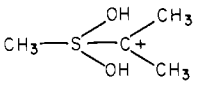
possible in the m/z 81 ion produced (Ia–c, Scheme II). A fragment (m/z 145, 40%) complementary to the sulfur moiety is also observed in the mass spectra.

Hence, unlike in aldicarb and aldicarb sulfoxide, the main CI fragmentation process in aldicarb sulfone parallels that found by pure EI (Benson and Damico, 1968). An additional characteristic cleavage observed in the CI-MS of aldicarb sulfone is that leading to the stable tertiary carbonium ion (m/z 123)



obtained in 70% abundance under set A conditions and in 12% abundance under the conditions of set B. The possibility of applying isobutane CI to the GC–MS of these compounds under mild conditions is of considerable practical importance and is presently being explored in this laboratory. At the same time, however, another simple possibility would be to introduce cleanup samples into the mass spectrometer via a liquid chromatography interface (LC–MS), which could obviate the sample heating and thermal decomposition inherent in the use of GC–MS.

ACKNOWLEDGMENT

We express sincere appreciation to Dr. S. Zitrin for his helpful comments. We thank Dr. I. Adato for providing us with the analytical samples used in these studies.

LITERATURE CITED

Benson, W. R.; Damico, J. N. *J. Assoc. Off. Anal. Chem.* **1968**, *51*, 4701.
Damico, J. N.; Barron, R. P.; Sphon, J. A. *Int. J. Mass Spectrom. Ion Phys.* **1969**, *2*, 161.
Fales, H. M.; Milne, G. A.; Winkler, H. U.; Beckey, H. D.; Damico, J. N.; Barron, R. P. *Anal. Chem.* **1975**, *47*, 207.
Hall, R. C.; Harris, D. E. *J. Chromatogr.* **1979**, *169*, 245.
Holmstead, R. L.; Casida, J. E. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 541.
U.S. Environmental Protection Agency "Substitute Chemical Program, Initial Scientific and Minieconomic Review of Aldicarb"; U.S. Environmental Protection Agency: Washington, DC, 1975.

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Received for review June 22, 1981. Accepted February 9, 1982.

Effect of Sesamex on the in Vivo Metabolism of Diflubenzuron in Larvae of Susceptible and Resistant Strains of the Housefly, *Musca domestica* L.

The effect of sesamex [5-[1-[2-(2-ethoxyethoxy)ethoxy]ethoxy]-1,3-benzodioxole] on the in vivo metabolism of diflubenzuron [1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl)urea] was investigated on a susceptible (NAIDM) and two resistant (R-diflubenzuron and R-OMS-12) strains of the housefly. Sesamex reduced significantly the in vivo metabolism of radiolabeled diflubenzuron in all strains. Recovery of internal unmetabolized diflubenzuron was 10–30 times higher in the mature larvae of the R strains when the compound was applied jointly with sesamex. Similarly, sesamex reduced markedly the recovery of polar and nonpolar metabolites of water-soluble conjugates of diflubenzuron both in the external and in the internal fractions of these strains. The recovery of radiolabel in the unextractable residue increased with sesamex treatment in all three strains. These results indicate that MFO enzymes play an important role in the resistance of houseflies to diflubenzuron.

Insecticides are metabolized within the insect system to less toxic or nontoxic polar products by three major types of enzyme systems, e.g., mixed-function oxidase (MFO), hydrolase, and glutathione-dependent transferase (GSAT) (Matsumura, 1976). Biochemical studies have demonstrated that resistant (R) strains usually possess increased ability to metabolize insecticides [reviews by Georghiou (1972), Oppenoorth (1976), and Plapp (1976)]. Since synergists act by inhibiting specific detoxication enzymes, they are frequently utilized as indicators of the possible biochemical mechanisms involved in resistance (Casida, 1970; Wilkinson, 1972).

In an earlier study we reported on the mechanisms of resistance to diflubenzuron [1-(2,6-difluorobenzoyl)-3-(4-chlorophenyl)urea] in a diflubenzuron-selected strain of the housefly (Pimprikar and Georghiou, 1979). It was shown that sesamex [5-[1-[2-(2-ethoxyethoxy)ethoxy]ethoxy]-1,3-benzodioxole] and piperonyl butoxide significantly increased the toxicity of diflubenzuron in the resistant strain, suggesting the involvement of MFO systems in resistance. Sesamex consistently produced higher synergism with diflubenzuron than did piperonyl butoxide. Here we report tests intended to show if the synergistic activity of sesamex was due to suppression of metabolism of diflubenzuron and the type of metabolic pathway affected by the synergist.

