Metabolism of *trans*- and *cis*-Permethrin, *trans*- and *cis*-Cypermethrin, and Decamethrin by Microsomal Enzymes

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Microsomal systems are used to compare the metabolism of *trans*- and *cis*-permethrin by mouse liver, rat liver, and housefly and cabbage looper preparations and of *trans*- and *cis*-cypermethrin and decamethrin by mouse liver. Esteratic cleavage is more extensive for trans pyrethroids than for cis pyrethroids, while the relative extent of oxidative metabolism of the two isomers depends on the enzyme source. Species and isomer differences are noted in the preferred sites of permethrin hydroxylation, i.e., trans or cis methyl group and 2′, 4′, or 6 position of the alcohol moiety. With the cyanopyrethroids, mouse microsomes hydroxylate the 5 position in addition to the 4′, trans methyl, and cis methyl sites. Several hydroxymethyl derivatives are further oxidized to the corresponding aldehydes and carboxylic acids. Four isomers of 2-carboxy-3-(2,2-dichlorovinyl)-2-methylcyclopropanecarboxylic acid are described. Thirteen to twenty-one metabolites of each pyrethroid are identified in the mouse microsomal systems.

Pyrethroid metabolites in microsomal esterase and oxidase systems are usually the same as those detected in vivo, except for conjugate formation (Casida et al., 1975/76; Miyamoto, 1976). These pyrethroid-hydrolyzing esterases are readily inhibited by tetraethyl pyrophosphate (TEPP) and the oxidases are only active when fortified with NADPH. This allows independent examination of products formed by esterase action (normal microsomes), oxidase action (TEPP-treated microsomes with NADPH), and esterase-plus-oxidase action (normal microsomes with NADPH) (Soderlund and Casida, 1977a,b,c; Ueda et al., 1975). Microsomal studies are therefore a useful adjunct to organismal investigations in understanding pyrethroid structure–biodegradability relationships and the sites most susceptible to metabolic attack.

Several chrysanthemates (e.g., allethrin, dimethrin, pyrethrin I, resmethrin, and tetramethrin) are hydroxylated at a methyl group in the isobutenyl substituent by liver and housefly oxidases (Elliott et al., 1972; Suzuki and Miyamoto, 1974; Ueda et al., 1975; Yamamoto and Casida, 1966; Yamamoto et al., 1969). The oxidatively susceptible isobutenyl moiety is replaced by a dihalovinyl group in several highly potent pyrethroids (Elliott, 1977; Itaya et al., 1977, Ruzo andd Casida, 1977), including *trans*- and *cis*-permethrin without a cyano group and *trans*- and *cis*-cypermethrin and decamethrin with a cyano group (Figure 1). It is therefore of interest to define the sites of microsomal metabolism in this series of structurally related dihalovinyl pyrethroids.

This report and a preliminary communication on this study (Shono and Casida, 1978) consider the metabolism of *trans*- and *cis*-permethrin in microsomal systems from mouse and rat liver, housefly (*Musca domestica* L.) thoraces plus abdomens, and cabbage looper (*Trichoplusia ni* Hübner) guts. It also uses the mouse liver microsomal system to compare the metabolism of *trans*- and *cis*-permethrin, *trans*- and *cis*-cypermethrin, and decamethrin.

MATERIALS AND METHODS

Spectroscopy. Infrared (IR) spectra reported in cm^{-1} were determined on a Perkin-Elmer Model 457 grating spectrophotometer using chloroform solutions. Nuclear magnetic resonance (NMR) spectra were recorded for chloroform-*d* solutions, using tetramethylsilane as the internal standard (δ = 0 ppm), with a Perkin-Elmer Model R32 spectrometer at 90 MHz. Chemical ionization–mass spectra (CI–MS) were obtained on the Finnigan Corp. Model 1015D mass spectrometer with the System Industries Model 150 control system, using a direct introduction probe and isobutane as the reactant gas at a source pressure of 0.6–1.0 torr. The quasimolecular ion, $[M + 1]^+$, is reported as *m/e* (relative intensity) based on the chlorine-35 isotope.

Thin-Layer Chromatography (TLC). Silica gel 60 F-254 chromatoplates (EM Laboratories Inc., Elmsford, NY) with 0.25- and 0.50-mm gel thickness for analysis and preparative isolations, respectively, were used with the following solvent systems: BC, benzene–carbon tetrachloride (1:1); BE, benzene–ethyl acetate (6:1); BE′, two developments with benzene–ethyl acetate (12:1); BEM, benzene–ethyl acetate–methanol (15:5:1); BFE, benzene (saturated with formic acid)–ether (10:3); BFE′, two developments with benzene (saturated with formic acid)–ether (10:3); CB, three developments with carbon tetrachloride–benzene (3:2); CE, carbon tetrachloride–ether (3:1); CE′, two developments with carbon tetrachloride–ether (10:1); CFE, chloroform (saturated with formic acid)–ether (10:3); EH, ether–hexane (1:1); HE, two developments with hexane–ether (4:1).

Chemicals. Figure 1 shows the labeling positions of the pyrethroids examined. Specific activities and radiochemical purities of the permethrin isomers are given by Shono et al. (1978) and of decamethrin by Ruzo et al. (1978). The labeled cypermethrin isomers were provided by D. H. Hutson (Shell Toxicology Laboratory, Sittingbourne, Kent, U.K.) with specific activities of 4.6 and 12–14 mCi/mmol for the ^{14}C-acid- and ^{14}C-alcohol-labeled preparations, respectively; their radiochemical purities were >99% after TLC cleanup (BC; R_f 0.61 and 0.68 for the trans and cis isomers, respectively).

Figures 2 and 3 give the structures and abbreviations for the compounds and metabolites considered. The suffixes Me and Et are used to designate methyl and ethyl esters, respectively. Most of the unlabeled standard compounds used for TLC cochromatography with ^{14}C metabolites are previously described (Ruzo et al., 1977, 1978; Shono et al., 1978; Unai and Casida, 1977). Additional chemicals utilized were 4′-HO-*t*- and -*c*-cyper and

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0021-8561/79/1427-0316$01.00/0

