Table III. Toxicity of Red Squill Aglycons to Charles River CD Rats (2-Day Criteria)

preparation	LD_{50},[a] mg/kg	% scilla compd[b]	ratio male to female
scillirosidin			
male	4.2 (3.7–4.7)		
desacetylscillirosidin			
female	>5.0		
aglycons 0-112-1[c]			
male	13.0 (9.8–17.3)	4.8	
female	8.1 (4.4–15)	3.0	1.6
aglycons 0-102-3[d]			
male	8.0 (4.4–14.1)	4.8	
female	2.8 (1.8–4.4)	1.7	2.8

[a] Numbers in parentheses represent confidence limits; $N = 5$ rats/dose level. [b] Total scillirosidin plus scilliroside. [c] Naringinase derived: scilliroside, 7%; scillirosidin, 30%; desacetylscillirosidin, 43%. [d] *A. niger* derived: scillirosidin, 60%; desacetylscillirosidin, 32%.

crystalline product containing 1.24 g of scillirosidin, 0.66 g of desacetylscillirosidin, and no unreacted scilliroside as determined by HPLC (Figure 3). Unidentified green and orange fluorescing spots were visible on TLC. It is clear that *A. niger* NRRL 3 produces large amounts of naringinase. Shorter reaction times decrease the amount of desacetylscillirosidin in the cultures.

The toxicity of the aglycon preparation was studied in Charles River CD rats following procedures previously reported (Verbiscar et al., 1986). For these tests, the preparations were administered to male and female rats orally at five dose levels ($N = 5$ rats per dose level). Behavioral effects and lethality were monitored for 2 days. For scillirosidin the dose levels for males ranged from 3.2 to 5.0 mg/kg. The LD_{50} was 4.2 mg/kg with a confidence level of 95% (Litchfield and Wilcoxon, 1949) (Table III). The aglycon preparations from naringinase (0-102-1) and *A. niger* (0-102-3) were administered at five dose levels from 2.5 to 20 mg/kg to both males and females. Toxicity is due to scillirosidin plus residual scilliroside. In accord with prior reports (Rothlin and Schalch, 1952; Verbiscar et al., 1986), the toxicity of scilliroside is higher in females than males. This is also the case for scillirosidin where male to female toxicity ratios are 1.6 and 2.8 for the two aglycon preparations (Table III). On the basis of higher toxicity to females than males, the absence of toxicity of desacetylscillirosidin at 5.0 mg/kg to females is notable. *A. niger* is an effective fungus for the production of naringinase to cleave scilliroside to scillirosidin, but reaction time should be minimized to limit hydrolysis of the 6-acetyl group, which contributes substantially to toxicity of scillirosidin.

ACKNOWLEDGMENT

We acknowledge with thanks the financial support this project received under Grant No. PCM 82-12322 from the National Science Foundation and the concerned interest and guidance of Dr. H. C. Huang of that agency.

Registry No. β-Glucosidase, 9001-22-3; scilliroside, 507-60-8; scillirosidin, 507-59-5; maringinase, 9068-31-9; desacetylscillirosidin, 7004-95-7.

LITERATURE CITED

Chitty, D. *Red Squill, Control of Rats and Mice*; Oxford University: London, 1954; pp 62–100.
Difco Manual, 9th ed.; Difco Laboratories, Inc.: Detroit, MI, 1953; p 245.
Gentry, H. S.; Verbiscar, A. J.; Banigan, T. F. *Econ. Bot.* **1987**, *41*.
Horowitz, R., USDA Laboratory, Pasadena, CA, 1981, personal communication.
Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.
Moyer, A. J. *Appl. Microbiol.* **1953**, *1*, 1–13.
Pazur, J. H.; Knull, H. R.; Cepure, A. *Carbohydr. Res.* **1971**, *20*, 83.
Raper, K. B.; Fennel, D. L. *Genus Asperigillus*; Williams and Wilkins: Baltimore, 1965.
Rothlin, E.; Schalch, W. R. *Helv. Physiol. Acta* **1952**, *10*, 427.
Stoll, A.; Renz, J. *Helv. Chim. Acta* **1942**, *25*, 43.
Stoll, A.; Renz, J. *Helv. Chim. Acta* **1950**, *33*, 286.
Stoll, A.; Renz, J.; Brack, A. *Helv. Chim. Acta* **1951a**, *34*, 397.
Stoll, A.; Renz, J.; Brack, A. *Helv. Chim. Acta* **1951b**, *34*, 2301.
Verbiscar, A. J.; Patel, J.; Banigan, T. F.; Schatz, R. A. *J. Agric. Food Chem.* **1986**, *34*, 973.
von Wartburg, A.; Renz, J. *Helv. Chim. Acta* **1959**, *42*, 1620.

Received for review May 27, 1986. Accepted March 2, 1987.