MATERIALS AND METHODS

Chemicals. [^{14}C]Diflubenzuron (aniline-U-^{14}C; specific activity 2.3 mCi/mmol) and [^{3}H]diflubenzuron (benzoyl-U-^{3}H; specific activity 68 mCi/mmol) were provided by Thompson-Hayward Chemical Co., Kansas City, KS. Unlabeled diflubenzuron and authentic standards of diflubenzuron metabolites were also supplied by the same company. Piperonyl butoxide, technical grade, was provided by FMC Corp., Philadelphia, PA, and sesamex by

0021-8561/82/1430-0615$01.25/0

Table I. In Vivo Metabolites of [^{14}C]Diflubenzuron in R and S Strains of Housefly following Treatment by Diflubenzuron Alone or with Sesamex[a,b]

		% ^{14}C radiolabel recovered[c]											
		external recovery[d]						internal recovery					
		S-NAIDM		R-OMS-12		R-diflu-benzuron		S-NAIDM		R-OMS-12		R-diflu-benzuron	
metabolites[e]	R_f	alone	+ses-amex	alone	+ses-amex	alone	+ses-amex	alone	+ses-amex	alone	+ses-amex	alone	+ses-amex
(4-chlorophenyl)hydroxy-diflubenzuron[f]	0.00	9.8	0.9	9.1	0.5	16.7	6.6	2.8	0.1	4.5	0.2	2.3	0.0
(2,6-difluorobenzoyl)hydroxy-diflubenzuron[f]	0.11	1.2	0.0	4.6	0.0	3.4	1.0	1.6	0.0	1.6	0.0	0.0	0.0
(4-chlorophenyl)urea	0.18	6.0	2.2	3.5	0.05	3.9	0.6	0.4	0.0	1.1	0.1	0.3	0.8
4-chloroaniline	0.55	4.1	3.9	5.2	3.3	6.5	4.0	5.3	3.1	2.2	2.7	0.7	1.2
diflubenzuron	0.70	25.8	38.3	41.4	51.4	41.0	51.8	18.2	31.3	0.6	28.9	1.4	9.7
4-chloro-*N*,*N*-dimethylaniline	0.83	3.2	1.7	0.1		0.5	0.7						2.6
polar[g]		6.5	1.4	10.5	4.0	12.6	6.9	2.3	0.2	11.3	1.6	5.3	5.0
conjugates		0.4	0.3	0.6	1.6	0.9	1.7	2.5	0.9	1.3	0.4	0.6	1.7
unextractable								9.7	16.6	2.9	7.1	3.2	5.8
total		57.0	48.7	75.0	60.85	85.5	73.3	42.8	52.20	25.50	41.0	13.8	26.8

[a] The TLC solvent system consists of benzene–dioxane–acetic acid (80:30:1). [b] Average recovery 79.6%. [c] Percent of the total radioactivity recovered. [d] External recovery includes flask rinse and larval rinse. [e] Metabolites identified by co-chromatography. [f] Metabolites identified by IR and GC-mass spectrometry. [g] Methanol-soluble metabolites.

the World Health Organization. All other chemicals and solvents used in this research were of analytical grade.

Insects. Three strains of the housefly were used, one susceptible (S-NAIDM) and two resistant (R-OMS-12 and R-diflubenzuron). The OMS-12 strain has been under selection pressure with *O*-ethyl *O*-(2,4-dichlorophenyl) phosphoramidothioate in this laboratory since 1960 and demonstrates 88× resistance to this chemical. The R-diflubenzuron strain has been under selection by diflubenzuron in this laboratory since June 1974. The level of resistance to diflubenzuron was >1000× in the R-diflubenzuron strain and ca. 395× in R-OMS-12.

In Vivo Metabolism of Diflubenzuron. One hundred postfeeding third instar larvae of each strain were individually treated with 0.5 μL of tetrahydrofuran (THF) solution containing ^{3}H- or ^{14}C-radiolabeled diflubenzuron alone or with sesamex at a ratio of 1:10. Each determination was repeated twice. The experimental procedure for extraction, cleanup, chromatography, and analysis of radiolabeled diflubenzuron metabolites was discussed in detail in a previous paper (Pimprikar and Georghiou, 1979). Briefly, exposure was for 24 h at which time the larvae were washed twice with THF. The holding flask was also rinsed with THF, methanol, and water in sequence. The larvae were homogenized in THF and centrifuged at 4000 rpm for 30 min. Methanol- and water-soluble metabolites were obtained by homogenizing the THF-extracted residue in sequence with 10 mL of methanol and 10 mL of distilled water. One milliliter of the extract was counted to determine total radioactivity in each fraction. The residue was subjected to combustion in a sample oxidizer.

