Metabolism of Fenvalerate by Resistant Colorado Potato Beetles

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Colorado potato beetles (*Leptinotarsa decemlineata* Say) were colonized from field collections made in potato-growing areas of Long Island, NY, previously treated with fenvalerate. These insects were 39-fold resistant to fenvalerate when compared to a susceptible reference colony, but the toxicity of fenvalerate to resistant beetles was increased 26-fold by coapplication of piperonyl butoxide. Resistant adult female beetles metabolized [^{14}C]fenvalerate, labeled in either the acid or alcohol moiety, to give 11 (acid label) or 10 (alcohol label) organosoluble metabolites in both free and conjugated forms. The metabolites tentatively identified by cochromatography with synthesized standards provide evidence for three major metabolic pathways: hydroxylation at methyl in the acid moiety; ester cleavage; diphenyl ether cleavage in the alcohol moiety. No metabolites arising from hydroxylation at the 4′-position of the alcohol moiety were detected.

The pyrethroid insecticide fenvalerate (fen) was introduced for the control of the Colorado potato beetle (CPB; *Leptinotarsa decemlineata* Say) on potatoes in the eastern United States in 1979. Reports of control failures in 1981 led to the use of fen with the synergist piperonyl butoxide (PB) in 1982 (Forgash, 1984). The effectiveness of PB in restoring the effectiveness of fen against resistant populations has been confirmed in controlled field trials (Ghidiu and Silcox, 1984) and laboratory bioassays (Forgash, 1981, 1984; Silcox et al., 1985).

These findings implicate enhanced metabolism of fen by cytochrome P_{450} dependent monooxygenases as a principal mechanism of resistance in CPB populations. fen is known to undergo hydrolysis of the central ester bond and oxidative attack at sites in both the acid and alcohol moieties in mammals (Ohkawa et al., 1979; Kaneko et al., 1981, 1984; Lee et al., 1985), in plants (Ohkawa et al., 1980a), soils (Ohkawa et al., 1978; Mikami et al., 1984; Lee, 1985), and in environmental microcosm systems (Ohkawa et al., 1980b; Caplan et al., 1984), but the information on the fate of this compound in insects is limited to two preliminary reports (Kogiso et al., 1982; Soderlund et al., 1983). We now describe the metabolism of fen in fen-resistant CPB adult females.

MATERIALS AND METHODS

Chromatography. Precoated 20 × 20 cm chromatoplates (silica gel 60 F_{254}; EM Laboratories, Elmsford, NY) having a gel thickness of 0.25 mm (analytical separations) or 0.5 mm (preparative separations) were used for thin-layer chromatography (TLC). Seven TLC solvent systems were used for metabolite separation and characterization: A, hexane–toluene–acetic acid, 3:15:2; B, toluene–ether–acetic acid, 75:25:1; C, hexane–ether, 6:1; D, toluene–hexane–ethyl acetate, 50:10:1; E, toluene–ether, 10:1; F, dichloromethane; G, hexane–acetone–acetic acid, 25:25:1. R_f values for fenvalerate, possible metabolites, and their derivatives are given in Table I. The following conventions are used to refer to specific TLC analyses: (A) indicates one-dimensional development in solvent A; (A,B×2) indicates two-dimensional TLC with development in solvent A in the first direction followed by development twice in solvent B in the second direction.

Table I. Chromatographic Properties of Fenvalerate, Possible Metabolites, and Their Derivatives

compd	TLC R_f in indicated solvent systems[a] A×2	B×2	other
Fenvalerate, Ester Metabolites, and Their Derivatives			
fen	0.77	0.86	
4′-HO-fen	0.39	0.64	(A) 0.24
4′-MeO-fen	0.75	0.84	(C×2) 0.30, (D×2) 0.52, (E) 0.61, (F) 0.48
2′-MeO-fen	0.74	0.83	(C×2) 0.26, (D×2) 0.49
desphenyl-fen-Me	0.74	0.83	(C×2) 0.36, (D×2) 0.53
α-$CONH_2$-fen	0.38, 0.33[b]	0.48, 0.41[b]	
CMBA, Metabolites of the Acid Moiety, and Their Derivatives			
CMBA	0.64	0.64	(A) 0.39
CMBA-Me	0.77	0.83	(A) 0.50
4-HO-CMBA (α)	0.11	0.07	
4-HO-CMBA lactone (α)	0.46	0.58	(G) 0.68
4-HO-CMBA (β)	0.20	0.20	
4-HO-CMBA lactone (β)	0.44	0.57	(G) 0.67
2-HO-CMBA	0.38	0.47	
2,4-$(HO)_2$-CMBA	0.00	0.00	
PBacid, Metabolites of the Alcohol Moiety, and Their Derivatives			
PBacid	0.59	0.54	(A) 0.37
PBacid-Me	0.73	0.82	
4′-HO-PBacid	0.23	0.34	
5-HO-PBacid	0.23	0.38	
6-HO-PBacid	0.39	0.54	
3-HO-Bacid	0.19	0.36	(A) 0.11
3-HO-Bacid-Me	0.25	0.58	

[a] See the Materials and Methods for compositions of TLC solvents. [b] Two pairs of diastereomers resolved in this system.

