Biological Activity of Pyrethroid Analogs in Pyrethroid-Susceptible and -Resistant Tobacco Budworms, *Heliothis virescens* (F.)†

Guomin Shan,‡ Robert P. Hammer,§ and James A. Ottea*,‡

Department of Entomology, Louisiana State University Agricultural Center, and Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

The phenoxybenzyl moiety of conventional pyrethroids is a major site of oxidative metabolism in resistant tobacco budworms, *Heliothis virescens* (F.). In this study, this group was replaced with known P450 monooxygenase-inhibiting or oxidatively blocked groups. A variety of isomers (1*R*/1*S*, *cis*/*trans*) of the resulting chrysanthemates were tested as insecticides or synergists against tobacco budworms that were insecticide-susceptible (LSU) or that expressed metabolic resistance to cypermethrin (Pyr-R). A number of compounds with pentafluorophenyl, methylenedioxyphenyl, and propargyloxyphenyl groups were insecticidal, and activity was dependent on both geometric and stereochemical configuration of the acid moiety. Both *trans* and *cis* isomers of 1(*R*)-fenfluthrin, which contains a pentafluorophenyl group, suppressed resistance to cypermethrin in Pyr-R insects, confirming that oxidative metabolism of the phenoxybenzyl moiety is a major mechanism of resistance in this strain. Of the methylenedioxyphenyl compounds, 1*R*, *trans*, and *cis* isomers were toxic and partially suppressed resistance in Pyr-R larvae. Similarly, both *trans* and *cis* isomers of α(*S*),1(*R*)-propargyloxyphenyl-containing compounds were insecticidal. Finally, α(*R*),1(*R*)-*cis*-methylenedioxyphenyl- and -propargyloxyphenyl- containing compounds were nontoxic but significantly enhanced toxicity of cypermethrin.

Keywords: *Tobacco budworm; pyrethroid analogs; Heliothis virescens*

INTRODUCTION

The tobacco budworm, *Heliothis virescens* (F.), was first recognized as a pest of cotton in 1934 (Folsom, 1936) and has become one of the most important insects attacking cotton in the United States (Sparks, 1981; Wolfenbarger et al., 1981). This pest, combined with the cotton bollworm, *Helicoverpa zea* (Boddie), has caused almost one-third of all insect damage to U.S. cotton during the 1990s (Head, 1992, 1993; Williams, 1994, 1995, 1996). Insecticide resistance is a major factor contributing to our inability to manage populations of *H. virescens* on cotton (Sparks, 1981; Sparks et al., 1993).

Insecticide resistance is due to the expression of one or more of three major mechanisms: reduced cuticular penetration, enhanced metabolic detoxication, and reduced target site sensitivity (Bull, 1981; Oppenoorth, 1985). Monitoring the susceptibility of tobacco budworms to insecticides and identifying actual or potential resistance mechanisms expressed during the cotton growing season are essential to maximizing the success of insecticide resistance management (IRM) strategies (ffrench-Constant and Roush, 1990; Plapp et al., 1990). In theory, if the mechanisms underlying resistance to an insecticide can be detected, it may be possible to manage metabolic resistance or reduced target site sensitivity by using synergists or insecticides from a different chemical class, respectively.

Studies with *H. virescens* suggest that resistance to pyrethroids, which are widely used against cotton pests in the United States, is associated with all three mechanisms (McCaffery et al., 1989; Gladwell et al., 1990; Ottea et al., 1995). Pyrethroid toxicity is enhanced in biological assays with field-collected tobacco budworms by cytochrome P450 monooxygenase inhibitors such as piperonyl butoxide (PBO) (Graves et al., 1991; Elzen et al., 1993) and propynyl ethers (Payne, 1987). In addition, biochemical and pharmacokinetic studies have shown the importance of cytochrome P450 monooxygenases in pyrethroid resistance in laboratory and field-collected strains of *H. virescens* (Nicholson and Miller, 1985; Little et al., 1989; McCaffery et al., 1991; Abd-Elghafar et al., 1994; Ottea et al., 1995) and demonstrated that oxidative metabolism of pyrethroids by these enzymes occurs predominantly at the 2′ and 4′ carbons of the phenoxybenzyl group that is prevalent in commercial pyrethroids.

Current methods for characterizing resistance mechanisms in field populations of the tobacco budworm include bioassays with combinations of insecticides and synergists (Raffa and Priester, 1985; Campanhola and Plapp, 1989) and biochemical assays that measure activities of enzymes associated with insecticide metabolism. However, multiple forms of these enzymes exist with differing substrate specificities and susceptibilities to inhibition by synergists (Payne, 1987; Scott, 1990; Feyereisen et al., 1991; Brown et al., 1996); thus, relevance of results from these assays is limited by the reliability of model substrates and synergists as indicators of toxicologically significant enzyme activities (Sawicki, 1987; Kirby et al., 1994; Ibrahim and Ottea, 1995). Furthermore, utility of results from bioassays with insecticide/synergist combinations is limited by nonmetabolic effects of these compounds and the lack of structural similarity between conventional synergists (such as PBO and propynyl ethers) and pyrethroid insecticides.

* Author to whom correspondence should be addressed [telephone (504) 388-1841; e-mail jottea@unix1.sncc.lsu.edu].

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‡ Department of Entomology.

§ Department of Chemistry.