Table I. Analytical Data for Four Isomers of 2-Carboxy-3-(2,2-dichlorovinyl)-2-methylcyclopropanecarboxylic Acid and Their Dimethyl Esters

condition or parameter	isomer of COOR-Cl_2CA-R′ (R = H or CH_3; R′ = CH_3 or none)			
	t-Cl_2CA-R′		c-Cl_2CA-R′	
	t-COOR	c-COOR	t-COOR	c-COOR
	Mp for Dicarboxylic Acids[a]			
°C	196-197	163-164	181-183	139-140
	TLC R_f Values for Dicarboxylic Acids			
solvent system				
BFE′	0.37	0.09	0.36	0.16
CFE	0.33	0.13	0.32	0.17
	TLC R_f Values for Dimethyl Esters			
CE	0.48[b]	0.34[b]	0.47	0.39
EH	0.46[b]	0.33[b]	0.46	0.39
	GLC t_R Values, Min., for Dimethyl Esters			
column				
OV 25[c]	18.2	20.1	18.6	19.1
	NMR Chemical Shifts (δ) and Coupling Constants (Hz) for Dimethyl Esters			
substituent[d]				
1-H	2.66 (d, J = 6.8)	1.87 (d, J = 6.8)	2.54-2.73 (m)	2.12-2.34 (m)
3-H	2.45 (dd, J = 8.5 and 6.8)	2.93 (dd, J = 8.5 and 6.8)		
1′-H	5.83 (d, J = 8.5)	5.61 (d, J = 8.5)	6.24 (dd, J = 8.5 and 1.5)	6.36 (d, J = 8.5)
CH_3	1.49	1.41	1.45	1.47
1-$COOCH_3$[e]	3.75	3.69	3.73	3.69
2-$COOCH_3$[e]	3.73	3.71	3.71	3.72

[a] Melting points are for compounds recovered from TLC without recrystallization. [b] Cochromatograph with methylated metabolites of *trans*-permethrin in mouse microsomal oxidase systems. [c] Glass column (2 m × 3 mm i.d.) of 5% OV 25 on Chromosorb W (A/W 80-100 mesh) with column temperature programming (80-180 °C, 4 °C/min), N_2 as carrier gas (30 mL/min), and flame ionization detector. [d] Substituent positions are designated as shown in Figure 4. [e] Carbomethoxy protons of the 1-substituent resonate at lower field than those of the 2-substituent with t-COOMe-Cl_2CA-Me and vice versa with c-COOMe-Cl_2CA-Me on analogy with the corresponding t- or c-CH_2OH-Cl_2CA-Me isomers (Unai and Casida, 1977).

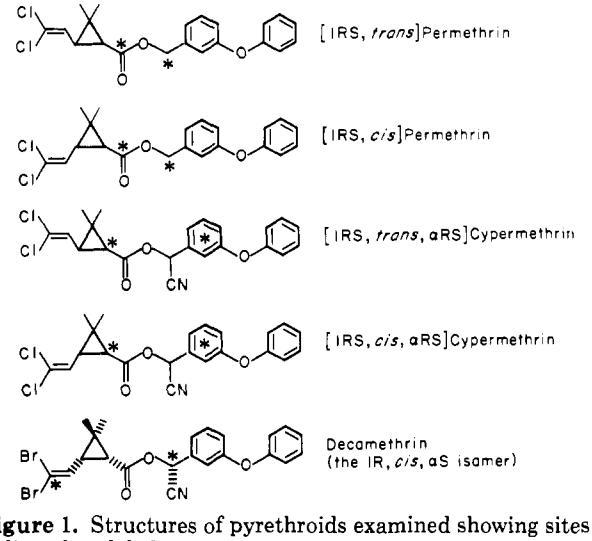

Figure 1. Structures of pyrethroids examined showing sites of radiocarbon labels.

t-CH_2OH-c-cyper provided by D. H. Hutson and the 3-HO-benzyl ester of t-Cl_2CA provided by T. Unai (formerly of this laboratory).

Synthesis of Four Isomers of 2-Carboxy-3-(2,2-dichlorovinyl)-2-methylcyclopropanecarboxylic Acid (COOH-Cl_2CA) and Their Dimethyl Esters (COOMe-Cl_2CA-Me). These dicarboxylic acids are possible metabolites of *cis*- and *trans*-permethrin and *cis*- and *trans*-cypermethrin, so procedures were developed for preparing or isolating them and their dimethyl esters. Appropriate analytical data and chromatographic properties are recorded in Table I. On CI–MS analysis, each of the four dimethyl esters gives $[M + 1]^+$ as the base peak and only the least stable cis,cis isomer (c-COOMe-c-Cl_2CA-Me) yields a prominent 235 ion corresponding to $M - OCH_3$.

The (1*RS*)-trans compounds (t- or c-COOH-t-Cl_2CA and t- or c-COOMe-t-Cl_2CA-Me) were obtained via the corresponding diethyl ester (COOEt-Cl_2CA-Et) (prepared by R. K. Huff, Plant Protection Division, Imperial Chemical Industries Limited, Jealott's Hill Research Station, Bracknell, Berkshire, U.K.) which consisted of t-COOEt-t-Cl_2CA-Et (60%), c-COOEt-t-Cl_2CA-Et (14%), and t-COOEt-c-Cl_2CA-Et (25%) based on NMR and <1% c-COOEt-c-Cl_2CA-Et based on gas–liquid chromatography (GLC) (conditions similar to those for the corresponding dimethyl esters in Table I). The isomeric mixture of the dicarbethoxy compounds (100 mg, 0.34 mmol) was hydrolyzed to the corresponding mixture of dicarboxylic acids on treatment with KOH (38 mg, 0.68 mmol) in water (4 mL) and methanol (8 mL) by stirring for 2 h at 50 °C. The diacids recovered by acidifying with HCl and ether extraction (70 mg, 78% yield) were esterified with diazomethane in ether–methanol mixture (COOMe-Cl_2CA-Me; 100% yield) and purified by repeated TLC (CB) to give isomerically pure t-COOMe-t-Cl_2CA-Me (R_f 0.38; 14% recovery) and c-COOMe-t-Cl_2CA-Me (R_f 0.12; 6% recovery) (Table I) each with IR 1740. Hydrolysis with KOH as above and TLC purification (BFE) gave t- and c-COOH-t-Cl_2CA in ~90% yields (Table I). A third isomer, t-COOMe-c-Cl_2CA-Me, was obtained in ~12% recovery and ~75% purity (the remainder being t-COOMe-t-Cl_2CA-Me) (R_f 0.37 in CB).