Spectroscopy. Proton nuclear magnetic resonance (NMR) spectra were determined for dilute solutions in chloroform-*d*, with tetramethylsilane as the internal standard (δ 0.00) on a Perkin-Elmer Model R600 spectrometer at 60 MHz. Electron impact mass spectra (MS) were recorded by a Hewlett-Packard Model 5985 mass spectrometer with data system operated in the direct-inlet mode at 20 eV. Spectra are reported as mass (relative intensity) for molecular ions (M^+) and all other fragments present at relative intensities >10%.

Chemicals. Two radiolabeled fenvalerate preparations were used. [*chlorophenyl*-U-^{14}C]Fenvalerate (designated ac*-fen; 14.9 mCi/mmol) and unlabeled fen were provided by Shell Development Co., Modesto, CA. [*methylene*-^{14}C]Fenvalerate (alc*-fen; 59.7 mCi/mmol) was a gift from J. O. Nelson, Department of Entomology, University of

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Maryland, College Park, MD. Prior to their use in metabolism experiments, these samples were purified by preparative TLC to give radiochemical purities in excess of 99%.

Shell Development Co. also provided the following compounds as standards for metabolite identification (trivial designations in parentheses): 2-(4-chlorophenyl)-3-methylbutyric acid (CMBA); α and β isomers of 2-(4-chlorophenyl)-3-(hydroxymethyl)butyric acid (4-HO-CMBA) and their corresponding lactones; 2-(4-chlorophenyl)-2-hydroxy-3-methylbutyric acid (2-HO-CMBA); 2-(4-chlorophenyl)-2-hydroxy-3-(hydroxymethyl)butyric acid (2,4-$(HO)_2$-CMBA); 3-(4-hydroxyphenoxy)benzoic acid (4'-HO-PBacid); α-cyano-3-(4-hydroxyphenoxy)benzyl 2-(4-chlorophenyl)-3-methylbutyrate (4'-HO-fen); and α-(aminocarbonyl)-3-phenoxybenzyl 2-(4-chlorophenyl)-3-methylbutyrate (α-$CONH_2$-fen). 3-Hydroxybenzoic acid (3-HO-Bacid) and 3-phenoxybenzoic acid (PBacid) were purchased from Aldrich Chemical Co., Milwaukee, WI. CMBA for use as an intermediate in syntheses was purchased from Frinton Laboratories, Vineland, NJ, and recrystallized three times from hexane–benzene (10:1) prior to use.

5-Hydroxy-3-phenoxybenzoic Acid (5-HO-PBacid) and 6-Hydroxy-3-phenoxybenzoic Acid (6-HO-PBacid). These compounds were synthesized as described previously (Unai and Casida, 1977). Analytical data for the products obtained were in agreement with those reported elsewhere (Ungnade and Rubin, 1951; Unai and Casida, 1977).

α-Cyano-3-methoxybenzyl 2-(Chlorophenyl)-3-methylbutyrate (desphenyl-fen-Me). This compound was synthesized by procedures similar to those described by Ohkawa et al. (1978). NaCN (59 mg, 1.2 mmol) and tetrabutylammonium chloride (7.7 mg, approximately 0.024 mmol at 85% purity; Sigma Chemical Co., St. Louis, MO) in 1 mL of water were added to a stirred solution of 4-(2-chlorophenyl)-3-methylbutyryl chloride (CMBA-Cl; 231 mg, 1 mmol; prepared from the reaction of CMBA with excess $SOCl_2$ in refluxing dry benzene for 4 h followed by removal of benzene and unreacted $SOCl_2$ under vacuum) and 3-methoxybenzaldehyde (136 mg, 1 mmol; Aldrich) in 5 mL of hexane. After reaction at room temperature with stirring for 19 h, the mixture was extracted with diethyl ether. Removal of solvent in vacuo yielded 294 mg of crude product, which was purified by preparative TLC (toluene–ethyl acetate, 6:1) to give 222 mg (62%) of the desired product. NMR: δ 0.67–1.12 [6 H, m, $CH(CH_3)_2$, two diastereomers]; 2.29 [1 H, m, $CH(CH_3)_2$]; 3.25 (1 H, d, J = 10 Hz, ArCHCO); 3.75 (1.5 H, s) and 3.80 (1.5 H, s), OCH_3, two diasteromers; 6.33 (0.5 H, s) and 6.36 (0.5 H, s), CHCN, two diastereomers; 6.84–7.23 (8 H, m, aromatic). MS: m/z 357 (7, M^+), 169 (32), 167 (41), 163 (51), 154 (14), 152 (43), 147 (15), 146 (50), 127 (26), 125 (100), 116 (13), 115 (11).