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The purpose of this research was to evaluate the utility of pyrethroid analogs as diagnostic compounds for detection of P450 monooxygenase-associated pyrethroid resistance. Esters containing 3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylic acid (permethric acid; PA) and enzyme-inhibiting side chains or groups blocking potential sites of oxidative metabolism were synthesized. Results are presented from biological assays measuring insecticidal activity and synergism of cypermethrin toxicity.

MATERIALS AND METHODS

Chemicals. Cypermethrin (technical grade; a racemic mixture of *trans*/*cis*, 1*R*/*S*, and α*R*/*S* isomers) was obtained from FMC Corp. (Princeton, NJ). The methyl ester of PA [methyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; *cis*/*trans* = 40/60] was purchased from Fisher Scientific (Pittsburgh, PA). Pentafluorobenzyl alcohol, piperonyl alcohol, piperonal, 2,3,6-trichlorophenol, sesamol, 3-hydroxybenzaldehyde, propargyl bromide, (+)-ephedrine, and (−)-ephedrine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Piperonyl butoxide (PBO) was purchased from ChemService (West Chester, PA), and *S*,*S*,*S*-tributyl phosphorotrithioate (DEF) was kindly provided by Bayer Corp. (Kansas City, MO). All other chemicals were of analytical quality and purchased from commercial suppliers.

^{1}H NMR spectra were obtained on a Bruker AC-200 spectrometer using tetramethylsilane as an internal standard. Optical rotations were determined on a Jasco digital polarimeter (Model DIP-370, Na 589 nm). The compounds were analyzed by gas chromatography using a capillary column (DB-5, 20 m × 0.18 mm) with the following temperature programming: T_{init} = 40 °C for 3 min, then raised at 20 °C/min to T_{final} = 280 °C; the compounds were detected by a Hewlett-Packard 5971A mass selective detector.

Insects. Pyrethroid-susceptible and -resistant laboratory strains of *H. virescens* were studied. The susceptible strain (LSU) was established in 1977 (Leonard et al., 1988) and has been reared in the laboratory since that time without exposure to insecticides. The resistant Pyr-R strain was derived from a field collection made in August 1995 from the Red River Research Station (Bossier City, LA). Insects from this collection were reared for one generation and then selected as fifth-stadium larvae with cypermethrin (1.75 μg/larva) for three generations. In an effort to dilute the contribution to resistance of reduced neuronal sensitivity, which is recessive in crosses between susceptible and resistant house flies [Milani, 1956, as cited in Oppenoorth (1965)], survivors of selection were crossed with LSU insects and F1 progeny were selected as third-stadium larvae with 1.0 μg of cypermethrin/larva, a dose corresponding to 21 times the LD_{50} for LSU larvae. Preliminary results from neurophysiological and molecular genetic assays suggest that reduced neuronal sensitivity is not a major resistance mechanism in this strain (Park and Taylor, unpublished results).

Preparation of PA. The methyl ester of PA (22.3 g, 0.1 mol), sodium hydroxide (12 g, 0.3 mol), and ethanol/water (1:1; 250 mL) were mixed and then refluxed overnight. The mixture was concentrated under reduced pressure, diluted with water, and extracted with ethyl ether (Et_2O, 2 × 40 mL). The aqueous layer was acidified with concentrated HCl, and the precipitate was extracted into Et_2O (2 × 50 mL), washed with water, and dried (Na_2SO_4) overnight. The Et_2O was removed to obtain a solid product, which was recrystallized from hexane/ether (1:1). Yield: 18.5 g, 83%, mp 55−58 °C.

Separation of *trans*- and *cis*-PA. Geometric isomers of PA were separated according to the method of Foggassy et al. (1986). PA (20.9 g, 0.1 mol) was mixed with benzene (100 mL) and stirred at 27 °C for 5 h. The suspension was filtered and then recrystallized from benzene to give 3.9 g (47%) of pure *cis*-PA, mp 94−96 °C. For isolation of the *trans* isomer, PA (20.9 g, 0.1 mol) was stirred with petroleum ether (100 mL) at 30 °C for 5 h. The resulting suspension was filtered to give 4.2 g of solid product, which was recrystallized from hexane to give 3.6 g (29%) of pure *trans*-PA, mp 84−87 °C.

Separation of 1*R*,*cis*- and 1*S*,*cis*-PA. The *cis* enantiomers of PA were resolved prior to esterification according to the method of Jolly et al. (1982). *cis*-PA (10 g, 0.05 mol) was dissolved in dichloroethane (100 mL), and then (−)- or (+)-ephedrine (8.25 g, 0.05 mol) was added. The mixture was stirred at 20 °C for 1 h and then filtered under reduced pressure. The solid crude product was recrystallized from dichloroethane to give 6.5 g (30%) of pure (−)-ephedrine (+)-*cis*-permethric salt or 6.6 g of (+)-ephedrine (−)-*cis*-permethric salt. The salts were dissolved in methylene chloride (25 mL) and stirred with HCl (2 M, 30 mL) at 20 °C for 15 min, and the aqueous phase was extracted with methylene chloride. The combined organic phases were washed with water, dried over Na_2SO_4, and evaporated to dryness to obtain 2.9 g (29%) of 1(*R*),*cis*-PA with an optical purity of 99.4% and a specific rotation of $[\alpha]_D^{20}$ = +32.1° (*c* 1.0, $CHCl_3$; lit. $[\alpha]_D$ = +32.2°; Nohira and Yoshida, 1989). For 1(*S*),*cis*-PA, yield was 2.7 g (27%) with an optical purity of 91.6% and a specific rotation of $[\alpha]_D^{20}$ = −29.5 (*c* 1.0, $CHCl_3$; lit. $[\alpha]_D$= −32.2°; Nohira and Yoshida, 1989).