Extracts from the flask rinse, larval rinse, and homogenates (except the water-soluble fraction) were evaporated to dryness under nitrogen. Hexane-saturated acetonitrile and acetonitrile-saturated hexane were used for solvent partition. The acetonitrile extracts were concentrated by evaporation under nitrogen and subjected to Florisil adsorption chromatography. Water-soluble metabolites from the flask rinse and homogenates were subjected to enzymatic hydrolysis by using citrate buffer containing β-glucosidase, β-glucuronidase, arylsulphatase, or acid phosphatase. After incubation for 6 h at 37 °C with continuous agitation, the reaction was stopped by addition of 2 mL of ether. The mixture was extracted with ether which was then evaporated and used for thin-layer chromatography (TLC).

All TLC analyses utilized silica gel HLF chromatoplates which were developed in one dimension and scanned on a Berthold LB 2723 scanner. Radioactive spots were identified by comparing R_f values of cochromatographed authentic standards. Unknown metabolites were determined by fragmentation GC–mass spectrometry and IR spectrometry. The radioactive spots were scraped from the plates and quantitated by liquid scintillation counting.

RESULTS AND DISCUSSION

The results of quantitative analytical determination of metabolites of ^{14}C- and ^{3}H-labeled diflubenzuron in the presence or absence of sesamex are presented in Tables I and II, respectively. The details on the metabolic pathways of diflubenzuron in the R and S strains were discussed elsewhere (Pimprikar and Georghiou, 1979).

A reduction in the concentration of metabolites in the presence of sesamex, both in internal and in external fractions, is evident in all three strains. The concentrations of major metabolites such as (4-chlorophenyl)urea and 4-chloroaniline (Table I) and 2,6-difluorobenzoic acid and 2,6-difluorobenzamide (Table II) were substantially reduced, as were the concentrations of hydroxylated diflubenzuron, methanol-soluble polar metabolites, and water-soluble conjugates (Tables I and II).

The synergistic effects of sesamex were greater in the R strains than in the S strain. However, sesamex did not appear to inhibit all the degradation systems completely, as is evident from the fact that the quantities of radiolabeled metabolites recovered from the R strains in the presence of sesamex were always higher than in the S strain. This suggested that in the R strains the mechanisms of diflubenzuron metabolism are not completely inhibited by sesamex and that some mechanisms continue to provide for the lower levels of degradation of diflubenzuron.

The levels of diflubenzuron and metabolites in each strain in the presence and absence of sesamex are summarized in Table III. The percentages of total recovered radioactivity as unmetabolized diflubenzuron in the internal fractions of larvae treated with [^{14}C]diflubenzuron

Table II. In Vivo Metabolites of [^{3}H]Diflubenzuron in R and S Strains of Housefly following Treatment by Diflubenzuron Alone or with Sesamex[a,b]

		% ^{3}H radiolabel recovered[c]									
		external recovery[d]						internal recovery			
		S-NAIDM		R-OMS-12		R-diflubenzuron		S-NAIDM		R-OMS-12		R-diflubenzuron	
metabolites[e]	R_f	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex
(4-chlorophenyl)hydroxydiflubenzuron[f]	0.00	9.1	0.11	7.7	0.08	16.0	7.90	1.1	0.03	3.0	0.02	2.1	0.02
(2,6-difluorobenzoyl)hydroxydiflubenzuron[f]	0.11	2.0	0.00	5.2	0.08	3.0	0.64	0.2				0.1	0.00
unknown I	0.33			1.4	0.00			0.3	0.00				
2,6-difluorobenzoic acid	0.40	2.5	0.006	5.6	0.60	4.9	1.2			1.1	0.06	5.5	0.06
2,6-difluorobenzamide	0.45	13.0	0.00	3.2	0.00	4.7	2.5	1.9	0.4	1.2	0.20	1.5	0.00
diflubenzuron	0.70	35.5	47.7	44.9	57.7	34.6	59.7	21.8	37.9	0.4	13.40	0.9	12.60
unknown II	0.78	0.4	1.0	1.7	6.5	1.0	0.13	0.1	0.04			0.1	0.20
polar[g]		1.1	0.5	8.2	5.9	16.9	5.3	2.6	0.60	8.4	1.70	1.9	1.30
conjugates		0.3	0.5	0.5	0.2	1.7	0.4	0.6	0.10	1.1	0.04	0.9	0.00
unextractable								9.3	11.2	7.7	12.80	4.2	8.3
total		63.9	49.82	78.40	71.06	82.83	77.77	37.9	50.27	22.90	28.22	17.20	22.48