α-Cyano-3-(2-methoxyphenoxy)benzyl 2-(4-Chlorophenyl)-3-methylbutyrate (2'-MeO-fen). 3-(2-Methoxyphenoxy)benzaldehyde (2'-MeO-PBald) was synthesized by reacting the potassium salt of 2-methoxyphenol [4.05 g, 0.025 mol; prepared by dissolving equimolar amounts of 2-methoxyphenol (Sigma) and KOH in water followed by removal of water as a binary azeotrope with benzene] and 3-bromobenzaldehyde (4.63 g, 0.025 mol; Aldrich) in the presence of $CuCl_2$ (0.5 g) in refluxing DMF (15 mL) under N_2 for 4.5 h. The mixture was cooled and partitioned between diethyl ether and water. The organosoluble product was concentrated by rotary evaporation and fractionated on Florisil (20 g) by elution with benzene. The crude product (462 mg) was purified by preparative TLC (hexane–ethyl acetate, 3:1) to give 187 mg (3%) of the desired product. NMR: δ 3.78 (3 H, s, CH_3O); 7.08–7.57 (8 H, m, aromatic); 9.93 (1 H, s, CHO). 2'MeO-fen was prepared from 2'-MeO-PBald (187 mg, 0.82 mmol), CMBA-Cl (175 mg, 0.82 mmol), and NaCN (48 mg, 0.98 mmol) in the presence of tetrabutylammonium chloride by the procedure described for desphenyl-fen-Me. Diethyl ether extraction of the reaction mixture yielded 388 mg of crude product. A portion (200 mg) of this was purified by preparative TLC (toluene) to yield 101 mg (overall yield 53%) of the desired product. NMR: δ 0.67–1.10 [6 H, m, $CH(CH_3)_2$, two diastereomers]; 2.30 [1 H, m, $CH(CH_3)_2$]; 3.23 (1 H, d, J = 10 Hz, ArCHO); 3.78 (1.5 H, s) and 3.80 (1.5 H, s), OCH_3, two diastereomers; 6.28 (0.5 H, s) and 6.32 (0.5 H, s, CHCN, two diastereomers); 6.9–7.4 (12 H, m, aromatic). MS: m/z 451 (12), 450 (10), 449 (30, M^+ for ^{35}Cl), 228 (15), 211 (10), 169 (28), 167 (45), 152 (14), 139 (11), 130 (26), 127 (29), 125 (100).

α-Cyano-3-(4-methoxyphenoxy)benzyl 2-(4-Chlorophenyl)-3-methylbutyrate (4'-MeO-fen). 4'-MeO-fen was synthesized from 3-(4-methoxyphenoxy)benzaldehyde (228 mg, 1 mmol; Aldrich), CMBA-Cl (231 mg, 1 mmol), and NaCN (59 mg, 1.2 mmol) in the presence of tetrabutylammonium chloride (8 mg) as described above for desphenyl-fen-Me. Diethyl ether extraction of the reaction mixture yielded the crude product (352 mg). Purification of 100 mg of this mixture by preparative TLC (toluene–ethyl acetate, 10:1, two developments) gave 50.5 mg (overall yield 40%) of the desired product. NMR: δ 0.66–1.10 [6 H, m, $CH(CH_3)_2$, two diastereomers]; 2.25 [1 H, m, $CH(CH_3)_2$]; 3.24 (1 H, d, J = 10 Hz, ArCHCO); 3.81 (3 H, s, OCH_3); 6.28 (0.5 H, s) and 6.32 (0.5 H, s), CHCN, two diastereomers; 6.8–7.5 (12 H, m, aromatic). MS: m/z 452 (11), 451 (37), 450 (32), 449 (93, M^+ for ^{35}Cl), 228 (32), 211 (19), 169 (25), 167 (39), 152 (13), 139 (12), 127 (23), 125 (100), 115 (17), 114 (10).

Preparation of Methyl Ester and Ether Derivatives. Synthesized potential metabolites and isolated radiolabeled metabolites were methylated by treatment with CH_2N_2 in dry diethyl ether for 0.5 h (carboxylic acids) or 24–48 h (phenols) at room temperature. Methyl ester or ether formation with synthetic standards was confirmed by NMR analysis of the product formed. Trivial designations incorporate the prefix MeO in place of HO for phenols and the suffix Me for methyl esters; thus, the permethylated derivative of 4'-HO-PBacid is designated 4'-MeO-PBacid-Me.

Insects and Bioassays. fen-resistant CPB, originally collected from potato fields adjacent to the Long Island Horticultural Research Laboratory, Riverhead, NY, in 1982, were obtained from M. Tauber, Department of Entomology, Cornell University, Ithaca, NY. Susceptible CPB were obtained from W. Tingey, Department of Entomology, Cornell University. Both strains were reared in continuous culture on potato plants at 27–29 °C and 50–60% relative humidity under a 16L:8D photoperiodic regime. Insects were sexed as pupae, and virgin adult females were held on potato plants for 7–14 days following emergence before use in bioassays or metabolism experiments. For bioassays, insects were treated with 1 μL of acetone solutions of fen or fen plus PB (Pfaltz and Bauer, Inc., Waterbury, CT) on the venter of the abdomen. PB was aministered at 20 μg/insect (susceptible strain) or 50 μg/insect (resistant strain). Preliminary studies established that these were the highest doses giving <5% mortality for each strain. Treated insects were held under rearing room conditions on bouquets of potato foliage for

48 h for the assessment of paralysis or death. Preliminary studies established that insects paralyzed at 48 h did not recover. LD_{50} values were obtained by computerized Probit analysis (Russell et al., 1977) of log dose–mortality data.