A similar approach was used to separate 1(*R*),*trans*- and 1(*S*),*trans*-PA. Whereas the 1*R*,*trans* enantiomer was not separated, a small quantity of 1(*S*),*trans*-PA was obtained (<10% yield) with an optical purity of 97.0% and a specific rotation of $[\alpha]_D^{20}$ = −34.6° (*c* 1.0, $CHCl_3$; lit. $[\alpha]_D$ = −35.6°; Nohira and Yoshida, 1989). This material was esterified and used as a standard for identification of *trans* isomers (see below).

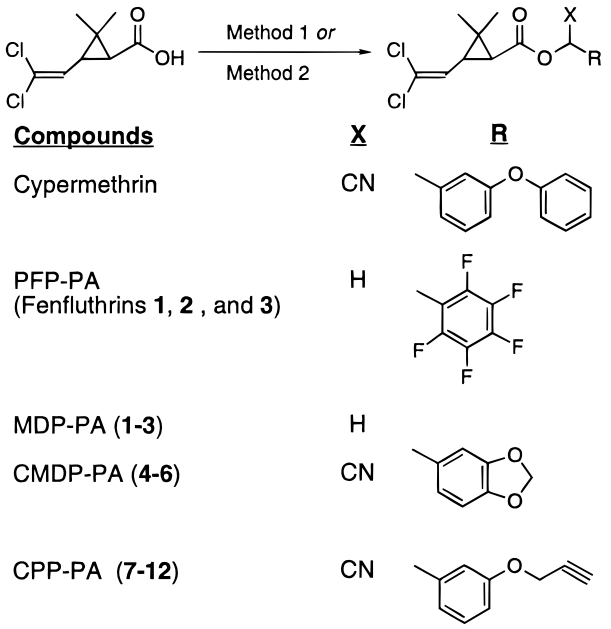
Synthesis of 3-Propargyloxybenzaldehyde. Potassium *tert*-butoxide (6.16 g, 0.055 mol), 3-hydroxybenzaldehyde (6.1 g, 0.05 mol), and dry dimethylformamide (DMF, over Na_2SO_4; 30 mL) were stirred briefly at 25 °C, and then propargyl bromide (6.6 g, 0.055 mol) was added in 30 mL of dry DMF (Albericio et al., 1990). The reaction mixture was heated at 110 °C for 8 h, and the solvent was removed under high vacuum. Ethyl acetate was added, inorganic salts were removed by filtration, and the organic extract was washed sequentially with water, 2 M NaOH, and saturated aqueous NaCl. The organic phase was dried ($MgSO_4$) overnight, and solvent was removed to give a yellow liquid that was purified by silica gel chromatography using hexane/ethyl acetate (8.5:1.5) as eluting solvent. Yield: 6.5 g (81%). ^{1}H NMR ($CDCl_3$): δ 2.54 (t, 1H, CH), 4.73 (t, 2H, $-OCH_2-$), 7.2−7.48 (m, 4H, aromatics), 9.96 (s, 1H, CHO). GC/MS (*m*/*z*): $M^{\bullet+}$ = 160.

General Procedure of Esterification. Two general routes of synthesis were used to esterify PA and various alcohols (Scheme 1).

Method 1. Mixed isomers of 3,4-methylenedioxyphenylmethyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (MDP-PA) and pentafluorophenylmethyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (PFP-PA) were made according to the method of Karanewsky and Badia (1986). PA (0.01 mol) and alcohols (0.011 mol) were dissolved in dry chloroform (20 mL) at room temperature, and then dicyclohexylcarbodiimide (DCC; 0.011 mol) and *N*,*N*-dimethylaminopyridine (DMAP; 0.001 mol) were added. After stirring at room temperature for 3 h, the mixture was diluted with chloroform (20 mL), filtered, and washed sequentially with 5% HCl, water, 10% sodium bicarbonate, and water. The organic phase was dried over Na_2SO_4, and solvent was removed to yield a crude product that was purified by silica gel chromatography using ethyl acetate/hexane (30:70, v/v) as eluting solvent. Yields: *trans*-MDP-PA, 74%; *cis*-MDP-PA, 70%; *trans*-PFP-PA, 75%; *cis*-PFP-PA, 68%.