An alternative method was used to prepare isomerically pure t-COOH-c-Cl_2CA and t-COOMe-c-Cl_2CA-Me via oxidation of the corresponding hydroxymethyl acid (Unai and Casida, 1977). Thus, a mixture of t-CH_2OH-c-Cl_2CA

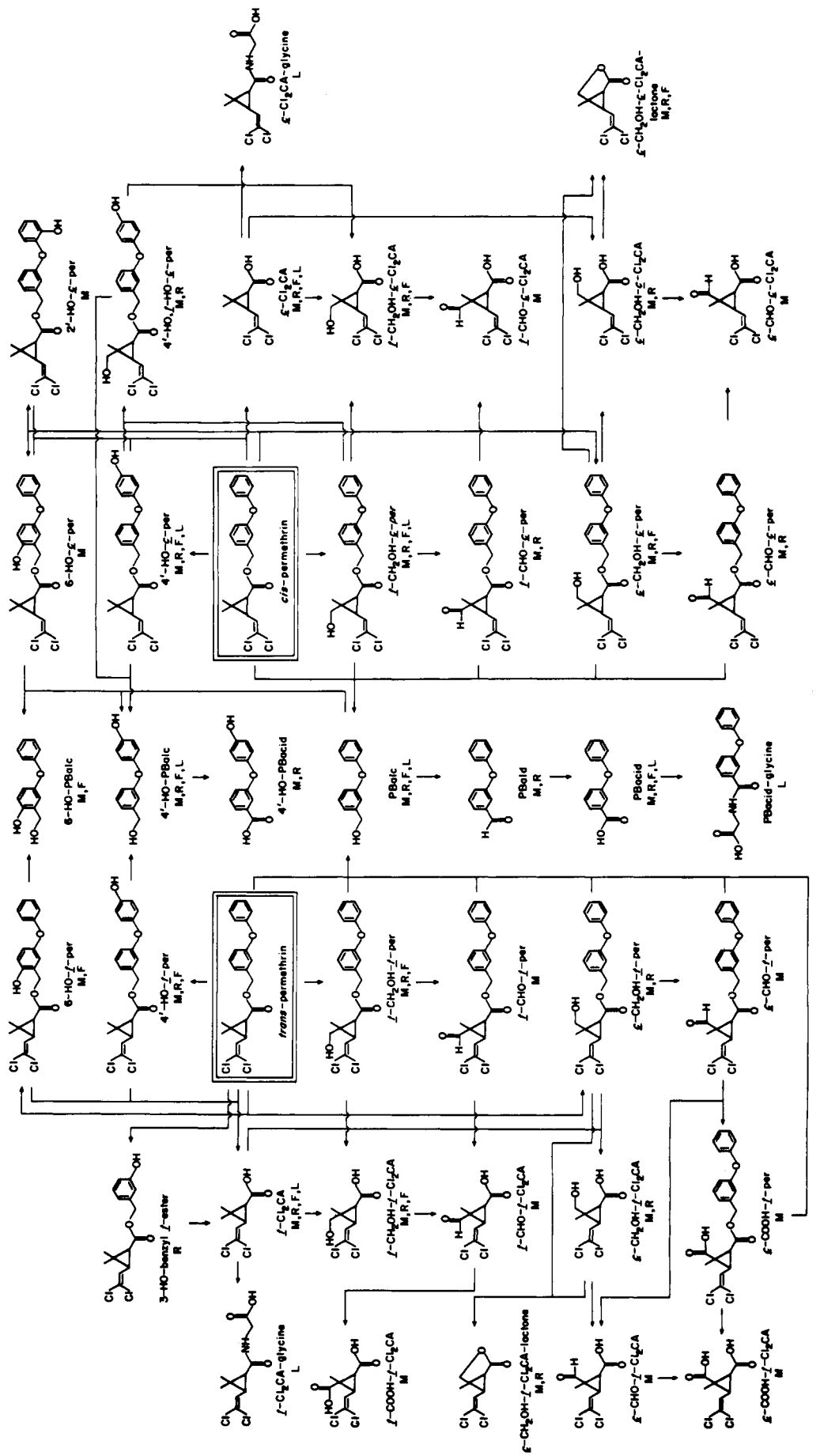

Figure 2. Metabolic pathways for permethrin isomers showing products detected in microsomal enzyme systems of mouse (M) and rat (R) liver, housefly abdomens-plus-thoraces (F), and cabbage looper guts (L).