Metabolism Studies. Resistant adult female CPB were treated as described above with either ac*-fen (14.9 mCi/mmol) or alc*-fen (specific activity adjusted to 15 mCi/mmol) at a dose of 5 μg/insect (310000 dpm/insect; 2 × LD_{50}) in 1 μL of 2-butanone. Following treatment, insects were placed in 20-mL glass scintillation vials, held at 20 ± 2 °C for 48 h, and then rinsed in hexane (10 mL, 2 min). Holding vials were rinsed with methanol (2 × 5 mL), and insects were extracted by homogenization (Polytron, Kinematica GmbH, Lucerne, Switzerland) in the methanol holding vial rinses to obtain extracts of insects plus excreta. Aliquots (0.1–0.5 insect equivalent) of each external rinse and combined methanol extract were evaporated under N_2 for liquid scintillation counting (LSC). Unextractable radiocarbon was determined by digestion of the methanol extract pellet with Protosol (0.5 mL; New England Nuclear, Boston, MA) for 6 h at 55 °C followed by LSC.

Metabolite Separation and Characterization. Aliquots (0.5–0.9 insect equivalents) of hexane rinses were fractionated by TLC (A×2). Unmetabolized fen and metabolite radiocarbon were located by exposure of plates to X-ray film (Kodak SB-5; Standard Medical Systems, Columbia, MD) and the relative amounts of parent and metabolites present were determined by LSC. No attempt was made to identify the metabolites found in this fraction. The methanol extracts of insects and excreta were concentrated to dryness under N_2, dissolved in 2 mL of 80% methanol, loaded onto a reversed-phase cleanup column (Baker-10 C-18; VWR Scientific, Rochester, NY), and eluted under vacuum with an additional 4 mL of 80% methanol, followed by 4 mL of 90% methanol and 4 mL of 100% methanol. The 100% and 90% methanol fractions were analyzed by TLC as described above for the hexane rinse fractions. The 80% methanol fractions (0.5–0.9 insect equivalents) were analyzed by two-dimensional TLC (A×2, B×2).

Organosoluble metabolites resolved by TLC were located by exposure of plates to X-ray film. Gel regions corresponding to metabolites were recovered and extracted with methanol. These isolated metabolites were further characterized by cochromatography with synthesized standards either by direct means or after derivatization with CH_2N_2.

Hydrolysis and Characterization of Conjugates. Radiocarbon remaining at the origin after initial TLC separation of 80% methanol fractions was recovered and extracted from the silica gel with methanol; typically, methanol extracts from several separations were pooled for further analysis. The residue obtained by evaporation of methanol under N_2 was incubated with 15 units of β-glucosidase (Sigma) in 3 mL of acetate buffer (pH 5.0) at 37 °C for 4 h, acidified to pH 2, and extracted with 3 × 3 mL of ethyl acetate. The combined ethyl acetate fractions were concentrated under N_2 and analyzed by two-dimensional TLC (A×2, B×2). Aglycons were recovered and characterized as described above for unconjugated metabolites. Radiocarbon remaining at the origin was recovered as described above, extracted from the silica gel, and incubated with 0.25 mL of 3 N HCl in a boiling-water bath for 30 min. The residue obtained after evaporation of the HCl solution was analyzed by two-dimensional TLC (A×2, B×2), and unconjugated metabolites liberated by acid hydrolysis were recovered and characterized as described above. In preliminary experiments, radiocarbon remaining at the origin after β-glucosidase incubations was incubated with 9 units of arylsulfatase (type H-1; Sigma) in 2 mL of acetate buffer (pH 4.5) for 6 h at 37 °C. However, no significant organosoluble radiocarbon was liberated by sulfatase treatment.

Table II. Toxicity and Synergism by Piperonyl Butoxide of Fenvalerate in Resistant Colorado Potato Beetles

strain	synergist	LD_{50}[a]	synergism ratio[b]	resistance ratio[c]
susceptible	none	0.06		
susceptible	piperonyl butoxide[d]	0.014	4.3	
resistant	none	2.36		39.3
resistant	piperonyl butoxide[e]	0.09	26.2	6.4

[a] Microgram/beetle by topical application. [b] LD_{50} alone/LD_{50} with synergist. [c] LD_{50} resistant/LD_{50} susceptible. [d] 20 μg/beetle; nontoxic to susceptible strain. [e] 50 μg/beetle; nontoxic to resistant strain.

Metabolite Quantitation. The hexane rinses and the 100% and 90% methanol fractions of the methanol extract, unextractable radiocarbon, and holding vials were quantified as described above. Metabolites in the 80% methanol fraction and those liberated by β-glucosidase or acid hydrolysis were recovered from two-dimensional TLC for LSC. The regions containing radiocarbon other than that resolved in distinct metabolite spots and aqueous radiocarbon following β-glucosidase incubation were also measured by LSC. The results presented are means of six determinations for each labeled preparation using individual insects for each determination, except that amounts of conjugated metabolites are calculated from pooled samples for each preparation.