Method 2. Isomers of PA were esterified with piperonal or 3-propargyloxybenzaldehyde using the method of Hu et al. (1985) to yield α-cyano-3,4-methylenedioxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (CMDP-PA) and α-cyano-3-propargyloxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (CPP-PA). PA (0.05 mol) was dissolved in $CHCl_3$ with 1 drop of DMF at 18 °C, and thionyl chloride ($SOCl_2$; 0.3 mol) was added dropwise over 5 min. The mixture was stirred at 40 °C for 2−3 h and cooled to room

Scheme 1. Generalized Methods for Synthesis of Pyrethroids



temperature, and then the solvent and excess $SOCl_2$ were removed at high vacuum to give a hazy oil of the acid chloride (yield > 95%). To this acid chloride were added benzaldehyde (0.05 mol), 18-crown-6 (2 mmol), and toluene (60 mL). This mixture was heated to 25−30 °C, and then NaCN (0.065 mol, in 10 mL of water) was added dropwise over 5 min. The mixture was stirred at 35−40 °C for 4−5 h, cooled, and diluted with water. The organic phase was washed sequentially with 10% HCl, water, 10% Na_2CO_3, water, and saturated NaCl. The organic phases were dried over Na_2SO_4 for 2 days, and solvent was removed at high vacuum to obtain a crude product, which was purified by silica gel chromatography using hexane/ethyl acetate (7:3) as eluting solvent. Yields: *trans*-CPP-PA, 75%; *cis*-CPP-PA, 72%; *trans*-CMDP-PA, 81%; *cis*-CMDP-PA, 73%.

All *trans* isomers and α(*RS*),1(*R*),*cis* enantiomers were separated following esterification by chiral HPLC using a Chiralcel OD HPLC column [cellulose tris(3,5-dimethylphenylcarbamate) on a 10 μm silica gel substrate, 250 × 20 mm; J. T. Baker, Inc., Phillipsburg, PA]. The mobile phase ranged from 90:10 to 70:30 (hexane/2-propanol), and the amount of compound per injection was between 10 and 100 mg (Table 1). Absolute configuration of the α-carbon was assigned according to the guidelines of Elliott et al. (1978a) and was based on retention times following separation by chiral HPLC and analysis of NMR spectra. The physical properties of isomers appear in Table 2.

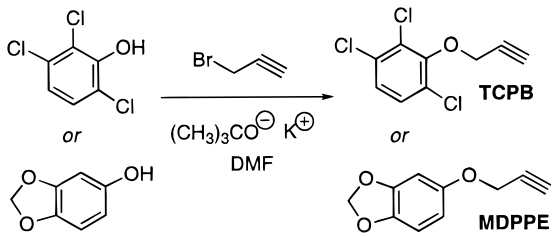
Synthesis of Propynyl Ethers. 1,2,4-Trichlorophenyl-3-(2-propynyloxy)benzene (TCPB) and 1-(2-propynyloxy)methylenedioxyphenyl ether (MDPPE) were synthesized according to the method of Albericio et al. (1990) (Scheme 2). A mixture of 2,3,6-trichlorophenol or methylenedioxyphenol (0.05 mol), potassium *tert*-butoxide (6.16 g, 0.055 mol), and propargyl bromide (6.6 g, 0.055 mol) in dry DMF (60 mL) was heated and stirred at 110 °C for 5 h, and then the solvent was removed under high vacuum. Ethyl acetate was added, the inorganic salts were removed by filtration, and the organic extract was washed sequentially with water, 2 M NaOH, and saturated aqueous NaCl. The organic phase was dried (Na_2SO_4) overnight and evaporated to dryness to give a crude product. MDPPE, a liquid product, was purified by silica gel chromatography using hexane/ethyl acetate (7:3) as eluting solvent. Yields: 6.5 g (74%). ^{1}H NMR ($CDCl_3$): δ 2.54 (t, 1H, CH), 4.61 (d, 2H, $-OCH_2-$), 5.92 (s, 2H, $-OCH_2O-$), 6.38−6.73 (m, 3H, aromatics). GC/MS (*m*/*z*): $M^{\bullet+}$ = 176. TCPB, a solid product, was recrystallized from ethyl ether/hexane. Yield: 7.6 g (65%). mp 58−59 °C (reference mp, 57−59 °C; Fellig et al., 1970). ^{1}H NMR ($CDCl_3$): δ 2.54 (t, 1H, CH), 4.80 (d, 2H, $-OCH_2-$), 7.24 (br, 2H, aromatics). GC/MS (*m*/*z*): $M^{\bullet+}$ = 236.

Table 1. Conditions for HPLC Separation of Enantiomers

compound	X	R[a]	stereochemistry in alcohol[b]	stereochemistry in acid	mobile phase[c]	flow rate[d]	t_R (min)
fenfluthrin **1**	H	PFP		*trans*,1*S*	80:20	3.0	12
fenfluthrin **2**	H	PFP		*trans*,1*R*	80:20	3.0	14
fenfluthrin **3**	H	PFP		*cis*,1*R*			
1	H	MDP		*trans*,1*S*	80:20	5.0	11.5
2	H	MDP		*trans*,1*R*	80:20	5.0	14.8
3	H	MDP		*cis*,1*R*			
4	CN	MDP	α*R*/*S*	*trans*,1*R*/*S*			
5	CN	MDP	α*S*	*cis*,1*R*	90:10	4.25	17.2
6	CN	MDP	α*R*	*cis*,1*R*	90:10	4.25	14.0
7	CN	PP	α*S*	*trans*,1*R*	70:30	5.0	13.2
8	CN	PP	α*R*	*trans*,1*R*	85:15	3.5	20.0[e]
9	CN	PP	α*R*	*trans*,1*S*	85:15	3.5	18.6[e]
10	CN	PP	α*S*	*trans*,1*S*	70:30	5.0	36.8
11	CN	PP	α*R*	*cis*,1*R*	70:30	5.0	14.4
12	CN	PP	α*S*	*cis*,1*R*	70:30	5.0	34.2

[a] PFP, pentafluorophenyl; MDP, 3,4-methylenedioxyphenyl; PP, 3-propargyloxyphenyl. [b] Assignment of absolute configuration at the α-carbon of compounds **5**−**12** was based on Elliott et al. (1978a). [c] Hexane/2-propanol. [d] mL/min. [e] A retention time of 7.2 min was measured for compounds **8** and **9** with 70:30 (hexane/2-propanol).