[a] The TLC solvent system consists of benzene–dioxane–acetic acid (80:30:1). [b] Average recovery 91.2%. [c] Percent of the total radioactivity recovered. [d] External recovery includes flask rinse and larval rinse. [e] Metabolites identified by cochromatography. [f] Metabolites identified by IR and GC–mass spectrometry. [g] Methanol-soluble metabolites.

Figure 1. Log dose–probit mortality lines for larvae of S-NAIDM, R-OMS-12, and R-diflubenzuron strains of houseflies treated as follows: S-NAIDM, diflubenzuron alone; R-diflubenzuron, diflubenzuron plus sesamex; R-OMS-12, diflubenzuron plus sesamex. [Data are in Pimprikar and Georghiou (1979).]

alone and in combination with sesamex were respectively S-NAIDM, 18% and 31%, R-OMS-12, 0.6% and 29%, and R-diflubenzuron, 1.4% and 10%. Relatively similar recoveries were found with [^{3}H]diflubenzuron (Table III).

The most probable metabolites of diflubenzuron obtained by MFO action could be 2,6-difluorobenzamide, 2,6-difluorobenzoic acid, (*p*-chlorophenyl)urea, and ring-hydroxylated forms of diflubenzuron. The results from Tables I and II indicate that the quantities of these diflubenzuron metabolites were significantly reduced by sesamex treatment in all three strains, indicating considerable inhibition of oxidative detoxifying enzymes. As a result of such inhibition, the internal recoveries of unmetabolized diflubenzuron were increased by 2-fold in S-NAIDM, 10-fold in R-diflubenzuron, and 30-fold in R-OMS-12. These data suggest that the inhibitory effects of sesamex are 5 and 15 times higher in the R-diflubenzuron and R-OMS-12 strains than in the S-NAIDM strain.

The amounts of unmetabolized diflubenzuron recovered from the external fractions (Table III) indicate that about one-third less diflubenzuron penetrated through the cuticle of larvae of all three strains in the presence of sesamex than in its absence. Most of the penetrated diflubenzuron was not metabolized in the presence of sesamex, leading to 10 and 30 times greater accumulation of the chemical in the internal fraction of the larvae of the R strains.

The recoveries of polar metabolites from both internal and external fractions were markedly reduced by sesamex; e.g., 2-fold, 4-fold, and 7-fold in the R-diflubenzuron, R-OMS-12, and S-NAIDM strains, respectively (Table III). The recoveries of organosoluble metabolites from the internal fractions were not reduced as much by sesamex as were those of the polar metabolites; however, the recoveries of organosoluble metabolites from the external fractions were reduced 2-fold in larvae of all three strains. These results suggest that sesamex treatment also reduced the excretion of diflubenzuron metabolites. The recoveries of radiolabel from the unextractable residue fractions also increased due to sesamex treatment of larvae of the S-NAIDM (1.7×), R-OMS-12 (2.5×), and R-diflubenzuron (1.8×) strains (Table III). The binding of unextractable radiolabel fraction was enhanced in the presence of sesamex in all three strains.

The results of this study indicate that treatment of larvae of R strains with a mixture of sesamex and diflubenzuron decreased the cuticular penetration of diflubenzuron and also its detoxication, presumably by inhibition of MFO enzymes. As a result, the excretion of metabolites also decreased. The ultimate consequence of these events was an increase in the internal concentration of diflubenzuron, resulting in higher toxic action and mortality. It must be noted that if detoxication is the sole