RESULTS

Toxicity of Fenvalerate to Resistant and Susceptible Colorado Potato Beetles. The LD_{50} values obtained by topical application of fen to adult virgin female CPB are shown in Table II. At the LD_{50} level, the Long Island derived strain was approximately 39-fold resistant to fen when compared with the susceptible strain. Coapplication of fen and sublethal doses of PB increased the sensitivity to fen of the resistant strain to a greater extent than in the susceptible strain and reduced the magnitude of resistance to approximately 6-fold. For metabolism studies, resistant beetles were treated with a dose of labeled fen equivalent to 5 μg/insect, approximately twice the LD_{50} for this strain. At this dose, insects became paralyzed within 4 h after treatment but remained alive for up to 72 h.

Radiocarbon Distribution and Recovery. Preliminary studies with ac*-fen established that more than 50% of a topical dose was lost from the cuticle surface within 24 h after treatment and that radiocarbon levels in extracts of solvent-rinsed beetles reached a maximum 24–48 h after treatment. Treated beetles were held for 48 h prior to extraction and analysis in all subsequent studies to allow maximum metabolite formation.

Table III summarizes the distribution of recovered radiocarbon in the external rinse, the three reversed phase cleanup subfractions of the methanol extract, and the digested pellet from methanol extraction for insects held 48 h after treatment with either ac>-fen or alc*-fen. During this period, approximately 60% of the applied dose was lost from the cuticle surface. Fen was metabolized more extensively in the studies using alc*-fen, which were not run concurrently with those for ac*-fen, and the overall recovery of radiocarbon with alc*-fen was lower. Labeled metabolites were efficiently recovered in the 80% methanol

Table III. Radiocarbon Balance for ac*-fen and alc*-fen 48 h after Topical Application to Adult Female Colorado Potato Beetles[a]

fraction	ac*-fen			alc*-fen		
	% rec	% fen	% other	% rec	% fen	% other
external rinse	38.2	98.2	1.8	41.4	95.9	4.1
extract (rinsed insects + excreta)[b]						
80% methanol	12.1	0.4	99.6	13.0	0.5	99.5
90% methanol	37.1	95.9	4.1	21.9	93.7	6.3
100% methanol	1.8	87.8	12.2	1.3	71.7	28.3
unextractable	2.4			1.8		
total recovery	91.6			79.4		

[a] Values are means for six insects for each labeled preparation. [b] Fractions of methanol extract eluted from reversed-phase cleanup column.

fraction from reversed-phase column cleanup at the initial extract, whereas unmetabolized fen was recovered predominantly in the 90% methanol fraction.

Ester Metabolites. Comparison of TLC patterns for metabolites of ac*-fen and alc*-fen in initial separation of 80% methanol fractions indicated the presence of one major ester metabolite, which had chromatographic properties similar but not identical with those of 4′-HO-fen (B×2, A). Hydrolysis of this metabolite in methanolic KOH followed by extraction and reanalysis by TLC (A×2, B×2) yielded CMBA from ac*-fen and a complex mixture of products from alc*-fen, suggesting that this compound was hydroxylated or otherwise modified in the alcohol moiety. The identity of this compound as the product of disphenyl ether cleavage [α-cyano-3-hydroxybenzyl 2-(4-chlorophenyl)-3-methylbutyrate; desphenyl-fen] was confirmed by methylation and cochromatography with synthesized desphenyl-fen-Me in three solvent systems (A×2, B×2; C×2, D×2; E, F). desphenyl-fen was similarly identified among conjugated metabolites following incubation with glucosidase or hydrolysis with acid. One additional minor ester metabolite was detected among the labeled products obtained from ac*-fen and alc*-fen. This product differed in chromatographic properties from all of the available ester standards.

Ester Cleavage Products. Adult female CPB metabolized ac*-fen to give nine resolved ester cleavage products. The major product was recognized as CMBA by cochromatography as the free acid nB×2, A) and as the methyl ester (B×2, A). CMBA was also identified among conjugated metabolites as the glucoside and as an acid-labile conjugate. The α and β isomers of 4-HO-CMBA were also identified by cochromatography (A×2, B×2) with standards. The identity of these products was confirmed by their lactonization under acidic conditions followed by cochromatography with lactone standards (B×2, G). The α and β isomers of 4-HO-CMBA were also detected as glucoside conjugates following hydrolysis by glucosidase, but they were not found among the acid-labile conjugates. Seven nonconjugated metabolites and five glucoside metabolites of ac*-fen did not cochromatograph with any of the available standards for hydroxylated derivatives of CMBA.

Eight chromatographically distinct ester cleavage products were recovered from CPB treated with alc*-fen. The major product was identified as PBacid by cochromatography with the free acid (B×2, A) and with the corresponding methyl ester after CH_2N_2 derivatization (A×2, B×2). PBacid was also identified among conjugated metabolites of alc*-fen following hydrolyses with glucosidase and acid. 3-HO-Bacid, the ester cleavage product