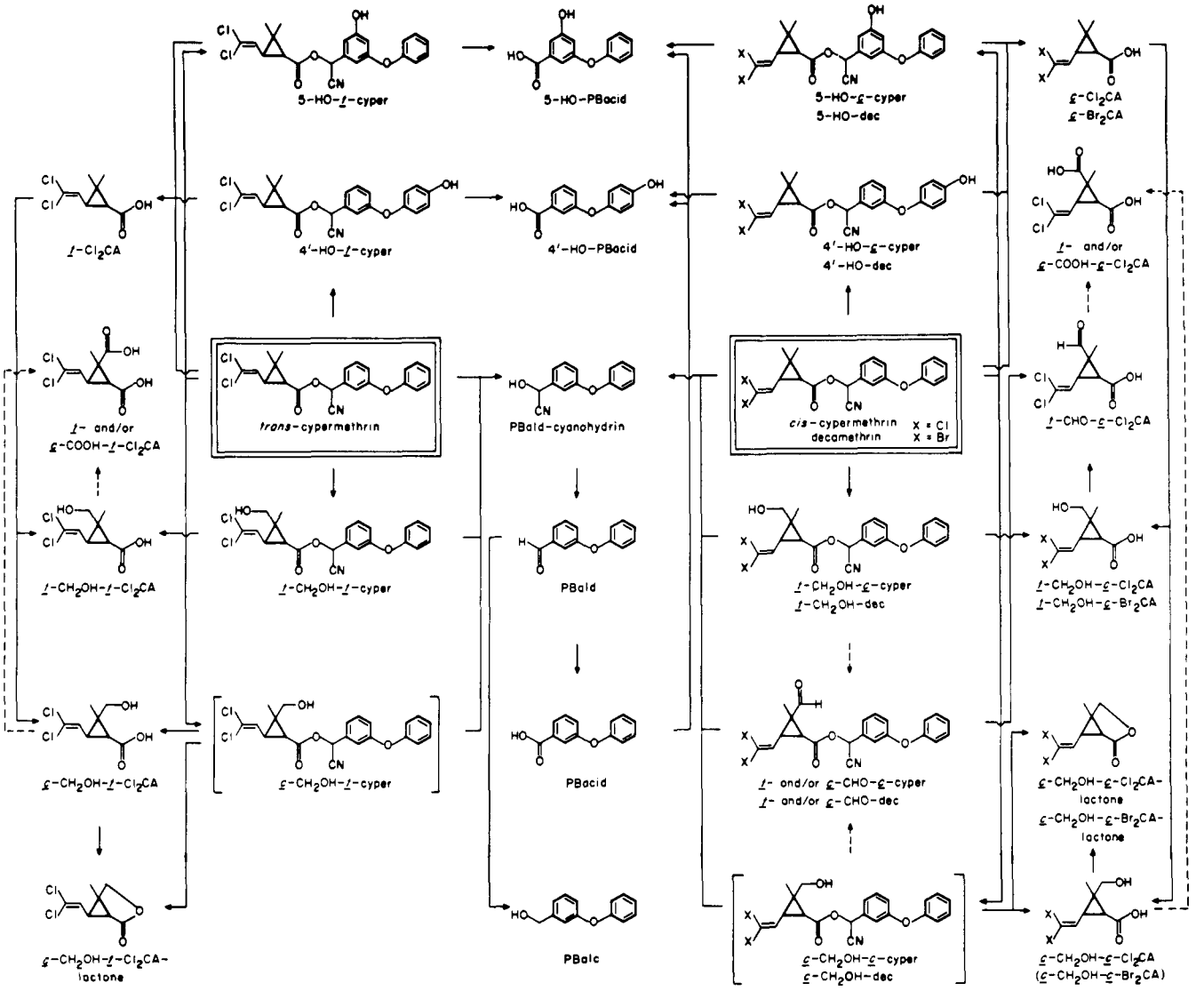

Figure 3. Metabolic pathways for cypermethrin isomers and decamethrin in microsomal enzyme systems of mouse liver. The 1*RS*,α*RS* isomers are shown but with decamethrin the configuration is 1*R*,α*S*. Compounds in brackets were not detected but are likely intermediates. Dotted lines are used when the reaction leads to a metabolite of undefined configuration.

(20 mg, 0.08 mmol) and $K_2Cr_2O_7$ (33 mg, 0.13 mmol) in 2 mL of 10% H_2SO_4 and 0.5 mL of glacial acetic acid was stirred at 25 °C for 48 h. TLC purification (BFE) gave *t*-COOH-*c*-Cl_2CA (25% yield) and *t*-CHO-*c*-Cl_2CA (5% yield) which were individually and quantitatively converted on treatment with diazomethane in ether–methanol mixture to *t*-COOMe-*c*-Cl_2CA-Me (Table I) (R_f 0.22) and *t*-CHO-*c*-Cl_2CA-Me [R_f 0.55; IR 1740 and 1700; CI–MS [M + 1]$^+$ *m/e* 237 (77%); NMR 1.55 (s, 3H), 2.50–2.80 (m, 2H), 3.70 (s, 3H), 6.28 (d, 1 H, J = 8.5 Hz) and 9.65 (s, 1 H)].

A several step reaction sequence (Figure 4) was used to obtain the cis,cis isomer (*c*-COOH-*c*-Cl_2CA and *c*-COOMe-*c*-Cl_2CA-Me) since the available sample of COOEt-Cl_2CA-Et contained no detectable cis,cis isomer (see above) and the corresponding isomer of the hydroxymethyl acid (*c*-CH_2OH-*c*-Cl_2CA or its lactone; Unai and Casida, 1977) was no longer available. Accordingly, *c*-CH_2OH-*t*-Cl_2CA-lactone (20 mg, 0.096 mmol) (Unai and Casida, 1977) was isomerized using isobutyrophenone (80 mg) as sensitizer by irradiation in benzene solution (2 mL) for 2 h at 25 °C with a 350-nm UV lamp. The *c*-CH_2OH-*c*-Cl_2CA-lactone (12 mg, 60% yield) recovered by TLC (EH) was hydrolyzed with KOH (4 mg, 0.07 mmol) in aqueous methanol (0.1 mL) at 50 °C for 2 h. The

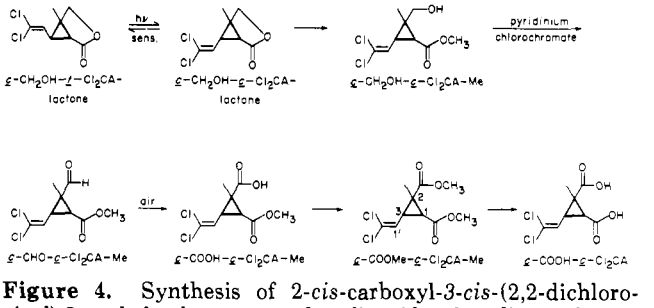

Figure 4. Synthesis of 2-*cis*-carboxyl-3-*cis*-(2,2-dichlorovinyl)-2-methylcyclopropanecarboxylic acid and its dimethyl ester.

hydroxymethyl acid, recovered by pouring the solution into saturated NaCl (1.5 mL), acidification with HCl to pH ~2, and immediate extraction with ether (5 mL × 3), was then methylated with diazomethane in ether–methanol mixture. *c*-CH_2OH-*c*-Cl_2CA-Me (5 mg, 0.021 mmol) was oxidized with pyridinium chlorochromate (9.1 mg, 0.04 mmol) (Corey and Suggs, 1975) in methylene chloride (0.2 mL) at 25 °C for 3 h. Purification by TLC (BFE) gave *c*-CHO-*c*-Cl_2CA-Me [61% yield; R_f 0.29; IR 1740 and 1700; CI–MS (M + 1)$^+$ *m/e* 237 (100%); NMR 1.35 (s, 3 H), 2.29–2.64 (m, 2 H), 3.74 (s, 3 H), 6.52 (d, 1 H, J = 8.5 Hz), and 9.72 (s, 1 H)]. On standing in air for 7 days, *c*-

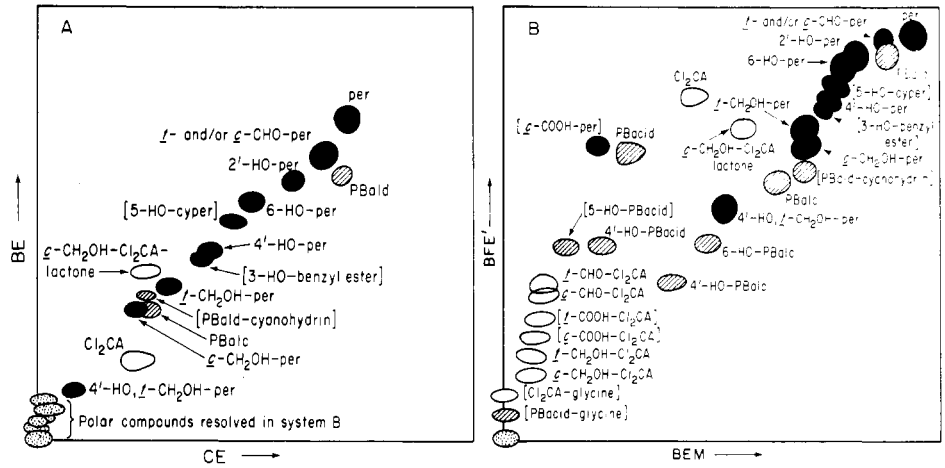

Figure 5. TLC patterns of metabolites of *cis*-permethrin and other pyrethroids separated in two-dimensional solvent systems optimal for esters and apolar compounds (A) and for cleavage products and polar compounds (B). The solvent fronts are the appropriate outlines of the figures. Compound designations refer to structures in Figures 2 and 3. Symbols for metabolite detection with different labeling positions are: (●) esters detected with both ^{14}C acid- and ^{14}C alcohol preparations; (○) metabolites from acid moiety detected with ^{14}C acid preparation only; (⌀) metabolites from alcohol moiety detected with ^{14}C alcohol preparation only. Compounds designated without brackets are metabolites of *cis*-permethrin in mouse microsome systems while those with brackets are metabolites of other pyrethroids or in other systems. Ester and acid moiety metabolites of *trans*-permethrin appear slightly below those of the corresponding *cis*-permethrin metabolites. Cypermethrin isomers, decamethrin, and their ester and acid moiety metabolites available as unlabeled standards appear in approximately the same positions as the corresponding permethrin derivatives.