Novel 1,3,4-Oxadiazol-2(3*H*)-ones as Potential Pest Control Agents

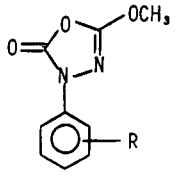
Jamin Huang* and Dean F. Bushey

The biological profile of a series of novel 5-methoxy-1,3,4-oxadiazol-2(3*H*)-ones with aliphatic moieties on nitrogen was determined. Relative to the known insecticidal aromatic analogue, these molecules are considerably less active. On the basis of chemical model studies with both aliphatic and aromatic substituted oxadiazolones, a reactive site is postulated that may be operational at the enzymatic level.

In recent years a number of 3-aryl-5-alkoxy-1,3,4-oxadiazol-2(3*H*)-ones have been claimed as anthelmintics (Boesch, 1978) and insecticides (Boesch, 1979; Tieman, 1981). Compound **1A**, a member of this class, has been widely tested under the code number RP 32,861 and is claimed to be particularly effective in controlling piercing and sucking insects such as aphids and rice hoppers (Ambrosi et al., 1979, 1980). This compound was reported to be an antiesterase agent (Ambrosi et al., 1980). Its enzymatic inhibitory activity on housefly head acetylcholinesterase was found to be $I_{50} = 8 \times 10^{-7}$ M. The oxadiazolone **2** (Tieman, 1981), which is a better inhibitor of

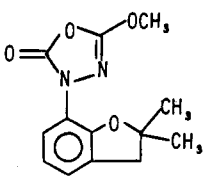
Union Carbide Agricultural Products Company, Inc., Research Triangle Park, North Carolina 27709.

0021-8561/87/1435-0368$01.50/0



1A R = 2-OCH$_3$

1B R = 4-OCH$_3$



2

acetylcholinesterase (I_{50} = 4 × 10^{-9} M), is also a more potent insecticide. This information led us to explore the general class of 5-methoxy-1,3,4-oxadiazol-2(3*H*)-one substituted with aliphatic groups at N-3 to determine whether this kind of activity could be extended to compounds with nonaromatic substituents. This report discusses the biological results of these studies. Also on the basis of chemical model studies on oxadiazolones substituted with both aromatic and aliphatic groups at N-3, a probable reactive site of oxadiazolones with acetylcholinesterase is also postulated.

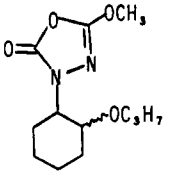
DISCUSSION AND RESULTS

A few selected 5-methoxy-1,3,4-oxadiazol-2(3*H*)-ones **3**–**16** were synthesized (Huang et al., 1987) and are listed in Table I. Some of these compounds have the oxadiazolone group and the alkoxy group (or the oxo functionality) in the similar connectivity relationship to that of compounds **1A** and **2**. Except for compounds **15** and **16**, little insecticidal activity is observed at 500 ppm. These materials inhibit housefly head acetylcholinesterase activity to varying degrees and are generally less potent than their aromatic analogue **1A** and **2**. In vitro inhibitory activities of **3**–**16** are grouped in Table I according to structure and oxidation state at the α- and β-carbons.

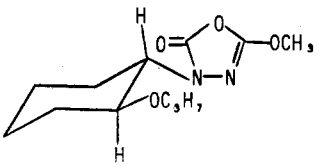
The acetylcholinesterase inhibitory activity of these compounds appears to be generally related to the nature of substituents on the α-carbon. For instance, inhibitory activity of oxadiazolones with an oxo or ketal functionality at the β-carbon in group B ($I_{50} \approx 10^{-5}$ M) is greater than for those compounds of group A with saturation at α- and β-carbons ($I_{50} \approx 10^{-3}$–10^{-4} M). The introduction of electron-withdrawing groups at the α-carbon also enhances the housefly head acetylcholinesterase inhibitory activity. For example, when a cyano group is introduced on the α-carbon of compound **5**, its I_{50} value increases from 6 × 10^{-4} to 5 × 10^{-5} M (for **14**). Enhanced inhibition is also observed when the methoxymethyl group of **15** is changed to a more electron-withdrawing ethyl ester moiety of **16**.

The relative position of the substituent on the phenyl ring and the 1,3,4-oxadiazolone moiety was reported to be critical to good insecticidal activity by Boesch (1979). This was supported by comparing the I_{50}, insecticidal, and synergism data of compound **1A** vs. that of its 4-methoxy analogue **1B** generated in our laboratories (Table II). Oxadiazolone **1A** with the ortho-substituted methoxy group is more potent than **1B**.