Table IV. Amounts of ac*-fen, alc*-fen, and Their Metabolites in Methanol Extracts of Rinsed Insects and Excreta 48 h after Topical Application to Adult Female Colorado Potato Beetles[a]

compd of fraction	% appl dose	
	ac*-fen	alc*-fen
Esters, Free and Conjugated		
fenvalerate	37.3	21.5
desphenyl-fen	0.6	0.9
desphenyl-fen-gluc	0.1	0.1
desphenyl-fen-conj[b]		0.1
unknown	0.1	0.1
Ester Cleavage Products, Free		
CMBA	0.9	
4-HO-CMBA (2 isomers)	0.8	
PBacid		0.7
3-HO-Bacid		0.2
major unknowns[c]	0.6	0.6
minor unknowns[d]	1.4	0.6
Ester Cleavage Products, Conjugated		
CMBA-gluc	2.2	
CMBA-conj	0.2	
4-HO-CMBA-gluc (2 isomers)	0.1	
PBacid-gluc		0.2
mPBacid-conj		0.2
3-HO-Bacid-gluc		0.1
3-HO-Bacid-conj		0.3
major unknowns	1.3	
minor unknowns	0.8	0.9
unhydrolyzed	0.9	3.3
other radiocarbon[e]	4.3	6.0

[a] Values are means for six insects for each labeled preparation. [b] Unknown conjugating moiety hydrolyzed by acid. [c] >0.5% of applied radiocarbon. [d] <0.5% of applied radiocarbon. [e] Radiocarbon recovered from TLC but not resolved into defined metabolite spots.

of desphenyl-fen, was also identified by cochromatography both as the free acid (B×2, A) and following methylation (A×2, B×2). 3-HO-Bacid was similarly detected as the glucoside conjugate following glucosidase treatment and as an acid-labeled conjugate. Six nonconjugated metabolites and six conjugates of alc*-fen did not cochromatograph with available standards.

Metabolite Quantitation. Table IV summarizes the amounts of fen and its metabolites recovered in extracts of adult female CPB and excreta 48 h after treatment with ac*-fen or alc*-fen. Most of the methanol-extractable radiocarbon was unmetabolized fen. Diphenyl ether cleavage to give desphenyl-fen was a major pathway with both labeled preparations. Ester cleavage of fen or ester metabolites to give CMBA, 4-HO-CMBA, PBacid, and 3-HO-Bacid were also major pathways based on the amounts of these metabolites recovered. In addition to these metabolites, two metabolites of ac*-fen and one metabolite of alc*-fen that individually comprised at least 0.5% of the applied dose were not identified in this study. The remaining unidentified metabolites were individually present in very small amounts, but collectively they represented a significant proportion of the recovered nonparent radiocarbon, particularly with ac*-fen.

DISCUSSION

Our data demonstrate significant resistance to fen in CPB from eastern Long Island. Moreover, much of the resistance was reversible by coapplication of PB, thus implicating enhanced oxidative metabolism as a mechanism of resistance. These findings are in good agreement with other studies of fen-PB combinations in the control of resistant CPB (Forgash, 1981; Ghidiu and Silcox, 1984; Silcox et al., 1985). Enhanced oxidative metabolism has

