral particle as discussed previously in the identification of the enanilide (Schlunegger, 1980). Ions at 99 amu indicated the ion fragment of $C(CH_3)(CH{=}CH_2)COOH$, and ions at 71 amu indicated the ion fragment of $OCH(CH_3)(CH{=}CH_2)$ resulting from loss of CO_2 from ion fragment $OC(CH_3)(CH{=}CH_2)COOH$, a product of α-cleavage along the carbonyl carbon (Creswell et al., 1972).

The mass spectral data of M1 were inconsistent with the carbamic acid (Figure 1B), which cannot undergo rearrangement to yield the 3,5-dichloroaniline ion fragment of 161 amu because there is no NH moiety in its structure (Schlunegger, 1980).

Table II. Bond Lengths (Å) and Angles (deg), with Estimated Standard Deviations in Parentheses

Cl(1)–C(3)	1.730 (6)	C(1)–C(6)	1.375 (7)
Cl(2)–C(5)	1.739 (6)	C(2)–C(3)	1.403 (7)
O(1)–C(7)	1.205 (6)	C(3)–C(4)	1.360 (8)
O(2)–C(7)	1.357 (6)	C(4)–C(5)	1.367 (8)
O(2)–C(8)	1.462 (5)	C(5)–C(6)	1.387 (7)
O(3)–C(9)	1.210 (6)	C(8)–C(9)	1.505 (7)
O(4)–C(9)	1.328 (7)	C(8)–C(12)	1.509 (8)
O(4)–C(10)	1.453 (6)	C(8)–C(13)	1.509 (8)
N–C(1)	1.407 (6)	C(10)–C(11)	1.452 (8)
N–C(7)	1.352 (6)	C(13)–C(14)	1.244 (14)
C(1)–C(2)	1.374 (6)	C(13)–C(14′)	1.13 (2)
N–H(N)	0.78 (4)		
C(7)–O(2)–C(8)	115.4 (4)	O(1)–C(7)–N	126.6 (5)
C(9)–O(4)–C(10)	117.6 (5)	O(2)–C(7)–N	108.9 (5)
C(1)–N–C(7)	127.4 (5)	O(2)–C(8)–C(9)	110.1 (4)
N–C(1)–C(2)	123.1 (5)	O(2)–C(8)–C(12)	110.6 (5)
N–C(1)–C(6)	115.4 (5)	O(2)–C(8)–C(13)	104.6 (4)
C(2)–C(1)–C(6)	121.5 (5)	C(9)–C(8)–C(12)	112.9 (5)
C(1)–C(2)–C(3)	117.3 (5)	C(9)–C(8)–C(13)	107.0 (5)
Cl(1)–C(3)–C(2)	117.8 (5)	C(12)–C(8)–C(13)	111.3 (5)
Cl(1)–C(3)–C(4)	119.2 (5)	O(3)–C(9)–O(4)	123.7 (6)
C(2)–C(3)–C(4)	123.0 (5)	O(3)–C(9)–C(8)	123.5 (6)
C(3)–C(4)–C(5)	117.4 (5)	O(4)–C(9)–C(8)	112.6 (5)
Cl(2)–C(5)–C(4)	119.0 (5)	O(4)–C(10)–C(11)	108.6 (5)
Cl(2)–C(5)–C(6)	118.5 (5)	C(8)–C(13)–C(14)	127.5 (10)
C(4)–C(5)–C(6)	122.5 (6)	C(8)–C(13)–C(14′)	129.0 (12)
C(1)–C(6)–C(5)	118.3 (5)	C(14)–C(13)–C(14′)	99.5 (14)
O(1)–C(7)–O(2)	124.5 (5)	C(7)–N–H(N)	114 (3)
Cl(1)–N–H(N)	118 (3)		