CHO-*c*-Cl_2CA-Me (3 mg) was oxidized to *c*-COOH-*c*-Cl_2CA-Me (~60% yield) which was esterified with diazomethane to give *c*-COOMe-*c*-Cl_2CA-Me (purified by TLC with CB, R_f 0.25; IR 1740) (Table I). On hydrolysis with KOH in aqueous methanol as above and TLC purification (BFE) it gave *c*-COOH-*c*-Cl_2CA (Table I) in good yield.

Enzyme Preparations, Incubation Conditions, and Metabolite Analysis. Tissues or body regions were homogenized in pH 7.5 phosphate buffer (50 mM except for housefly preparations where 200 mM was used) as follows: liver of male albino mice and rats and housefly abdomens-plus-thoraces from the Rutgers-R strain (Shono et al., 1978) at a concentration of 20% (fresh weight/volume); the midgut portion with some hindgut obtained from fifth instar cabbage looper larvae (Shono et al., 1978) and washed free of contents prior to homogenization at six guts/mL. The microsomal fraction was obtained by centrifuging the homogenate at 10000*g* for 15 min, the recovered supernatant at 100000*g* for 60 min, and the pellet resuspended in buffer at 100000*g* for 60 min. With looper guts, the microsome-plus-soluble fraction (10000*g* supernatant) was used without further purification.

Each reaction mixture in a 25-mL Erlenmeyer flask contained 1.0 mL of enzyme, 1.2 mL of phosphate buffer as above, NADPH (0 or 2.2 μmol), and ^{14}C substrate (1 μg except with looper where 0.1 μg was used) added last in acetone (10 μL) with immediate mixing. To inhibit esterase activity, TEPP (200 nmol) in acetone (10 μL) was added to the enzyme preparation at 25 °C, and, after 10 min of incubation, NADPH and substrate were added. Four types of incubations in air with shaking were carried out with each enzyme source and substrate: (1) TEPP-treated microsomes (no esterase or oxidase activity, i.e., control); (2) normal microsomes (esterase activity); (3) TEPP-treated microsomes plus NADPH (oxidase activity); (4) normal microsomes plus NADPH (esterase plus oxidase activity). Liver microsome preparations were incubated at 37 °C for 30 min and insect preparations at 30 °C for 120 min. Each reaction mixture was extracted with ether (5 mL × 3) following addition of 0.2 mL of 1 N HCl and 1 g of $(NH_4)_2SO_4$. The ^{14}C content was determined for the organosoluble fraction and the remaining water-soluble fraction (after combustion) by liquid scintillation counting (LSC).

Each organosoluble fraction was dried (Na_2SO_4) and analyzed by two-dimensional TLC as shown in Figure 5. Additional solvent systems for special purposes were: EH and CE for dicarbomethoxy compounds from methylation (diazomethane) of dicarboxylic acids (see above) and for carbomethoxy compounds from methylation (diazomethane) of monocarboxylic acids (all relevant compounds are well separated); two-dimensional development with first BE′ and then CE′ for the 3-HO-benzyl ester of *t*-Cl_2CA (R_f 0.60 and 0.28, respectively); HE for separation of (*R*)-α- and (*S*)-α-decamethrin and their 4′-hydroxy derivatives (see Ruzo et al., 1978).

The ^{14}C content of each product, detected by radioautography and determined by scraping the appropriate gel region and direct LSC, was related on a percentage basis to that of the original substrate. The tabulated results for the substrates and ester metabolites are the averages of those with the ^{14}C acid and ^{14}C alcohol preparations. The remaining ^{14}C metabolites, which are ester cleavage products, were then apportioned between the individual compounds and fractions for the appropriate labeled preparation.

Metabolite Identification. Metabolites were identified by two-dimensional cochromatography with unlabeled standards detected with UV visualization or phosphomolybdic acid reagent (Ueda et al., 1974). The BE × CE solvent system was used for esters and apolar compounds and the BFE′ × BEM solvent system for cleavage products and polar compounds (Figure 5). Unlabeled standards were not available for *t*-CH_2OH-dec, *t*-CH_2OH-Br_2CA, and *c*-CH_2OH-Br_2CA-lactone, so these metabolites were cochromatographed with the corresponding dichloro analogues since the dibromo and dichloro compounds give essentially identical R_f values (Ruzo et al., 1978). Mono- and dicarboxylic acids after methylation were cochromatographed with the appropriate carbomethoxy compounds in the CE and EH solvent systems. The compound designated as 3-HO-benzyl ester was cochromatographed with the standard in solvent systems (see above) optimal to separate it from 4′-HO-per.

Metabolites with formyl groups (CHO-per, -cyper, -dec,

Table II. Metabolism of *trans*- and *cis*-Permethrin, *trans*- and *cis*-Cypermethrin and Decamethrin by Esterase, Oxidase, and Esterase-plus-Oxidase Systems of Mouse and Rat Liver Microsomes, Housefly Abdomens-plus-Thoraces and Cabbage Looper Guts

		extent of metabolism, %[a]					
		trans			cis		
substrate	species	est[b]	ox[c]	est + ox[c]	est[b]	ox[c]	est + ox[c]
permethrin	mouse	91.0	83.8	95.2	9.1	74.3	83.1
	rat	89.3	9.5	72.0	6.0	20.7	41.5
	housefly	38.0	4.6	44.5	9.1	4.2	14.0
	looper[d]	37.0	1.5	38.1	12.5	11.4	16.5
cypermethrin	mouse	93.2	17.3	89.6	41.5	37.6	62.1
decamethrin	mouse				28.3	41.0	75.1

[a] No metabolism (<1%) when preparation treated with TEPP and NADPH not added, i.e., no esterase or oxidase activity. [b] Only ester cleavage products are detected with all esterase preparations except that from the cabbage looper which gives the same products as the esterase plus oxidase preparation. [c] Quantitative data on individual metabolites are given in Tables III, IV, and V. [d] Microsome-plus-soluble rather than microsome fraction as in the other cases.

and -Cl_2CA) were reduced to the corresponding alcohols (CH_2OH-per, -cyper, -dec, and -Cl_2CA or the lactone derivatives of the cis hydroxymethyl compounds) by treatment with $NaBH_4$ (10 mg in 0.5 mL of ethanol at 25 °C for 1 h) prior to cochromatography (BE × CE or BFE′ × BEM, as appropriate). *c*-COOH-*t*-per was hydrolyzed on incubation at 50 °C for 20 min in a mixture of 0.02 N KOH in methanolic solution (0.1 mL) and hexane (4 mL), and the cleavage products were cochromatographed with PBalc (BE and CE) and, after methylation (diazomethane), with *c*-COOMe-Cl_2CA-Me (CE and EH).