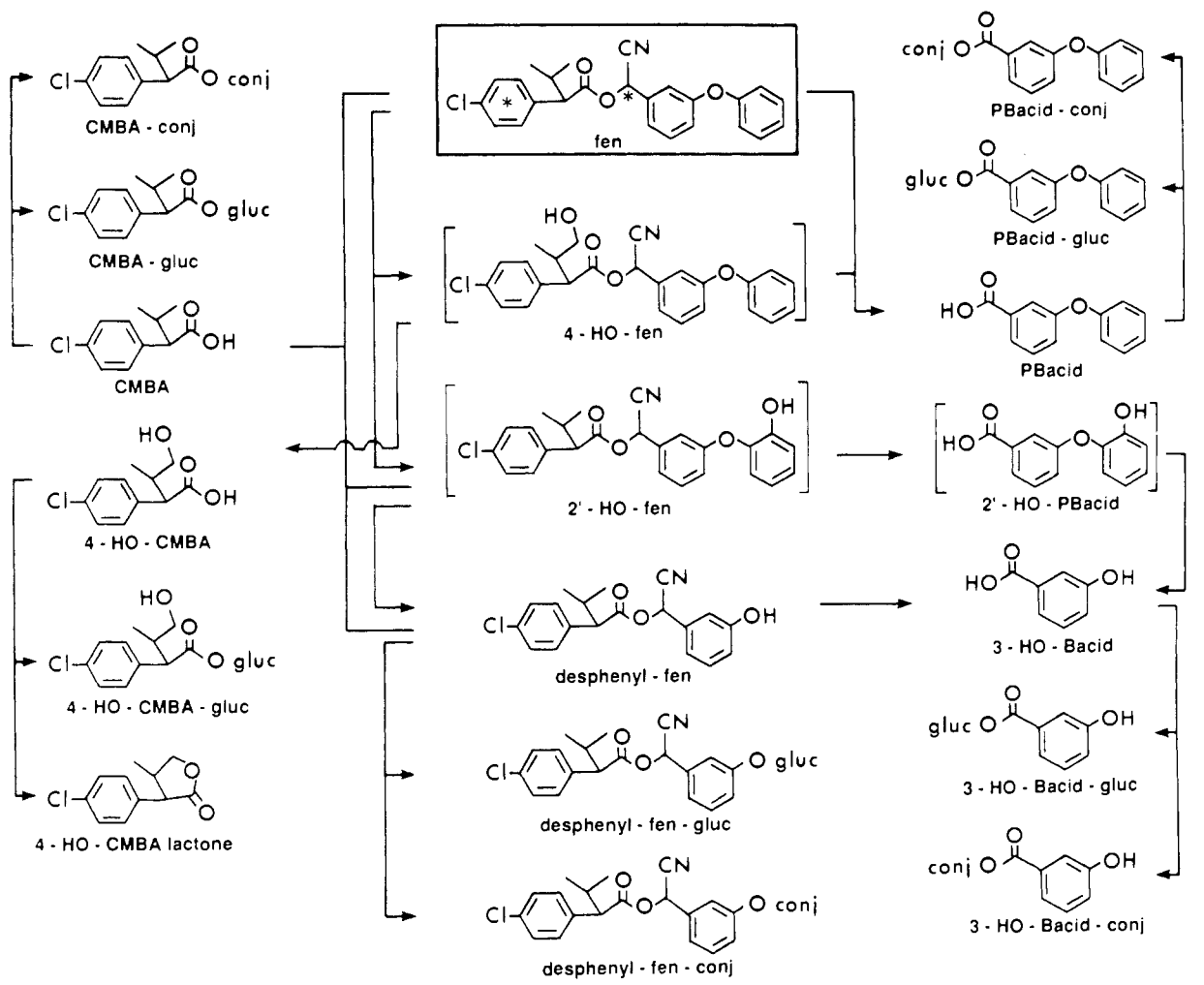

Figure 1. Proposed pathways for fenvalerate metabolism in resistant Colorado potato beetles based on identified metabolites. Asterisks indicate positions of radiolabeling in the parent molecule. Structures in brackets are possible intermediates not identified in this study. Aldehyde intermediates to benzoic acids are not shown. Conjugates are designated as gluc for glucosides and conj for uncharacterized acid-labile conjugates. Conjugates of dihydroxy compounds (e.g., 3-HO-Bacid) are arbitrarily shown with the conjugating moiety at the carboxyl position.

also been implicated in the resistance of CPB to carbofuran (Rose and Brindley, 1985). Our objective in this study was to define the pathways of fen metabolism in an insect species in which extensive oxidative biotransformation was anticipated.

On the basis of the metabolites tentatively identified in this study, there are three major pathways of fenvalerate metabolism in resistant CPB: ester cleavage, acid methyl hydroxylation, diphenyl ether cleavage (Figure 1). All of these pathways involve reactions previously identified from other biological systems. Ester cleavage is an ubiquitous pathway, found in all organisms examined to date (Ohkawa et al., 1978, 1979, 1980a, 1980b; Kaneko et al., 1981, 1984; Kogiso et al., 1982; Soderlund et al., 1983; Mikami et al., 1984; Caplan et al., 1984; Lee, 1985; Lee et al., 1985). Hydroxylation of one methyl group of the isopropyl substituent in the acid moiety has been found in mammals (Ohkawa et al., 1979; Kaneko et al., 1981, 1984; Lee et al., 1985) and in house flies (Kogiso et al., 1982; Soderlund et al., 1983). Diphenyl ether cleavage, the major pathway in resistant CPB, has been reported previously only in soils (Ohkawa et al., 1978; Mikami et al., 1984) and house flies (Kogiso et al., 1982). The mechanism of diphenyl ether cleavage is not clear. On the basis of the reported instability of phenoxybenzyl esters hydroxylated in the 2′-position (Gaughan et al., 1977; Ohkawa et al., 1978), we have proposed 2′-hydroxyfen as the initial product in this pathway (Figure 1). However, the intermediacy of the 2′-hydroxy derivative remains to be confirmed.

The hydroxy esters formed by monooxygenase attack on the parent compound undergo ester cleavage as a secondary reaction. 3-HO-Bacid may be formed by enzymatic hydrolysis of desphenyl-fen, or it may arise by nonenzymatic degradation of 2′-HO-PBacid following hydrolysis if 2′-HO-fen is the initial product in the diphenyl ether cleavage pathway. Significant ester cleavage may also occur in the nonenzymatic degradation of 4-HO-fen, which is unstable under analytical conditions. It is not clear from our data whether the ester linkage is primarily cleaved before or after hydroxylation. However, since several esterase inhibitors do not synergize fen in bioassays with resistant CPB (Soderlund, D. M., unpublished observations), extensive cleavage of the parent compound is of doubtful significance as a detoxication reaction. Two major metabolites of alc*-fen and one major metabolite of ac*-fen, all of which are ester cleavage products, remain unidentified. Consequently, one or more major pathways of fen metabolism in resistant CPB in addition to those identified in this study may exist.

All of the identified metabolites were recovered in part as glucoside conjugates, and all but the isomers of 4-HO-CMBA were conjugated to glucosidase- and sulfatase-insensitive moieties that were hydrolyzed by acid treatment. It is likely that the latter compounds were conjugated to

one or more amino acids, a pathway of secondary metabolism characterized for permethrin in other insect species (Shono et al., 1978).