Scheme 2. Generalized Method for Synthesis of Propynyl Ethers



Biological Assays. Fifth-stadium larvae (day 1) weighing 180 ± 20 mg were treated on the midthoracic dorsum with 1 μL of compound (in acetone) or acetone alone (control). Dose−mortality relationships for each compound were measured using triplicate assays with five doses and 10 insects per dose. Median lethal dose (LD_{50}) and 95% fiducial limits (FL) were computed by probit analysis using SAS (1985). Resistance ratios (RRs) were calculated as LD_{50} of Pyr-R larvae/LD_{50} of LSU larvae. For synergist bioassays, nontoxic isomers of pyrethroids, as well as PBO, TCPB, MDPPE, and DEF were applied to the dorsal surface of the midabdomen 30 min prior to application of cypermethrin, which was applied to the midthoracic dorsum. Control larvae were treated with the appropriate concentration of synergist only or acetone alone. Synergism ratios (SRs) were calculated as LD_{50} of toxin/LD_{50} of toxin plus synergist. After treatment, larvae were maintained at 27 °C, and mortality was recorded after 72 h. The criterion for mortality was absence of coordinated movement within 30 s after being prodded with a pencil.

RESULTS

Biological Activity of Pyrethroids. Susceptibility to pyrethroids was measured in bioassays with both pyrethroid-susceptible (LSU) and -resistant (Pyr-R) larvae (Table 3). For LSU larvae, fenfluthrin isomers and pyrethroid analogs were less toxic than cypermethrin, with LD_{50} values ranging from 1.06 (for fenfluthrin **3**) to 57.5 μg/larva (for compound **7**) as compared with 0.05 μg/larva for cypermethrin. For 8 of the 15 compounds, no toxicity was measured following treatment with 100 μg/larva.

The biological activity of compounds was dependent upon chemical conformation. Stereochemistry about C-1 was a major determinant of toxicity: 1*R* enantiomers were toxic, whereas 1*S* enantiomers were not

Table 2. Physical Data for Pyrethroid Analogs

				^{1}H NMR peaks in $CDCl_3$								
				alcohol			acid					
										C_2-CH_3's	
	$[\alpha]_D$[a]	GC t_R (min)	MS (m/z) ($M^{\bullet+}$)	aromatics (m)	H_α (s)	others	H_1 (d)	H_3 (q)	vinyl (d)	1 (s)	2 (s)
fenfluthrin **1**	14.6	12.8	389		5.19		1.60	2.20	5.60	1.18	1.27
fenfluthrin **2**	−13.9	12.8	389		5.19		1.60	2.20	5.60	1.18	1.27
fenfluthrin **3**	−3.0	12.7	389		5.19		1.80	2.10	6.20	1.24	
1	22.2	15.3	343	6.7−6.9	5.0	6.0 (s, 2H, OCH_2O)	1.62	2.25	5.60	1.19	1.28
2	−21.9	15.3	343	6.7−6.9	5.0	6.0 (s, 2H, OCH_2O)	1.62	2.25	5.60	1.19	1.28
3	−0.7	15.4	343	6.7−6.9	5.0	6.0 (s, 2H, OCH_2O)	1.84	2.05	6.3	1.26	
4		16.3	368	6.8−7.0	6.3 (d)	6.0 (s, 2H, OCH_2O)	1.60	2.30	5.60	1.18−1.27[b]	
5	35.4	16.2	368	6.8−7.0	6.17	6.0 (s, 2H, OCH_2O)	1.85	2.10	6.29	1.19	1.28
6	−46.1	16.2	368	6.8−7.0	6.15	6.0 (s, 2H, OCH_2O)	1.85	2.10	6.25	1.19	
7	−13.0	16.2	368	7.1−7.4	6.40	2.5 (d, 1H, CH), 4.7 (s, 2H,CH_2)	1.70	2.30	5.60	1.19	1.26
8	−21.6	16.2	378	7.1−7.4	6.40	2.5 (d, 1H, CH), 4.7 (s, 2H, CH_2)	1.70	2.30	5.60	1.22	1.33
9	12.0	16.2	378	7.1−7.4	6.40	2.5 (d, 1H, CH), 4.7 (s, 2H, CH_2)	1.70	2.30	5.60	1.19	1.26
10	25.8	16.2	378	7.1−7.4	6.40	2.5 (d, 1H, CH), 4.7 (s, 2H, CH_2)	1.70	2.30	5.60	1.22	1.33
11	−19.5	16.0	378	7.0−7.4	6.30	2.5 (d, 1H, CH), 4.7 (s, 2H, CH_2)	1.90	2.10	6.10	1.30	
12	25.4	16.0	378	7.0−7.4	6.30	2.5 (d, 1H, CH), 4.7 (s, 2H, CH_2)	1.90	2.10	6.10	1.21	

[a] c = 1.0, $CHCl_3$, 20 °C. [b] Containing four singlets: 1.18, 1.22, 1.26, and 1.33.