Ester metabolites (4′-HO, 5-HO, and *t*-CH_2OH) of the cyano compounds were cleaved and the acid and alcohol fragments were derivatized for cochromatography. The method used was established in preliminary studies to give high yields of the appropriate derivatives from *trans*- and *cis*-cypermethrin and from decamethrin. Metabolites designated as the 4′- and 5-hydroxy derivatives of each cyanopyrethroid were cleaved with KOH (conditions as above) and the products were recovered by acidification with 0.03 N HCl (0.1 mL), evaporation to dryness, and washing with methanol. Cleavage products from ^{14}C acid preparations were methylated (diazomethane) and cochromatographed (CE and EH) with Cl_2CA-Me or Br_2CA-Me. Those from ^{14}C alcohol preparations were reduced (overspotting on the TLC plates with saturated $NaBH_4$ ethanolic solution) and cochromatographed with 4′-HO- or 5-HO-PBalc (two-dimensional development with BFE′ and BEM; for R_f values, see Unai and Casida, 1977). *t*-CH_2OH-*t*-cyper was detected by the same method, cochromatographing with PBalc in the BE and CE solvent systems and with *t*-CH_2OH-*t*-Cl_2CA-Me in the CE and EH solvent systems.

RESULTS

Relative Importance of Esteratic and Oxidative Metabolism. Each esterase preparation hydrolyzes *trans*-permethrin and -cypermethrin to a much greater extent than the corresponding cis isomer (Table II). In contrast, oxidative metabolism is more extensive for *cis*- than for *trans*-permethrin except with mouse and housefly microsomes where little or no isomer difference is evident. Each trans/cis comparison was made with the same enzyme preparation whereas different batches of mouse microsomes were used for the cypermethrin isomers and decamethrin. Mouse microsomal oxidase metabolism is less extensive with the cyanopyrethroids than with the permethrin isomers. The combined esterase and oxidase action always metabolizes the trans isomers more than the cis isomers.

Oxidative Metabolites of *trans*- and *cis*-Permethrin. Housefly enzymes hydroxylate the permethrin isomers at the 4′ position and the trans methyl group and there is also hydroxylation of *trans*-permethrin at the 6 position and *cis*-permethrin at the cis methyl group (Table III). The cleavage products are consistent with the sites of metabolic attack on the esters, i.e., *c*-CH_2OH-Cl_2CA-lactone from *cis*- but not from *trans*-permethrin and more 6-HO-PBalc from *trans*- than from *cis*-permethrin. It is not known whether the lactone formed in this case and with other enzymes and substrates is a metabolite or an artifact due to lactonization during analysis (Unai and Casida, 1977).

Looper enzymes yield relatively large amounts of 4′-HO- and *t*-CH_2OH-per from *cis*-permethrin but no ester metabolites from *trans*-permethrin (Table III). It was surprising to find Cl_2CA- and PBacid-glycine as major metabolites in the esterase-plus-oxidase system (and in the esterase system which is not tabulated). Further, the esterase preparation (without NADPH) carries out similar reactions to the esterase-plus-oxidase system. These findings indicate that the microsome-plus-soluble preparations contain appropriate enzymes and endogenous cofactors to accomplish the relevant oxidations and conjugations.

Larger amounts of permethrin oxidation products are detected with rat and particularly mouse liver enyzmes (Table IV) than with insect preparations (Table III) under the assay conditions used. Aryl hydroxylation occurs at the 4′ and 6 positions with mouse enzymes but only at the 4′ position with rat enzymes (Table IV). Hydroxylation at the 2′ position is observed only with *cis*-permethrin and the mouse oxidase system. An unusual ester derivative, the 3-HO-benzyl ester detected with *trans*-permethrin and rat enzyme, is possibly formed by degradation of 2′-HO-*t*-per (for related discussion, see Unai and Casida, 1977). The amount of trans hydroxymethyl ester metabolites exceeds that of the corresponding cis hydroxymethyl compounds except with rat enzymes acting on *trans*-permethrin. The dihydroxy ester metabolite (4′-HO,*t*-CH_2OH-per) is evident only with *cis*-permethrin. The cis hydroxymethyl ester derivative of *trans*-permethrin is further oxidized to the corresponding aldehyde and carboxylic acid by mouse enzymes and analogous aldehyde formation is evident with *cis*-permethrin acted on by both mouse and rat enzymes. Aldehydes and acids from hydroxymethyl oxidation are also prominent among the mouse metabolites from the acid moiety and with *trans*-permethrin their amount exceeds that of the direct cleavage product, Cl_2CA. The alcohol moiety cleavage products are those anticipated from the ester metabolites, i.e., 4′- and 6-hydroxy derivatives in mouse enzymes and 4′-hydroxy derivatives in rat enzymes. The mouse preparations are more effective than the rat preparations

Table III. Metabolites of *trans*- and *cis*-Permethrin in Oxidase and Esterase-plus-Oxidase Systems of Housefly Abdomens-plus-Thoraces and Cabbage Looper Guts