Some significant pathways for fen metabolism previously identified in other systems were not found in our studies with resistant CPB. In particular, we found no evidence for the formation of 4′-HO-fen or its hydrolysis product, 4′-HO-PBacid. Hydroxylation at the 4′-position has been observed in all other studies of fen metabolism where the fate of the alcohol moiety of fen has been characterized (Ohkawa et al., 1978, 1979, 1980a, 1980b; Kaneko et al., 1981, 1984; Kogiso et al., 1982; Mikami et al., 1984; Caplan et al., 1984; Lee, 1985; Lee et al., 1985). On the basis of the chromatographic properties of available standards (Table I), we also did not find metabolites resulting from benzylic hydroxylation at the 2-position of the acid moiety, a pathway found in mammals (Ohkawa et al., 1979; Kaneko et al., 1981, 1984; Lee et al., 1985), nor did we find ester metabolites resulting from the hydration of the cyano substituent to an amide. The latter pathway is well characterized only in metabolism studies with soils (Ohkawa et al., 1978; Mikami et al., 1984; Lee, 1985) and aquatic sediments (Ohkawa et al., 1980b; Caplan et al., 1984).

In view of the significance of oxidative detoxication implied by the bioassay data, the large amounts of unmetabolized fen recovered in insect extracts is surprising. It is likely that much of the penetrating dose of fen is sequestered by the large mass of fat body and reproductive organs found in adult female CPB, as has been described previously in pharmacokinetic studies of pyrethroids in cockroaches (Soderlund, 1979). If the gut rather than the fat body is the principal site of oxidative metabolism, a situation common in phytophagous insects (Wilkinson and Brattsten, 1972; Brattsten, 1979), then the portion of dose sequestered in these tissues may be unavailable for either biotransformation or interaction with target sites in the insect nervous system. Thus, the relatively small amount of biotransformation evident in the extracts of whole insects may in fact represent extensive metabolism of that part of the applied dose available for uptake by the nervous system. These findings suggest that apparently subtle changes in the rate constants for partitioning and detoxication events can produce profound alterations in the susceptibility of an insect to toxicants.

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LITERATURE CITED

Brattsten, L. B. *Drug Metab. Rev.* **1979**, *10*, 35.

Caplan, J. A.; Isensee, A. R.; Nelson, J. O. *J. Agric. Food Chem.* **1984**, *32*, 166.

Forgash, A. J. In *Advances in Potato Pest Management*; Lashomb, J. H., Casagrande, R., Eds.; Hutchinson Ross: Stroudsburg, PA, 1981; p 34.

Forgash, A. J. *Pestic. Biochem. Physiol.* **1984**, *22*, 178.

Gaughan, L. C.; Unai, T.; Casida, J. G. *J. Agric. Food Chem.* **1977**, *25*, 9.

Ghidiu, G. M.; Silcox, C. A. *J. Agric. Entomol.* **1984**, *1*, 360.

Kaneko, H.; Ohkawa, H.; Miyamoto, J. *J. Pestic. Sci.* **1981**, *6*, 317.

Kaneko, H.; Izumi, T.; Matsuo, M.; Miyamoto, J. *J. Pestic Sci.* **1984**, *9*, 269.

Kogiso, S.; Kawanishi, Y.; Ohkawa, H.; Matsuo, M. Abstracts of the Fifth International Congress of Pesticide Chemistry (IUPAC), Kyoto, Japan, Aug 1982; Abstract Va-13.

Lee, P. W. *J. Agric. Food Chem.* **1985**, *33*, 993.

Lee, P. W.; Stearns, S. M.; Powell, W. R. *J. Agric. Food Chem.* **1985**, *33*, 988.

Mikami, N.; Sakata, S.; Yamada, H.; Miyamoto, J. *J. Pestic. Sci.* **1984**, *9*, 697.

Ohkawa, H.; Nambu, K.; Inui, H.; Miyamoto, J. *J. Pestic. Sci.* **1978**, 3, 129.

Ohkawa, H.; Kaneko, H.; Tsuji, H.; Miyamoto, J. *J. Pestic. Sci.* **1979**, *4*, 143.

Ohkawa, H.; Nambu, K.; Miyamoto, J. *J. Pestic. Sci.* **1980a**, *5*, 215.

Ohkawa, H.; Kikuchi, R.; Miyamoto, J. *J. Pestic. Sci.* **1980b**, *5*, 11.

Rose, R. L.; Brindley, W. A. *Pestic. Biochem. Physiol.* **1985**, *23*, 74.

Russell, R. M.; Robertson, J. L.; Savin, N. E. *Bull. Entomol. Soc. Am.* **1977**, *23*, 209.

Shono, T.; Unai, T.; Casida, J. E. *Pestic. Biochem. Physiol.* **1978**, *9*, 96.

Silcox, C. A.; Ghidiu, G. M.; Forgash, A. J. *J. Econ. Entomol.* **1985**, *78*, 1399.

Soderlund, D. M. *Pestic. Biochem. Physiol.* **1979**, *12*, 38.

Soderlund, D. M.; Sanborn, J. R.; Lee, P. W. In *Progress in Pesticide Biochemistry and Toxicology*; Hutson, D. H., Roberts, T. R., Eds.; Wiley: New York, 1983; Vol. 3, p 401.

Unai, T.; Casida, J. G. *J. Agric. Food Chem.* **1977**, *25*, 979.

Ungnade, H. Z.; Rubin, L. *J. Org. Chem.* **1951**, *16*, 1311.

Wilkinson, C. F.; Brattsten, L. B. *Drug Metab. Rev.* **1972**, *1*, 153.

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