Table III. Effect of Sesamex on in Vivo Metabolism of Diflubenzuron in Larvae of S-NAIDM, R-OMS-12, and R-diflubenzuron Strains of Housefly[a]

	external fraction						internal fraction							
	diflubenzuron metabolites						diflubenzuron metabolites							
	organo-soluble		polar[b]		unmetabolized diflubenzuron		organo-soluble		polar[b]		unmetabolized diflubenzuron		unextractable radiolabel	
	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex	alone	+sesamex
						[*aniline*-^{14}C]Diflubenzuron								
S-NAIDM	13.3	7.8	17.9	2.6	25.8	38.3	5.7	3.1	9.2	1.2	18.2	31.3	9.7	16.6
R-OMS-12	8.8	3.4	24.8	6.1	41.4	51.4	3.3	2.8	18.7	2.2	0.6	28.9	2.9	7.1
R-diflubenzuron	10.9	5.3	33.6	16.2	41.0	51.8	1.0	4.6	8.2	6.7	1.4	9.7	3.2	5.8
						[*benzoyl*-^{3}H]Diflubenzuron								
S-NAIDM	15.9	1.0	12.5	1.1	35.5	47.7	2.3	0.5	4.5	0.7	21.8	37.9	9.3	11.2
R-OMS-12	11.9	7.1	21.6	6.3	44.9	57.7	2.3	0.8	12.5	1.8	0.4	13.4	7.7	12.8
R-diflubenzuron	10.6	3.8	34.2	14.2	38.0	59.7	7.1	0.2	5.0	1.3	0.9	12.6	4.2	8.3

[a] Percent of the total radioactivity recovered. [b] Polar metabolites constitute hydroxylated diflubenzuron, methanol-soluble metabolites, and water-soluble conjugates.

mechanism of diflubenzuron resistance, then the levels of synergism (SR ratios) should correspond to the levels of diflubenzuron resistance (RR ratios) exhibited by the R strains. This was not found to be entirely the case. The log dose–probit mortality lines for the S-NAIDM (without synergist), R-OMS-12, and R-diflubenzuron strains (with synergist) (Figure 1) demonstrate that resistance to diflubenzuron in the R strains is not completely abolished by MFO inhibitors. This suggests that other factors such as esterases, glutathione-dependent transferases, slower cuticular penetration, or decreased effectiveness at the site of action may also be contributing to diflubenzuron resistance.

ACKNOWLEDGMENT

We are indebted to Thompson-Hayward Chemical Company, Kansas City, KS, for providing the radiolabeled diflubenzuron and the authentic diflubenzuron metabolites. We are also thankful to Dr. M. A. H. Fahmy and Professor R. B. March for advice during this project.

LITERATURE CITED

Casida, J. E. *J. Agric. Food Chem.* **1970**, *18*, 753.
Georghiou, G. P. *Annu. Rev. Ecol. Syst.* **1972**, *3*, 133.
Matsumura, F. "Toxicology of Insecticides"; Plenum Press: New York and London, 1976; pp 1–503.
Oppenoorth, F. J. In "Insecticide Biochemistry and Physiology"; Wilkinson, C. F., Ed.; Plenum Press: New York, 1976; pp 1–507.
Pimprikar, G. D.; Georghiou, G. P. *Pestic. Biochem. Physiol.* **1979**, *12*, 10.
Plapp, F. W., Jr. *Annu. Rev. Entomol.* **1976**, *21*, 179.
Wilkinson, C. F. *Drug Metab. Rev.* **1972**, *1*, 153.

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Received for review June 8, 1981. Accepted January 11, 1982.

Oxidative Cleavage of the Disulfide Bond of Cystine by Allyl Isothiocyanate

Interaction of isothiocyanate with L-cystine under mild conditions was studied in detail. Electrophilic attack of allyl isothiocyanate to cystine resulted in scission of the sulfide bond to give two thiazoline derivatives, 2-amino- (3) and 2-(allylamino)-2-thiazoline-4-carboxylic acid (4), and their formation mechanisms were proposed.

It is well-known that many kinds of alkyl isothiocyanates are formed from glucosinolates by the action of myrosinase in crushed tissues of *Brassica* species such as rapeseed, radish root, etc., and they usually show strong pungent tastes. Most of isothiocyanates are not so stable and gradually decomposed to unpungent products in the presence of water (Gmelin et al., 1960; Gmelin and Virtanen, 1962; Kawakishi and Muramatsu, 1966; Kawakishi et al., 1967; Kawakishi and Namiki, 1969). Those are strong electrophilic reagents and easily react with some nucleophiles such as amines, water, and alcohols under mild condition to give the corresponding adducts. On the other hand, synthetic phenyl isothiocyanate has been shown to readily react with the N-terminal amino acid of protein to afford phenyl thiocarbamoyl protein and subsequently phenylthiohydantoin of the amino acid on dehydration (Edman, 1949, 1950). Therefore, isothiocyanates formed in the crushed *Brassica* tissues may arise from some chemical modification of tissue proteins under physiological condition.

0021-8561/82/1430-0618$01.25/0