The ^{1}H NMR spectrum of M1 was consistent with the butenoic acid (Figure 2b) as indicated by the following chemical shifts (ppm): 1.72, singlet, 3 H (aliphatic CH_3); 5.26–5.48, doublet of doublets (vinylic CH_2); 6.24–6.30, doublet of doublets (vinylic CH); 7.10, triplet, 1 H (aromatic CH at the para position); 9.22, singlet (NH); 2.80 and 11.4, singlet (carboxylic proton) (Figure 2b). The peaks of NH and carboxylic proton were very broad. The fact that the carboxylic proton peaks appeared at varying positions, namely 2.80 and 11.4 ppm, may be attributed to the occurrence of both intra- and intermolecular hydrogen bonding. Similarly the ^{13}C NMR spectrum of M1 was also consistent with the butenoic acids as indicated by the following chemical shifts (ppm): 22.7 (C_{10}); 115.9 (C_{12}); 142.5 (C_{11}); 80.6 (C_8); 152.9 (C_7); 172.7 (C_9); 138.5 (C_1); 117.4 (C_2 and C_6); 123.0 (C_4); 135.8 (C_3 and C_5) (Figure 2b). The ^{13}C NMR spectrum of M1 was inconsistent with the carbamic acid (Figure 1B). The chemical shift of the ortho carbons of the carbamic acid would be in the range 125–128 ppm. Many *N*-(3,5-dichlorophenyl)dicarboximides showed chemical shifts at about 125–128 ppm for carbons ortho to the nitrogen (Bremser, 1985; Bremser and Fachinger, 1985). For example, the chemical shift for the two ortho carbons (C_2 and C_6) was 125.6 ppm for vinclozolin (Figure 1A). The actual measurement of the chemical shift of the two ortho carbons (C_2 and C_6) of M1 (Figure 2b) was 117.4 ppm, which did not agree with the predicted chemical shift for the carbamic acid (Figure 1B).

Based on the spectral data of MS and ^{1}H and ^{13}C NMR, the degradation product M1 isolated from hydrolysis of vinclozolin was identified as 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid. Our findings differed from those reported by Clark (1983). He synthesized *N*-(2-hydroxy-2-methyl-1-oxobuten-3-yl)-3,5-dichlorophenyl-1-carbamic acid (Figure 1B) by modifying the method of Sumida et al. (1973) for its 1-oxopropan-2-yl analogue. On the basis of the method of synthesis by Clark, it is highly likely that the compound he synthesized is the same compound as M1. Indeed, the mass spectrum and the proton NMR spectrum Clark presented are similar to those of M1. It is apparent that there are disagreements in the structure elucidation. The proton NMR data are consistent with either the carbamic acid or the butenoic acid. The chemical shifts of the OH proton and NH proton may appear at varying positions due to intra- and intermolecular hydrogen bonding. However, the ^{13}C NMR and MS data are consistent with the butenoic acid but inconsistent with the carbamic acid as discussed previously. In order to confirm the identity of M1, the crystal structure of ethylated M1 was determined by X-ray crystallography.

Crystal Structure. The molecule of the ethyl ester of M1 (Figure 3) consists of two approximately planar parts: (i) the aromatic ring and the extended side chain through to the vinyl group; (ii) the carbethoxy group through to the C(12) methyl group. These two molecular fragments make an angle of about 70°. In more detail, the aromatic ring is essentially planar, maximum displacement of C atoms 0.0137 (7) Å; Cl(1), Cl(2), and N are slightly displaced from the ring plane by 0.048 (2), 0.018 (2), and 0.031 (5) Å, respectively, all in the same direction. The C(1)–NH–C(7)O_2 grouping is also planar and makes an angle of only 3° with the plane of the aromatic ring. The carboxyl group C(8)–C(9)O_2 is slightly nonplanar, C(9) displaced 0.026 (7) Å, with larger displacements of C(10), C(11), and C(12), 0.062 (7), −0.180 (9), and 0.396 (7) Å, respectively.

The molecular conformation is such that the O(1)···C(9) intramolecular distance is very short, at 2.617 (6) Å. This conformation requires a rotation of 180° about O(2)–C(7) to produce the geometry with N in proximity to C(9), which would result from the breaking of the N–C(9) bond in the oxazolidine ring of vinclozolin. Bond lengths and angles (Table II) in the molecule are generally close to expected values, except for those in the vinyl group, which are unreliable as a result of the disorder of C(14). In the crystal the molecules are linked into chains along *b* by N–H···O(3) hydrogen bonds: N–H = 0.78 (4), H···O = 2.19 (4), N···O = 2.941 (6) Å, N–H···O = 163°. The shortest Cl···Cl intermolecular contact is 3.922 (3) Å.

The X-ray crystallography of ethylated M1 confirmed the structure of M1 to be 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid.

ACKNOWLEDGMENT

We thank the Natural Sciences and Engineering Research Council of Canada for financial support, the University of British Columbia Computing Centre for assistance, and U. Ohnsorge of BASF Aktiengesellschaft for providing ^{13}C NMR data of various dichlorophenyl dicarboximides.

Registry No. 1A, 50471-44-8; **2a**, 83792-61-4; **2b**, 119209-27-7; **2c**, 121013-71-6; *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine, 4245-77-6.