	yield in oxidase system (and esterase plus oxidase system), %			
	housefly		looper[b]	
compound[a]	trans	cis	trans	cis
unmetabolized	95.4 (55.5)	95.8 (86.0)	98.5 (61.9)	88.6 (83.5)
Ester Metabolites				
4′-HO	0.8 (1.0)	0.8 (1.6)	0.0 (0.0)	1.6 (1.5)
6-HO	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
t-CH_2OH	0.9 (1.2)	0.4 (0.5)	0.0 (0.0)	5.5 (6.2)
c-CH_2OH	0.0 (0.0)	0.3 (0.8)	0.0 (0.0)	0.0 (0.0)
total	1.8 (2.3)	1.5 (2.9)	0.0 (0.0)	7.1 (7.7)
Metabolites from Acid Moiety				
Cl_2CA	2.2 (40.3)	0.9 (5.7)	0.7 (32.4)	1.4 (2.3)
t-CH_2OH-Cl_2CA	0.2 (1.5)	0.3 (0.9)	0.0 (0.0)	0.0 (0.0)
c-CH_2OH-Cl_2CA-lactone	0.0 (0.0)	1.2 (2.3)	0.0 (0.0)	0.0 (0.0)
Cl_2CA-glycine	0.0 (0.0)	0.0 (0.0)	0.0 (3.6)	0.0 (0.7)
unknowns				
organosoluble	0.0 (0.0)	0.0 (1.3)	0.0 (1.2)	1.7 (4.3)
water-soluble	0.4 (0.4)	0.3 (0.9)	0.8 (0.9)	1.2 (1.5)
total	2.8 (42.2)	2.7 (11.1)	1.5 (38.1)	4.3 (8.8)
Metabolites from Alcohol Moiety				
PBalc	1.0 (29.3)	1.2 (6.0)	0.3 (10.2)	0.7 (1.2)
PBacid	0.3 (10.4)	0.3 (4.2)	0.2 (5.3)	0.0 (0.0)
4′-HO-PBalc	0.1 (0.4)	0.0 (0.1)	0.0 (0.0)	0.0 (0.6)
6-HO-PBalc	0.1 (0.5)	0.1 (0.2)	0.0 (0.0)	0.0 (0.0)
PBacid-glycine	0.0 (0.0)	0.0 (0.0)	0.0 (16.9)	0.6 (4.0)
unknowns				
organosoluble	1.0 (1.0)	0.7 (0.2)	0.0 (3.0)	2.1 (1.9)
water-soluble	0.3 (0.6)	0.4 (0.4)	1.0 (2.7)	0.9 (1.1)
total	2.8 (42.2)	2.7 (11.1)	1.5 (38.1)	4.3 (8.8)

[a] Metabolites detected in liver microsome systems (Table IV) but not tabulated here were not detected with the insect systems. [b] Microsome-plus-soluble rather than microsome fraction as with houseflies.

in oxidation of PBalc to PBald and PBacid.

There are large amounts of unknown organosoluble and water-soluble metabolites in the liver microsome assays (Table IV) but not with the insect preparations (Table III). These products are largely polar compounds and may be formed in part by more extensive oxidation than in the identified derivatives. In some cases they also include metabolites in which the methyl group is oxidized to the carboxylic acid and appropriate standards were not available at the time for identification of these compounds.

Higher yields of ester metabolites are detected in four cases with the esterase-plus-oxidase system than with the oxidase system, i.e., both permethrin isomers with housefly enzymes and *cis*-permethrin with looper and rat enzymes. This is unexpected since the additional esterase action should decrease the amount of ester metabolites. It therefore appears that TEPP partially inhibits oxidases of the housefly, looper, and rat preparations.

Oxidative Metabolites of *trans*- and *cis*-Cypermethrin and of Decamethrin. These substrates were examined only with mouse enzyme preparations (Table V). The major site of ring hydroxylation is the 4′ position, but in contrast to the permethrin isomers the secondary site is the 5 position. The trans methyl group is an important site of hydroxylation of the esters and cis methyl oxidation is evident from the acid moiety metabolites. The hydroxymethyl derivatives are further oxidized to the corresponding aldehydes and carboxylic acids. The alcohol moiety metabolites include the expected 4′- and 5-HO-PBacids. PBald-cyanohydrin is detected as a minor metabolite in all cases (except TEPP-treated microsomes without NADPH) but due to its ease of cleavage to PBald the tabulated amounts are probably below the actual levels. The unknown metabolites, mostly polar compounds, are likely to include derivatives hydroxylated at more than one position and unidentified esters and cleavage products in which one methyl group has been oxidized to the carboxylic acid.

The studies on decamethrin provide an opportunity to examine possible epimerization at the α position, yielding an (*RS*)-α mixture from the (*S*)-α substrate since the (*R*)-α and (*S*)-α epimers are separable by TLC in the HE solvent system. In all relevant cases, decamethrin and its 4′-hydroxy derivative were recovered as single products, establishing that epimerization does not occur under the enzyme incubation and analysis conditions.

DISCUSSION

Metabolic pathways in the enzyme systems examined are given in Figure 2 for *trans*- and *cis*-permethrin and in Figure 3 for *trans*- and *cis*-cypermethrin and for decamethrin. The preferred sites of hydroxylation, based on all identified metabolites in the oxidase and esterase-plus-oxidase systems, are generalized as shown in Chart I. Relative to the acid moiety, mouse and rat enzymes preferentially hydroxylate the cis methyl group of the trans pyrethroids and the trans methyl group of the cis pyrethroids. The opposite relationship in the preferred methyl group is observed with housefly microsomes. Looper enzymes hydroxylate only the trans methyl group of *cis*-permethrin. The preferred site of hydroxylation in the alcohol moiety is the 4′ position with all substrates, but the secondary site perference varies with the pyrethroid, i.e., 6 position with phenoxybenzyl esters and 5 position with α-cyanophenoxybenzyl esters. The cyano group appears to influence the substrate orientation at the oxidase site or the reactivity of various positions in the benzyl moiety. Alternatively, the cyanopyrethroids may

Table IV. Metabolites of *trans*- and *cis*-Permethrin in Oxidase and Esterase-plus-Oxidase Systems of Mouse and Rat Liver Microsomes