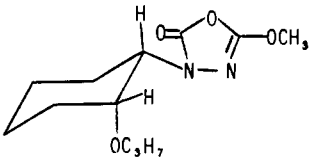
Since the relative position of the methoxy group and the oxadiazolone moiety was demonstrated to be important for insecticidal activity of compound **1A**, the isolation of the trans and cis isomers of **17** (Huang et al., 1987) as compounds **3** and **4**, respectively, permitted us to evaluate the difference in biological activity, if any, between the two geometric isomers. The stereochemical mixture **17** had an I_{50} value of 3.6 × 10^{-4} M. Although the in vitro inhibitory activity of **3** and **4** appeared to be comparable, it was interesting to note that, with the synergist *N*-methylbicyclo[2.2.1]-5-heptene-2,3-dicarboximide (500 ppm), the



17



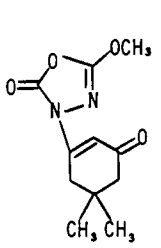
3



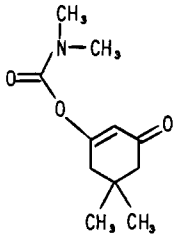
4

trans isomer **3** was found to be slightly more potent than **4** in the housefly bait test (LC_{50} = 170 ppm for **3** and ≈500 ppm for **4**).

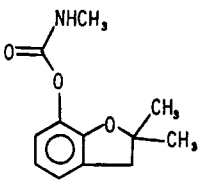
Compound **12** may be considered the oxadiazolone analogue of the insecticide dimetan (**18**), as **2** is the analogue of carbofuran. Although oxadiazolone **2** and carbofuran exhibited similar insecticidal activity in our screen, compound **12** was found to be considerably less active than was dimetan (Table III).



12



18

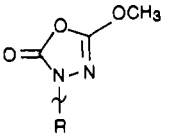


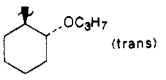
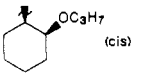
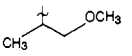
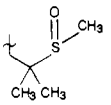
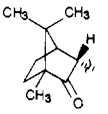
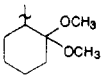
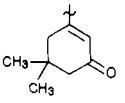
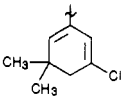
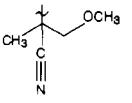
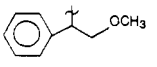
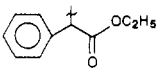
Carbofuran

Oxadiazolone **16** (in Table I) exhibited modest insecticidal activity against aphids, houseflies, and Mexican bean beetles. Six additional compounds **19**–**24** (Huang et al., 1987) were also studied (Table IV), but none were superior to compound **16**. Although these oxadiazolones were quite active in vitro with I_{50} values in the range of 10^{-6}–10^{-7} M, their in vivo insecticidal activity was considerably less than for compounds **1A** and **2**, probably because of metabolic lability.

Proposed Reaction Mechanism of Oxadiazolones with AChE. Hydrazinedicarboxylic acid esters **26a** (Hai et al., 1973) and **26b** (Pilgram, 1982) have been isolated from the reaction of oxadiazolones **25a** and **25b**, respectively, with sodium methoxide (Scheme I). Similar products were also obtained when oxadiazolone **2** was treated with either sodium *tert*-butyl mercaptide/tetrahydrofuran or methanol/sodium carbonate. Ring-opened materials **27a** and **27b** were the only products isolated from these reactions (Scheme II). Oxadiazolone **10** reacted in a similar manner to afford **28a** when treated with methanol in the presence of sodium carbonate at room temperature. When **16** was subjected to a similar treatment, a ring-opened and transesterified compound **29** was the sole isolated product as depicted in Scheme II. All of these examples strongly suggest that the carbonyl group of the oxadiazolone heterocycle is the reactive center with nucleophiles. The rate of heterocyclic ring opening of **2** (or **10**) with perdeuteriomethanol in the presence of sodium carbonate (1.275 equiv) at 30 °C was also determined. The reaction

Table I



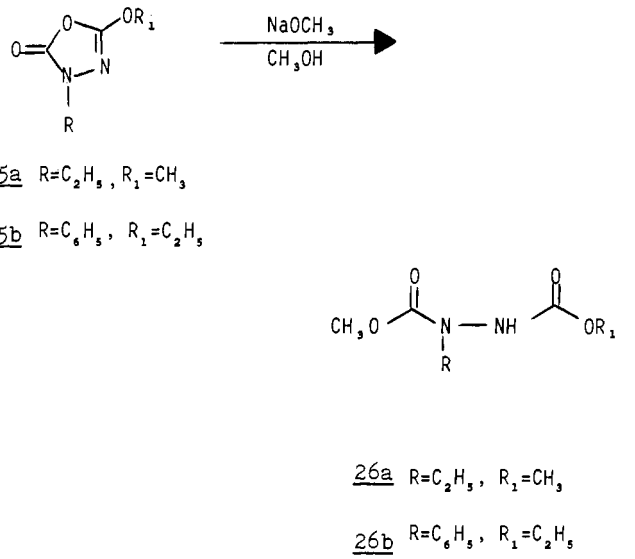
gp		R	I_{50}(AChE), M	remarks
A	3		3.2×10^{-4}	inactive[a]
	4		5×10^{-4}	inactive[a]
	5		6×10^{-4}	inactive[a]
	6		$>1 \times 10^{-3}$	inactive[a]
	7		1×10^{-3} (44%)	inactive[a]
	8		$>1 \times 10^{-3}$	inactive[a]
	9		$>1 \times 10^{-3}$	inactive[a]
B	10		2×10^{-5}	inactive[a]
	11		6×10^{-5}	inactive[a]
C	12		3×10^{-5}	inactive[a]
	13		2×10^{-5}	inactive[a]
D	14		5×10^{-5}	inactive[a]
	15		2×10^{-5}	aphid, 300[b] housefly, 410[b]
	16		3×10^{-6}	aphid, 38[b] housefly, 200[b] MBB, 600[b,c]