Table 3. Toxicity of Pyrethroids in Topical Bioassays with Pyrethroid-Susceptible (LSU) and -Resistant (Pyr-R) Strains of *H. virescens*[a]

	LSU			Pyr-R			
compound	LD_{50}[b]	FL[c]	slope	LD_{50}	FL	slope	RR[d]
cypermethrin	0.05	0.04−0.06	2.52	2.91	2.36−4.06	2.20	58.2
fenfluthrin **2**	1.46	1.19−1.86	2.34	4.16	3.57−4.92	3.40	2.85
fenfluthrin **3**	1.06	0.92−1.24	3.55	2.92	2.62−3.31	4.67	2.75
2	9.44	7.57−12.5	2.36	>130[e]			>13.8
3	4.85	3.85−6.39	2.17	117	91.4−171	2.65	24.1
5	>150[e]			NT			
7	57.5	39.3−112	1.44	NT			
12	1.14	0.91−1.42	2.32	51.3	38.3−84.7	1.69	45.0

[a] Fenfluthrin **1** and compounds **1**, **4**, **6**, and **8**−**11** were nontoxic (NT) at a dose of 100 μg/larva in bioassays with both LSU and Pyr-R insects. [b] LD_{50} values are expressed as μg/larva and were computed by probit analysis. [c] FL, 95% fiducial limits. [d] RR, LD_{50} for Pyr-R strain/LD_{50} for LSU strain. [e] For compounds **2** and **5**, levels of mortality were 13.3 and 58% at the highest doses tested (130 and 150 μg/larva, respectively).

(Table 3). Similarly, for α-cyano-containing compounds (**4**, **5**, **7**−**12**), α*S*,1*R* enantiomers were toxic, whereas α*R*,1*R* and α*R*,1*S* compounds were not. In addition, susceptibility was always greater for 1*R*,*cis* than for 1*R*,*trans* isomers of toxic compounds. For the noncyano, methylenedioxyphenyl (MDP)-containing compounds, biological activity of **3** (the *cis* isomer) was almost twice as high as that of **2** (the *trans* isomer). Likewise, for toxic propargyloxyphenyl (PP) compounds, the *cis* isomer (**12**) was over 50 times more toxic than the corresponding *trans* isomer (**7**). In contrast, for toxic fenfluthrin isomers, there were no significant differences in LD_{50} values between the *trans* and *cis* isomers (1.46 and 1.06 μg/larva for fenfluthrin **2** and **3**, respectively).

Cypermethrin also was the most toxic pyrethroid in tests with Pyr-R insects, but resistance was greater to cypermethrin than to the other toxic pyrethroids tested (Table 3). Whereas the LD_{50} for cypermethrin in this strain (2.91 μg/larva) was 58-fold higher than that measured for LSU-S larvae, RRs were low for fenfluthrin isomers [2.85 and 2.75 for 1(*R*),*trans*- and 1(*R*),*cis*-fenfluthrin, respectively], intermediate for the 1(*R*),*cis*-MDP compound (**3**; RR = 24), and high for 1(*R*),*trans*-MDP (**2**; RR = 41) and α(*S*),1(*R*)-PP (**12**; RR = 45) compounds. As in tests with LSU larvae, susceptibility of Pyr-R insects was greater to *cis* than *trans* isomers of toxic compounds.

Synergism of Pyrethroid Toxicity. No significant synergism was measured with nontoxic isomers of pyrethroids or conventional synergists in bioassays with LSU-S larvae. In contrast, all compounds tested increased the susceptibility of Pyr-R larvae to cypermethrin (Table 4). The propynyl ether, TCPB, was the most effective synergist with an SR of 4.69. Of the other PP-containing compounds tested, only **11** [α(*R*),1(*R*),*cis*-PP] significantly enhanced toxicity of cypermethrin (SR = 2.55). Coapplication of cypermethrin and **6**, the α-(*R*),1(*R*),*cis*-MDP compound, increased cypermethrin toxicity by 2.69-fold, which was greater than synergism with PBO (SR = 1.97). Relative to PBO and TCPB, synergism with MDPPE, which contains both MDP and PP side chains, was intermediate (SR = 2.65). Finally, significant levels of synergism (4.04-fold) were measured with the esterase inhibitor, DEF.

Synergism of cypermethrin toxicity by nontoxic pyrethroids in Pyr-R larvae varied depending on the isomer (Table 4). Among the four PP-containing compounds evaluated, only **11** [α(*R*),1(*R*),*cis*-PP] synergized cypermethrin toxicity (SR = 2.55), whereas the corresponding *trans* isomer (**8**) did not. In addition, the structurally related compounds, **9** [α(*R*),1(*S*),*trans*-PP] and **10** [α-(*S*),1(*S*),*trans*-PP], were inactive as synergists. For MDP-containing compounds, synergism of cypermethrin toxicity was greater with **6** (α*R*,1*R*,*cis*; SR = 2.69) than PBO (SR = 1.97), and no synergism was measured with compound **1** (1*S*,*trans*, no αCN) in Pyr-R *H. virescens*. Finally, in tests with isomers of fenfluthrin and conventional synergists in Pyr-R insects, toxicity of the 1*R*,*cis* isomer (fenfluthrin **3**) was not increased by coapplication of either PBO or DEF (SR = 0.86 or 0.91, respectively) but was increased slightly by TCPB (SR