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Received for review October 4, 1988. Accepted January 31, 1989.

Absorption, Distribution, and Metabolism of [^{14}C]Chlorpyrifos Applied Dermally to Goats

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Radiolabeled chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] was applied dermally to two male weanling goats, at a dose of 22 mg/kg of body weight. A blood sample was drawn before dosing and every 4 h thereafter. The animals were sacrificed approximately 18 h after dosing, when blood radioactivity levels at 16 h had declined from maximum values (12 h) in both animals. Radioanalysis of blood and selected tissues (liver, kidney, heart, fat, muscle) indicated that radioactivity levels were, in general, very low, ranging from 0.04 ppm (chlorpyrifos equivalents) in muscle to 0.90 ppm in omental fat. Tissue extracts contained 80–96% of the ^{14}C residue, most of which was organosoluble. High-performance liquid chromatography analysis of tissue extracts showed that the predominant ^{14}C residue in liver and kidney was [^{14}C]-3,5,6-trichloro-2-pyridinol (chlorpyridinol) whereas [^{14}C]chlorpyrifos was predominant in fat and heart extracts. In muscle, in addition to approximately equal amounts of [^{14}C]chlorpyridinol and [^{14}C]chlorpyrifos, 18.6% of the radioactivity was unidentified; alkaline hydrolysis quantitatively converted the latter radioactivity to pyridinol.

Chlorpyrifos, *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate, is the active insecticidal ingredient in Lorsban and Dursban insecticides, products of Dow Chemical U.S.A. (Midland, MI). These products have a variety of applications including dermal administration to ruminants for the control of various insect pests (Kenaga, 1974). Since such dermal exposure may result in residues of chlorpyrifos and its metabolites in edible tissues of ruminants, it is of interest to determine the magnitude and nature of those residues.

Ivey et al. (1978) reported the magnitude of residues of chlorpyrifos and its metabolite 3,5,6-trichloro-2-pyridinol (chlorpyridinol) in tissues of cattle that had a 10% chlorpyrifos-impregnated plastic band attached to each ear. Additional studies of the dermal absorption of chlorpyrifos include that of Ivey and Palmer (1979), in which residues of chlorpyrifos and pyridinol were measured in tissues of swine treated with Dursban 44 insecticide formulation and that of Nolan et al. (1983) in which the kinetics of the absorption of chlorpyrifos and pyridinol was studied following dermal administration to human volunteers. In addition, Shah et al. (1981) included [^{14}C]chlorpyrifos in their study of the comparative rates of dermal penetration of insecticides in mice.

The present report contains the results of a study conducted to determine the magnitude and nature of ^{14}C residues in tissues of goats following dermal application of [^{14}C]chlorpyrifos.

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MATERIALS AND METHODS

Chemicals. [^{14}C]Chlorpyrifos (A) (specific activity 15.7 mCi/mmol), blank Dursban 44 formulation, and nonlabeled reference standards chlorpyridinol (B), *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphate (C), 3,5,6-trichloro-2-methoxypyridine (D), and sodium *O*-ethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (E) were provided by the Agricultural Products Department, Dow Chemical U.S.A. The chemical structures are in Figure 1.

All solvents used were high-performance liquid chromatography (HPLC) grade; other chemicals were reagent grade from standard sources. Insta-Gel and Perma-Fluor V liquid scintillation cocktails, Carbo-Sorb carbon dioxide absorber, and Spec-Chec-^{14}C radiocarbon standard were products of Packard Instrument Co. (Downers Grove, IL).

Experimental Animal Procedures. Two male goats, weighing 15–18 kg, were purchased from Sunshine Farms, Portage, WI. The animals were acclimated to the test environment (temperature 22–25 °C, humidity 48–68%, light 12 h/day), diet, and management program for 19 days; the goats were gradually acclimated to metabolism cages during this period. From 24 h before application of chlorpyrifos, the goats were housed continuously in metabolism cages.

Approximately 10 h before dose application, an 8-in. × 10-in. patch of hair was shaved behind the right shoulder of each goat. A 4-in.-diameter rubber ring (approximately 0.5 in. in thickness) was glued to the skin with 3M CA-8 cyanoacrylate adhesive (St. Paul, MN). A sealant of Silastic medical adhesive silicone Type A, Dow Corning (Midland, MI), was applied around the outside of the rubber ring.

An appropriate amount of [^{14}C]chlorpyrifos (specific activity 15.7 mCi/mmol) was combined with nonlabeled chlorpyrifos and a blank formulation to yield a dose containing 42.2% active

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