[a] 3–14 are inactive against aphid, mite, and housefly at 500 ppm. [b] LC_{50} value, ppm. [c] MBB = Mexican bean beetle.

was monitored by measuring the relative intensities of the methoxy group of **2** and **27c** (or **10** and **28b**) in the proton NMR spectra taken at various intervals. No reaction was observed in the absence of sodium carbonate. In the case of compound **10**, H–D exchange at the α-carbon of the carbonyl group was completed within 40 min and the half-life ($t_{1/2}$) was approximately 7 h for the ring opening. The heterocyclic moiety of **2** opened at a slower rate with $t_{1/2} \approx 14$ h.

The chemical reactions demonstrated above may be indicative of a parallel reaction at the enzymatic level with the serine hydroxy group of acetylcholinesterase, leading to the formation of enzyme-bound intermediate **30**, as

Scheme I



illustrated in Scheme III. Unlike the other classes of acetylcholinesterase inhibitors such as *N*-methylcarbamates and organophosphates (Fuchs et al., 1983), which act by transferring only the carbamoyl and phosphoryl moieties, respectively, to the enzyme, the oxadiazolones may be acting differently in that the entire ring-opened molecule is attached to the enzyme.

CONCLUSION

The N-3-substituted aliphatic series is not as biologically active as aromatic analogues such as **1A** and **2**. Although the steric requirement for activity is unclear in the N-3 aliphatic area, the difference in activity seems to be related to the potential susceptibility of the oxadiazolone carbonyl group toward nucleophiles. In general, compounds with better in vitro activity have more electron-withdrawing substituents at the α-carbon attached to N-3 nitrogen. There is a suggestion in the aliphatic series that the spatial relationship between the oxadiazolone ring and the alkoxy substituent may have some effect; however, it is of minor importance relative to the electronic effect.

Based on the chemical model studies, the mode-of-action of oxadiazolones is postulated to be a mixture of carbamate and organophosphate characteristics. The initial ring opening by nucleophilic attack at the carbonyl moiety of oxadiazolones resembles the enzymatic interaction with the carbamate group, while the end result would be "organophosphate-like" involving a transfer of a more bulky substituent to the enzyme. The formation of stable enzyme-bound intermediate **30** would lead to irreversible inhibition of enzyme.

Very recently, enzymatic studies with compounds **1A** and **2**, conducted in Dr. Eldefrawi's laboratory, did reveal that these two oxadiazolones produced progressive and irreversible inhibition of the membrane-bound AChE of Torpedo and electric eel organs, red blood cells, fly brain, and horse serum BuChE (Bakry et al., 1986). These findings are supportive of our hypothesis on the mode of action of oxadiazolones (see Scheme III), which was proposed based on chemical model studies.

Biology—Test Methods. The biological evaluations on various organisms were carried out as described by Payne et al. (1966) with the exception that the foliage to which the Mexican bean beetle larvae was exposed was treated by spraying plants on a turntable (Hansberry, 1943) instead of dipping excised leaves. I_{50}'s were determined against housefly head acetylcholinesterase by the