Table 4. Synergism of Pyrethroid Toxicity in Pyrethroid-Susceptible (LSU) and -Resistant (Pyr-R) *H. virescens*[a]

	LSU				Pyr-R			
	LD_{50}[b]	FL	slope	SR[c]	LD_{50}	FL	slope	SR
cypermethrin +								
PBO	0.04	0.03−0.05	2.18	1.25	1.48*	1.08−2.34	2.14	1.97
TCPB	0.04	0.03−0.07	1.60	1.25	0.62*	0.48−0.78	2.46	4.69
MDPPE	0.06	0.04−0.09	1.70	0.83	1.10*	0.83−1.67	2.31	2.65
DEF	0.05	0.03−0.08	1.31	1.00	0.72*	0.57−0.92	2.62	4.04
fenfluthrin **1**	0.05	0.04−0.07	1.59	1.00	2.07	1.48−5.61	2.49	1.41
1	0.05	0.04−0.06	2.71	1.00	1.74	1.37−2.82	3.32	1.67
6	0.05	0.04−0.06	2.37	1.00	1.08*	0.79−1.71	2.12	2.69
8	0.06	0.05−0.08	2.39	0.83	1.84	1.35−4.11	2.49	1.58
9	0.04	0.03−0.05	2.76	1.25	1.80	1.22−8.54	1.81	1.62
10	0.04	0.03−0.06	2.81	1.25	2.55	1.66−5.61	2.22	1.14
11	0.04	0.03−0.04	3.28	1.25	1.14*	0.86−1.74	2.33	2.55
fenfluthrin **2** +								
TCPB	1.21	1.01−1.44	3.62	1.21	2.27	1.78−2.73	3.57	1.83
DEF	1.17	0.97−1.45	3.43	1.24	3.12	2.51−4.11	3.31	1.33
fenfluthrin **3** +								
PBO	1.39	1.15−1.75	3.59	0.76	3.41	2.95−4.20	4.62	0.86
TCPB	1.23	1.02−1.49	3.93	0.86	1.98	1.62−2.64	4.37	1.47
DEF	0.94	0.78−1.12	4.27	1.13	3.21	2.74−3.93	4.40	0.91

[a] Compounds were applied to the third abdominal dorsum 30 min prior to application of fenfluthrin or cypermethrin. Doses of compounds were 50 μg/larva except for compound **11** (25 μg/larva). [b] μg of cypermethrin or *cis*,1(*R*)-fenfluthrin per larva. Asterisks signify values that are significantly different from those measured in tests with toxin only. [c] SR, synergism ratio (LD_{50} of insecticide/LD_{50} of insecticide with synergist).

= 1.47). However, in tests with the 1*R*,*trans* isomer (fenfluthrin **2**), slightly higher synergism was measured with TCPB and DEF (SR = 1.83 and 1.33, respectively).

DISCUSSION

Enhanced metabolism is a major mechanism of resistance to pyrethroids. Oxidative metabolism in a number of insects has been shown to occur at the 2′-, 4′-, and 6-positions of the phenoxybenzyl moiety or the geminal dimethyl groups of the cyclopropane ring (Shono et al., 1978, 1979; Casida and Ruzo, 1980; Leahey, 1985). In addition, oxidative diphenyl ether cleavage also was observed in a study with fenvalerate and the Colorado potato beetle, *Leptinotarsa decemlineata* (Soderlund et al., 1987). In studies with *H. virescens*, Lee et al. (1989) and Little et al. (1989) suggested that the 2′- and 4′-positions on the phenoxybenzyl moiety of cypermethrin are the main sites of metabolism, whereas oxidation at geminal dimethyl groups is less important. The objective of this study was to evaluate effects of structural modifications at some metabolically sensitive sites of the pyrethroid molecule on toxicity and resistance.

The insecticidal activity and synergism of cypermethrin toxicity measured in bioassays with fenfluthrin and structurally modified pyrethroids confirm that P450 monooxygenases are associated with pyrethroid resistance in the Pyr-R strain. An isomer of fenfluthrin, in which potential sites for oxidative metabolism have been blocked, was as toxic as cypermethrin to pyrethroid-resistant *H. virescens*. These results are consistent with those reported in studies with mixed isomers of fenfluthrin by Scott and Georghiou (1986) in the house fly, *Musca domestica*, by Scott et al. (1986) with the southern house mosquito, *Culex quinquefasciatus*, and by Forrester et al. (1993) with the cotton bollworm, *Helicoverpa armigera*. In addition, resistance ratios were higher for cypermethrin than with compounds in which the metabolically labile phenoxyphenyl group was replaced with MDP or PP, although, for reasons not known, compounds with these groups were not as effective as fenfluthrin.