Scheme II

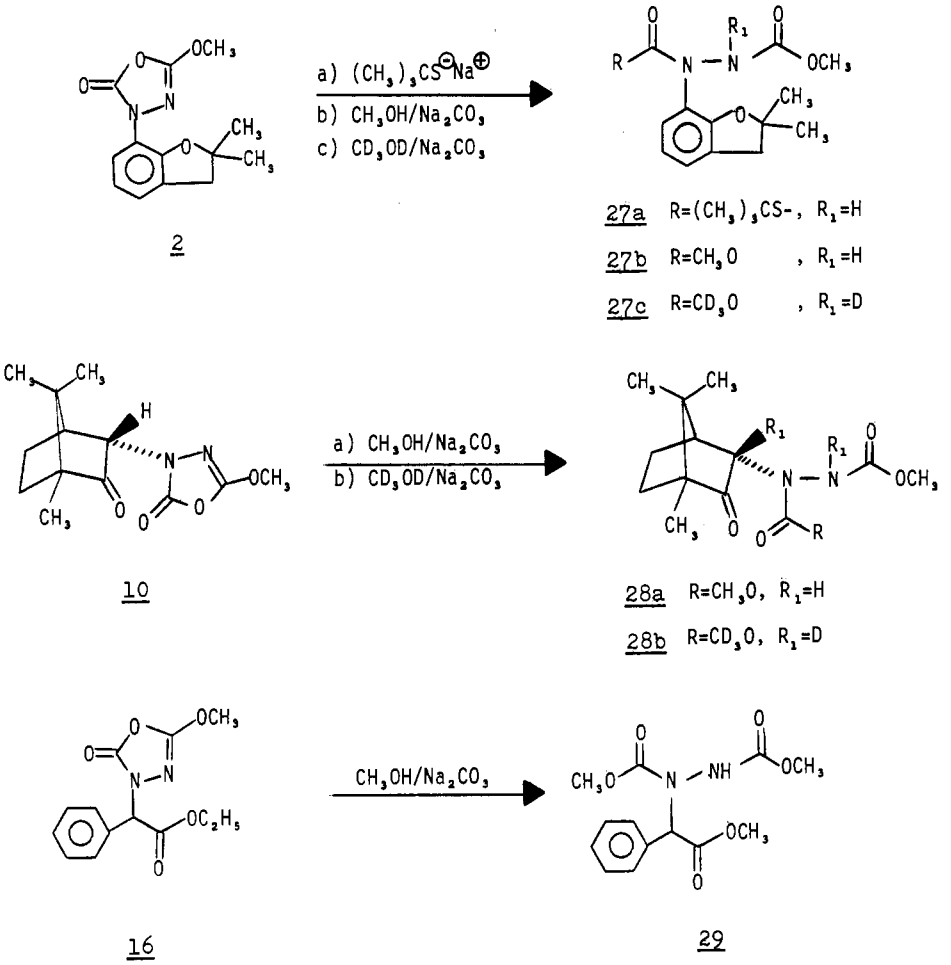
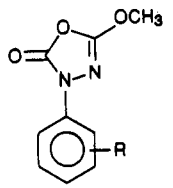


Table II



	R	LC50, ppm: buckthorn aphid	two-spotted mite	Southern armyworm	Mexican bean beetle	housefly	LC50,[a] ppm (synergism): housefly	I_{50}(AChE), M
1A	2-OCH_3	4	>500	150	41	50	0.9	8×10^{-7}
1B	4-OCH_3	>500	>500	>500	250	>1000	260	7×10^{-6}

[a] With the synergist *N*-methylbicyclo[2.2.1]-5-heptene-2,3-dicarboximide (500 ppm).

Table III

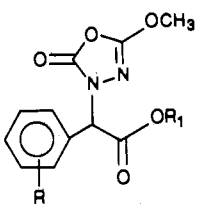
compd	LC50, ppm: buckthorn aphid	housefly	I_{50}(AChE), M
12	>500	>500	3×10^{-5}
18	<15	80	5×10^{-7}

Warburg manometric method (Payne et al., 1966).

EXPERIMENTAL SECTION

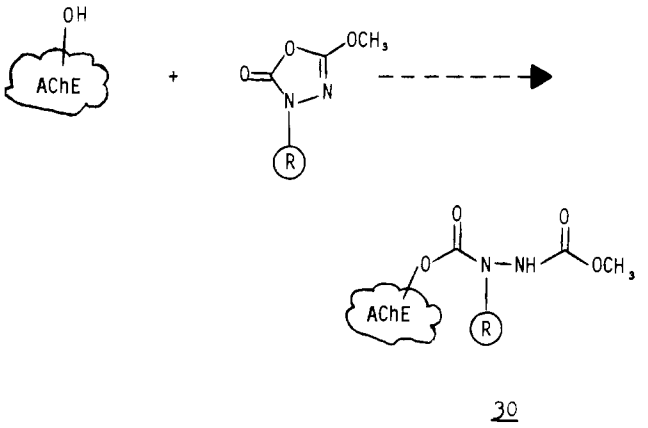
The synthesis of all novel oxadiazolones except for compounds **7** and **19** has been reported in another journal (Huang et al., 1987). Hydrazinedicarboxylic acid esters that were isolated have been characterized by NMR and IR spectral analyses. The melting points are uncorrected. ^1H NMR spectra were obtained with a Varian Associates EM-360L spectrometer using Me_4Si as an internal standard. ^{13}C NMR spectra were recorded at 22.5 MHz with

Table IV



	R	R_1	I_{50}(AChE), M	LC50, ppm: aphid	housefly
16	H	C_2H_5	3×10^{-6}	38	200
19	H	*i*-C_3H_7	1×10^{-6}	>100	148
20	2-CF_3	C_2H_5	6×10^{-6}	>100	480
21	2-F	C_2H_5	2×10^{-6}	>100	190
22	4-F	C_2H_5	5×10^{-6}	>100	175
23	4-OCH_3	C_2H_5	6×10^{-6}	>100	>100
24	2-Cl, 6-F	C_2H_5	3×10^{-7}	>100	>100

Scheme III



30

a JEOL FX-90Q Fourier transform spectrometer. Microanalyses were performed by Galbraith Laboratories, Inc. Infrared spectra were recorded on a Perkin-Elmer 197 spectrometer.

5-Methoxy-3-(4-methoxyphenyl)-1,3,4-oxadiazol-2-(3*H*)-one (1B). Compound **1B** was prepared from (4-methoxyphenyl)hydrazine hydrochloride according to the procedure reported by Boesch (1979): mp 87–90 °C; ^{1}H NMR ($CDCl_3$) δ 7.60 (d, J = 9 Hz, 2 H), 6.82 (d, J = 9 Hz, 2 H), 4.03 (s, OCH_3, 3 H), 3.78 (s, OCH_3, 3 H); ^{13}C NMR ($CDCl_3$) δ 157.4, 155.7, 148.6, 129.4, 119.8, 114.2, 57.6, 55.5; IR (CH_2Cl_2) 1795, 1670 cm^{-1}.

3-[2-Methyl-2-(methylsulfinyl)propyl]-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one (7). To a solution of 3-[2-methyl-2-(methylthio)propyl]-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one (**6**: 1.1 g) and methylene chloride (60 mL) was added *m*-chloroperbenzoic acid (1.08 g, 80% purity) at room temperature. The reaction mixture was washed with water and saturated sodium bicarbonate solution, dried over $MgSO_4$, filtered, and concentrated in vacuo. The desired product as an oil [1.0 g (86% yield)] was obtained: ^{1}H NMR ($CDCl_3$) δ 4.06 (s, OCH_3, 3 H), 3.93 (s, CH_2, 2 H), 2.55 (s, CH_3S=O, 3 H), 1.40 (s, CH_3, 3 H), 1.32 (s, CH_3, 3 H); IR (neat) 1800, 1660 cm^{-1}.

Isopropyl α-[3-(5-Methoxy-2(3*H*)-oxo-1,3,4-oxadiazolyl)]phenylacetate (19). This compound was prepared according to the procedure reported by Huang et al. (1987): ^{1}H NMR ($CDCl_3$) δ 7.40 (s, aromatic H, 5 H), 5.72 (s, 1 H), 5.12 (septet, OC*H*$(CH_3)_2$, 1 H), 3.93 (s, OCH_3, 3 H), 1.24 (d, J = 6 Hz, 3 H), 1.19 (d, J = 6 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 167.3, 155.5, 151.6, 133.0, 129.3, 129.0, 128.6, 70.3, 61.6, 57.5, 21.64, 21.60; IR (neat) 1805, 1745, 1660 cm^{-1}.

2-[(*tert*-Butylthio)carbonyl]-2-(2,3-dihydro-2,2-dimethylbenzofuran-7-yl)hydrazinecarboxylic Acid Methyl Ester (27a). To a solution of sodium *tert*-butyl mercaptide in THF, prepared by the addition of 2-methyl-2-propanethiol (0.45 mL) and sodium hydride (0.24 g, 60% in oil dispersion), was added a solution of 3-(2,3-dihydro-2,2-dimethylbenzofuran-7-yl)-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one [**2**; 1.03 g, prepared according to the procedure reported by Tieman (1981)] and THF (50 mL). This solution was refluxed for 2 days. THF was removed in vacuo, and the residue was partitioned between CH_2Cl_2 and saturated NH_4Cl solution. The CH_2Cl_2 concentrate contained only the product **27a** according the ^{1}H NMR spectrum. It was further chromatographed to yield **27a** [0.8 g (58%)] as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.48–6.75 (m, aromatic H and NH, 4 H), 3.70 (s, OCH_3, 3 H), 2.98 (s, CH_2, 2 H), 1.48 (s, $SC(CH_3)_3$, 9 H), 1.43 (s, $C(CH_3)_2$, 6 H); IR (CH_2Cl_2) 1750, 1665 cm^{-1}.

2-(Methoxycarbonyl)-2-(2,3-dihydro-2,2-dimethylbenzofuran-7-yl)hydrazinecarboxylic Acid Methyl Ester (27b). Compound **27b** was isolated from the reaction of **2** and methanol in the presence of sodium carbonate (3 equiv): mp 108–111 °C; ^{1}H NMR ($CDCl_3$) δ 7.48–6.75 (m, aromatic H and NH, 4 H), 3.73 (s, OCH_3, 3 H), 3.71 (s, OCH_3, 3 H), 3.00 (s, CH_2, 2 H), 1.45 (s, $C(CH_3)_2$, 6 H); IR (CH_2Cl_2) 1755, 1730 cm^{-1}. Anal. Calcd for $C_{14}H_{18}N_2O_5$: C, 57.14; H, 6.16; N, 9.52. Found: C, 56.93; H, 6.35; N, 9.47.