Involvement of P450 monooxygenases in resistance was further supported by studies with synergists. Cypermethrin toxicity was increased by coapplication of compounds containing PP or MDP functionalities, and synergism of toxicity was greatest with the propynyl ether, TCPB. These results agree with those from previous studies demonstrating the activity of this compound against pyrethroid-resistant *H. armigera* (Forrester et al., 1993) and *H. virescens* (Brown et al., 1996). In addition, synergism was lower in tests with PBO than with TCPB, which supports the conclusion of Brown et al. (1996) that different classes of P450 monooxygenase are involved in resistance-associated metabolism of pyrethroids. In contrast, Forrester et al. (1993) found that both PBO and TCPB fully suppressed resistance to fenvalerate in *H. armigera*. Finally, toxicity of cypermethrin was increased significantly following coapplication of DEF, which suggests additional involvement of esterases in pyrethroid resistance as was reported by Graves et al. (1991) for adult *H. virescens* and by Gunning et al. (1996) for larval *H. armigera*. However, further studies with pyrethroid substrates will be required to confirm that esterases contribute to pyrethroid resistance in the tobacco budworm.

Insecticidal and synergistic activity of pyrethroids was dependent on chemical configuration about C-1 and the α-carbon. No significant toxicity or synergism was measured with 1*S* isomers, and 1*R*,*cis* isomers were always more toxic than the corresponding *trans* compounds. These findings are similar to those of Ackermann et al. (1980), who studied the effects of stereochemical conformation on the toxicity of a brominated analog of cypermethrin to pyrethroid-susceptible *H. virescens*. In addition, the toxicity of 1(*R*)-fenfluthrin was slightly higher with the *cis* isomer (fenfluthrin **3**) than with the *trans* isomer (fenfluthrin **2**) in both pyrethroid-susceptible and -resistant insects. Finally, only 1*R*,*cis* (but not *trans*) chrysanthemates containing MDP (**6**) or PP (**11**) groups significantly synergized cypermethrin toxicity. At the α-carbon, only *S* enantiomers (**5**, **7**, **12**) were toxic, which is consistent with previous studies by Elliott et al. (1978b), whereas the

corresponding α*R*,*cis* enantiomers (**6** and **11**) were synergists. Assuming that synergism results from P450 monooxygenase inhibition (Feyereisen et al., 1991), these results suggest that interaction between these enzymes and synergists is stereospecific. The stereospecific nature of catalysis by mammalian P450 monooxygenases has been shown previously [see, for example, Atkins and Sligar (1989) or Fruetel et al. (1992)]. Finally, whereas cypermethrin toxicity was enhanced by DEF, no significant synergism was observed when DEF was coapplied with either of the toxic fenfluthrin isomers (fenfluthrin **2** or fenfluthrin **3**). This suggests either that fenfluthrin is a poor substrate for resistance-associated esterases or that oxidation of the phenoxybenzyl alcohol precedes hydrolysis.

Previous comparisons of biological activity between pyrethroid isomers have provided few generalizations [reviewed in Elliott (1977), Tessier (1985), and Vijverberg and Oortgiesen (1988)]. Relationships between stereochemical configuration and toxicity appear to be dependent upon the insect and pyrethroid studied. For example, in tests with pyrethroid-susceptible house flies, Elliott et al. (1975) found that 5-benzyl-3-furylmethyl esters of 1*R*,*trans* isomers of dichlorovinyl chrysanthemates were slightly more toxic than the corresponding *cis* isomers, but the opposite was true for pyrethroids containing a 3-phenoxybenzyl ester. In our studies, the higher toxicity of 1*R*,*cis* diastereomers and slightly higher synergism of the toxicity of *trans* than *cis* isomers by TCPB and DEF may reflect preferential metabolism of *trans* isomers of pyrethroids, as was reported previously (Soderlund and Casida, 1977; Soderlund et al., 1983).

Fenfluthrin toxicity was not synergized significantly by PBO, TCPB, or DEF, confirming that the phenoxybenzyl group is a primary site of attack in metabolically resistant *H. virescens* (Little et al., 1989; Lee et al., 1989). In previous studies, PBO completely suppressed resistance to cypermethrin in *H. armigera* (Forrester et al., 1993) and to permethrin in house flies (Scott and Georghiou, 1986) and the southern house mosquito, *C. quinquefasciatus* (Scott et al., 1986) but was relatively ineffective as a synergist of fenfluthrin toxicity.

Whereas the relatively low toxicities of compounds tested in this study would probably preclude their use as pest control agents, these results suggest that fenfluthrin, which lacks the metabolically sensitive sites of phenoxybenzyl-containing pyrethroids, may be a useful diagnostic compound to monitor P450 monooxygenase-associated resistance in field populations of *H. virescens*. In addition, these findings provide a further illustration that modification of insecticide structure can be used to circumvent metabolic resistance to pyrethroid insecticides (Forrester et al., 1993).

ABBREVIATIONS USED

CMDP, α-cyanomethylenedioxyphenyl methyl; CPP, α-cyanopropargyloxyphenyl methyl; DCC, dicyclohexylcarbodiimide; DEF, *S*,*S*,*S*-tributyl phosphorotrithioate; DMAP, *N*,*N*-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; FL, 95% fiducial limits; IRM, insecticide resistance management; MDP, methylenedioxyphenyl; PA, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid or permethric acid; PBO, piperonyl butoxide; MDPPE, methylenedioxyphenyl propynyl ether; PFP, pentafluorophenyl; PP, propargyloxyphenyl; TCPB, 1,2,4-trichlorophenyl-3-(2-propynyloxy)benzene; RR, resistance ratio; SR, synergism ratio.

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