Table V

no.	time int, h	% 10 converting to **28b**	no.	time int, h	% 10 converting to **28b**
1	0	0	5	11$^1/_2$	71.43
2	1$^1/_2$	22.22	6	16$^1/_2$	81.94
3	2$^1/_2$	30.70	7	28$^1/_2$	>99.9
4	6$^1/_2$	53.13			

Table VI

no.	time int, h	% 2 converting to **27c**	no.	time int, h	% 2 converting to **27c**
1	0	0	4	4	26
2	1	7.5	5	8	42
3	2	14	6	23	68

2-(Methoxycarbonyl)-2-α-camphorylhydrazinecarboxylic Acid Methyl Ester (28a). Compound **28a** [0.38 g (69.2%)] as a viscous oil was isolated from the reaction of 3-α-camphoryl-1,3,4-oxadiazol-2(3*H*)-one (**10**; 0.49 g) and methanol (50 mL) in the presence of sodium carbonate (0.59 g, 3.0 equiv): ^{13}C NMR ($CDCl_3$) δ 156.6 (s), 156.4 (s), 156.3 (s), 66.6 (d), 58.6 (s), 53.9 (q), 48.3 (d), 43.7 (s), 31.0 (t), 19.5 (q), 19.5 (t), 18.7 (q), 9.3 (q); ^{1}H NMR suggested it to be an endo isomer on the basis of the coupling constant (J = 4.5 Hz) of the methine proton α to the carbonyl group of **28a** at 4.63 ppm; IR (CH_2Cl_2) 1750, 1725 cm^{-1}; high-resolution MS at m/e 298 (molecular ion), 298.1528 (calcd for $C_{14}H_{22}O_5N_2$, 298.1528).

Reaction of Ethyl α-[3-(5-Methoxy-2(3*H*)-oxo-1,3,4-oxadiazolyl)]phenylacetate (16) with Methanol in the Presence of Sodium Carbonate. A suspension mixture of **16** (2.17 g), sodium carbonate (2.47 g), and methanol (100 mL) was stirred at room temperature for 8 h. Compound **29** [1.84 g (80%)] as a white solid was obtained: mp 103–104 °C; ^{1}H NMR ($CDCl_3$) δ 7.45 (s, aromatic H, 5 H), 6.03 (s, methine H, 1 H), 3.86 (s, OCH_3, 3 H), 3.82 (s, OCH_3, 3 H), 3.42 (br, OCH_3, 3 H) (broad peak at δ 3.42 becomes sharper in Me_2SO-d_6); ^{13}C NMR ($CDCl_3$) δ 171.2 (s), 156.6 (s), 155.8 (s), 132.5 (s), 129.8 (d), 128.9 (d), 128.4 (d) 64.4 (d), 53.9 (q), 52.6 (q), 52.5 (q); high-resolution MS at m/e 296 (molecular ion), 296.1006 (calcd for $C_{13}H_{16}N_2O_6$, 296.1008); high-resolution MS at m/e 264 ($M^+ - CH_3OH$), 264.0742 (calcd for $C_{12}H_{12}N_2O_5$, 264.0746).

Measuring the Reaction Rate of 10 with Methanol-d_4 in the Presence of Sodium Carbonate. The suspension mixture of **10** (0.1 g), sodium carbonate (51 mg, 1.275 equiv), and methanol-d_4 (1.96 g) was maintained in a 10-mm NMR tube at 30 °C. The reaction was monitored by ^{1}H NMR spectra taken at various intervals for up to 30 h. The percentage of **10** converting to **28b** at any given interval was calculated on the basis of the integration ratio of the methoxy group of **10** (at δ 3.97) and **28b** (at δ 3.71). The results are shown in Table V. It was estimated that 50% conversion required 7 h and complete conversion occurred after 28 h. The methine proton α to the carbonyl group of **10** (at δ 4.63) was completely exchanged with deuterium within 40 min.

Measuring the Reaction Rate of 2 with Methanol-d_4 in the Presence of Sodium Carbonate. The suspension

mixture of **2** (0.1 g), sodium carbonate (51.5 mg, 1.275 equiv), and methanol-d_4 (1.96 g) in a 10-mm NMR tube was monitored according to the same methodology used for **10**. The results are shown in Table VI. The conversion percentage was calculated on the basis of the integration ratio of the methoxy group of **10** (at δ 3.98) and **27c** (at δ 3.71).

ACKNOWLEDGMENT

We gratefully acknowledge Dr. H. H. Moorefield and C. R. Phillabaum for generating AChE I_{50} values and conducting synergism tests. Particular thanks are due to Dr. H. H. Moorefield for many valuable discussions throughout the project and to Dr. M. H. J. Weiden for consultations during the preparation of this manuscript. We also thank M. D. Graves, D. D. Singleton, B. J. Johnson, E. C. Bailey, A. A. Sousa, and the Biological Evaluation Group for their contributions.

LITERATURE CITED

Ambrosi, D.; Bic, G.; Desmoras, J.; Gallinelli, G.; Roussel, G. *Proc.-Br. Crop Prot. Conf.-Pests Dis.* **1979**, 533.

Ambrosi, D.; Boesch, R.; Desmoras, J. *J. Phytiatrie-Phytopharmacie* **1980**, 199.

Bakry, N. M.; Sherby, S. M.; Eldefrawi, A. T.; Eldefrawi, M. E. *Neurotoxicology* **1986**, 7(3), 1.

Boesch, R. U.S. Patent 4 076 824, 1978; *Chem. Abstr.* **1976**, *85*, 143115g.

Boesch, R. U.S. Patent 4 150 142, 1979; *Chem. Abstr.* **1976**, *85*, 143115g.

Fuchs, R. A.; Schroder, R. *Chemistry of Pesticides*; Buchel, K. H., Ed.; Wiley: New York, 1983; pp 95–97, 148–151.

Hai, S. M. A.; Lwowski, W. *J. Org. Chem.* **1973**, *38*, 2442.

Hansberry, R. In *Laboratory Procedures in Studies of the Chemical Control of Insects*; Campbell, F. L., Monlton, F. R., Eds.; AAAS: Boulder, CO, 1943; Publ. No. 20, p 85.

Huang, J.; Bushey, D. F.; Graves, M. D.; Johnson, B. F.; Singleton, D. D. *J. Heterocycl. Chem.* **1987**, *24*, 1.

Payne, L. K., Jr.; Stansbury, H. A., Jr.; Weiden M. H. J. *J. Agric. Food Chem.* **1966**, *14*, 356.

Pilgram, K. H. *J. Heterocycl. Chem.* **1982**, 823.

Tieman, C. H. U.S. Patent 4 302 592, 1981; *Chem. Abstr.* **1982**, *96*, 104254j.

Received for review June 19, 1986. Accepted November 10, 1986.

Persistence of Deltamethrin and Its Isomers on Pasture Forage and Litter

Bernard D. Hill* and Daniel L. Johnson

The deposition, persistence, and isomeric conversion of deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-*cis*-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropanecarboxylate] were measured after a 7.2 g/ha aerial application to control grasshoppers in two pastures. Mean deposition was 75.9% of applied, with initial deltamethrin residues of 2.06 ppmd (d = dry-weight basis) on the forage and 146 $\mu g/m^2$ on the litter. Deltamethrin dissipation was biphasic, and a two-compartment model was fitted to the residue data. The DT_{50} was 5.9 days for deltamethrin residues on forage and 17 days on litter. Small amounts of the αR diastereomer of deltamethrin were detected on the forage (0.06 ppmd) and on the litter (0.65 $\mu g/m^2$). The concentration of the trans isomers increased exponentially until, within 14 days after application, asymptotic levels of 0.26 ppmd on the forage and 22.7 $\mu g/m^2$ on the litter were reached.

The synthetic pyrethroid deltamethrin is registered for the control of grasshoppers in cereal crops in western Canada with the restriction that fields must not be treated within 40 days of harvest. Deltamethrin also has potential for controlling grasshoppers in pastures (Johnson et al., 1986).

There have been few published reports on the persistence of deltamethrin in field crops or forage. Ruzo and Casida (1979) reported that, under greenhouse conditions, [^{14}C]deltamethrin had a half-life of 1.1 weeks on cotton and a time for 90% loss of 4.6 weeks. Cole et al. (1982) found that less than 50% [^{14}C]deltamethrin remained on cotton and beans after 4–5 days outdoors. Khan et al. (1984) investigated the formation of bound residues of deltamethrin in bean plants. Ten days after treatment with [^{14}C]deltamethrin, 3–10% of the ^{14}C label was in the form of bound residues. In summarizing deltamethrin residue levels found in various crops, L'Hotellier (1982) reported that residues in oat straw were less than 0.05 ppm 1 month after treatment at 12.5 g/ha. Two to three weeks after treatment at 17.5 g/ha, the residue levels in fresh or dried alfalfa were 0.10–0.15 ppm.

Table I. Name, Numbering, and Structures of Deltamethrin Isomers[a]

common name	numerical designation	stereochemical configuration: α-cyano C	stereochemical configuration: C_1,C_3 of cyclopropane
deltamethrin	1	*S*	1*R*,3*R*-cis[b]
	1′	*R*	1*S*,3*S*-cis
(αR)-deltamethrin	2	*R*	1*R*,3*R*-cis
	2′	*S*	1*S*,3*S*-cis
trans-deltamethrin	3	*S*	1*R*,3*S*-trans[b]
	3′	*R*	1*S*,3*R*-trans
	4	*R*	1*R*,3*S*-trans
	4′	*S*	1*S*,3*R*-trans

[a] As designated by Ruzo et al. (1977). [b] Most biologically active isomers.

The purpose of this study was to obtain residue data for the establishment of a minimum time interval between treatment of pastures and grazing by cattle. We were particularly interested in the isomeric nature of the "deltamethrin" residues. There are eight possible isomers that may be found after chemical transformations of

Agriculture Canada Research Station, Lethbridge, Alberta T1J 4B1, Canada.

0021-8561/87/1435-0373$01.50/0 Published 1987 by the American Chemical Society