	yield in oxidase system (and esterase plus oxidase system), %			
	mouse		rat	
compound	trans	cis	trans	cis
unmetabolized	16.2 (4.8)	25.7 (16.9)	90.5 (28.0)	79.3 (58.5)
	Ester Metabolites			
2′-HO	0.0 (0.0)	0.4 (0.0)	0.0 (0.0)	0.0 (0.0)
4′-HO	0.5 (0.1)	3.9 (1.8)	0.9 (0.2)	5.0 (9.2)
6-HO	0.8 (0.1)	1.6 (0.5)	0.0 (0.0)	0.0 (0.0)
t-CH_2OH	9.3 (0.2)	7.6 (2.3)	0.9 (0.4)	4.9 (5.4)
c-CH_2OH	3.9 (0.6)	0.5 (0.4)	2.0 (0.9)	0.5 (0.6)
4′-HO, *t*-CH_2OH	0.0 (0.0)	3.8 (2.1)	0.0 (0.0)	0.4 (0.7)
t- and/or *c*-CHO[a]	11.9 (0.0)	4.8 (1.7)	0.0 (0.0)	1.0 (1.4)
c-COOH	16.8 (7.9)	*b*	*b*	*b*
3-HO-benzyl ester[c]	0.0 (0.0)	0.0 (0.0)	1.1 (0.4)	0.0 (0.0)
total	43.2 (8.9)	22.6 (8.8)	4.9 (1.9)	11.8 (17.3)
	Metabolites from Acid Moiety			
Cl_2CA	7.0 (25.9)	18.3 (23.4)	2.4 (62.3)	1.7 (7.0)
t-CH_2OH-Cl_2CA	7.6 (15.9)	3.7 (6.9)	0.4 (1.0)	0.5 (3.1)
t-CHO-Cl_2CA	2.0 (6.4)	2.1 (3.6)	0.0 (0.0)	0.0 (0.0)
t-COOH-Cl_2CA	0.3 (1.2)	*b*	*b*	*b*
c-CH_2OH-Cl_2CA	2.3 (2.4)	2.6 (3.8)	0.2 (0.5)	0.3 (0.4)
c-CH_2OH-Cl_2CA-lactone	1.5 (1.3)	3.4 (3.3)	0.2 (1.7)	0.6 (1.6)
c-CHO-Cl_2CA	6.7 (25.6)	1.7 (4.7)	0.0 (0.0)	0.0 (0.0)
c-COOH-Cl_2CA	0.9 (0.8)	*b*	*b*	*b*
unknowns				
organosoluble				
origin	4.3 (2.0)	8.6 (6.8)	1.2 (0.0)	2.9 (5.1)
higher R_f	7.0 (4.5)	10.6 (20.4)	0.0 (4.3)	2.6 (6.2)
water-soluble	1.0 (0.3)	0.7 (1.4)	0.2 (0.3)	0.3 (0.8)
total	40.6 (86.3)	51.7 (74.3)	4.6 (70.1)	8.9 (24.2)
	Metabolites from Alcohol Moiety			
PBalc	5.5 (20.5)	4.6 (7.2)	1.4 (55.6)	0.8 (6.9)
PBald	0.8 (5.3)	0.8 (1.2)	0.2 (5.2)	0.7 (1.1)
PBacid	4.2 (38.0)	7.5 (14.2)	0.2 (3.0)	0.2 (0.6)
4′-HO-PBalc	2.1 (2.4)	0.8 (4.1)	0.1 (2.8)	0.5 (2.0)
4′-HO-PBacid	0.0 (1.6)	0.0 (0.0)	0.1 (0.6)	0.2 (0.3)
6-HO-PBalc	2.0 (2.4)	1.1 (1.2)	0.0 (0.0)	0.0 (0.0)
unknowns				
organosoluble				
origin	8.6 (4.3)	17.3 (14.5)	1.8 (0.1)	3.8 (5.8)
higher R_f	14.4 (9.1)	18.6 (29.3)	0.5 (2.1)	2.2 (6.3)
water-soluble	3.0 (2.7)	1.0 (2.6)	0.3 (0.7)	0.5 (1.2)
total	40.6 (86.3)	51.7 (74.3)	4.6 (70.1)	8.9 (24.2)

[a] Both *t*-CHO- and *c*-CHO-esters are present since $NaBH_4$ reduction yields both *t*-CH_2OH- and *c*-CH_2OH-esters or the corresponding *c*-CH_2OH-Cl_2CA-lactone. [b] These metabolites, if present, are included in unknown organosoluble products since, at the time of these experiments, authentic standards were not available. [c] May be an artifact from decomposition of a metabolite hydroxylated on the phenoxy group, e.g., 2′-HO-*t*-per.

Chart I

trans-permethrin	mouse	cis > trans > 4′ = 6
	rat	4′ = cis > trans
	housefly	trans > 4′ > 6
cis-permethrin	mouse	trans > cis = 4′ > 6 > 2′
	rat	4′ = trans > cis
	housefly	cis > 4′ = trans > 6
	looper	trans > 4′
trans-cypermethrin	mouse	cis > 4′ > trans > 5
cis-cypermethrin	mouse	trans > cis > 4′ > 5
decamethrin	mouse	trans > 4′ > cis = 5

be acted on in part by an oxidase not involved with the noncyano compounds.

It is of interest to relate the enzyme studies to in vivo investigations, e.g., permethrin isomers in rats (Gaughan et al., 1977) and housefly adults and cabbage looper larvae (Shono et al., 1978) and decamethrin in rats (Ruzo et al., 1978). The in vitro and in vivo studies are in agreement on the greater extent of hydrolysis of *trans*- than of *cis*-permethrin and on the major sites of hydroxylation of each pyrethroid. Several metabolites detected in the in vitro studies are not previously reported in vivo, e.g., the CHO-per, CHO-dec, COOH-per, CHO-X_2CA, and COOH-X_2CA derivatives. An earlier study also established *c*-CHO-per derivatives as mouse microsomal metabolites of *trans*- and *cis*-permethrin (Unai and Casida, 1977). The intermediate hydroxymethyl metabolites are possibly conjugated and/or excreted sufficiently fast under in vivo conditions that they are not further oxidized to a major

Table V. Metabolites of *trans*- and *cis*-Cypermethrin and Decamethrin in Oxidase and Esterase-plus-Oxidase Systems of Mouse Liver Microsomes

compound (X = Cl or Br)	yield in oxidase system (and esterase plus oxidase system), %: cypermethrin (X = Cl) trans	cypermethrin (X = Cl) cis	decamethrin (X = Br)
unmetabolized	82.7 (10.4)	62.4 (37.9)	59.0 (24.9)[a]
Ester Metabolites			
4'-HO	0.8 (0.4)	3.8 (3.2)	3.3 (1.8)[a]
5-HO	0.3 (0.0)	2.6 (2.4)	0.8 (0.5)
t-CH_2OH	0.9 (0.0)	8.9 (8.5)	1.6 (1.0)
t- and/or *c*-CHO[b]	0.0 (0.0)	2.4 (1.2)	2.1 (0.3)
total	2.0 (0.4)	17.7 (15.3)	7.8 (3.6)
Metabolites from Acid Moiety			
X_2CA	3.8 (74.8)	6.6 (26.4)	10.5 (31.6)
t-CH_2OH-X_2CA	0.0 (1.2)	1.8 (4.6)	3.5 (7.0)
t-CHO-X_2CA	0.0 (0.0)	0.2 (1.5)	*c*
c-CH_2OH-X_2CA	1.5 (1.5)	1.1 (2.2)	*c*
c-CH_2OH-X_2CA-lactone	2.6 (3.4)	6.0 (5.6)	0.7 (2.8)
t- and/or *c*-COOH-X_2CA	1.7 (1.1)[d]	1.5 (1.5)[d]	*c*
unknowns			
organosoluble			
origin	3.6 (3.1)	1.2 (3.1)	7.9 (9.3)
higher R_f	1.6 (3.5)	1.1 (1.1)	8.9 (19.2)
water-soluble	0.5 (0.6)	0.4 (0.8)	1.7 (1.6)
total	15.3 (89.2)	19.9 (46.8)	33.2 (71.5)
Metabolites from Alcohol Moiety			
PBalc	1.8 (20.3)	1.9 (8.0)	2.4 (6.7)
PBald	1.3 (16.2)	2.3 (9.9)	1.4 (7.4)
PBacid	4.4 (43.1)	7.3 (19.0)	15.7 (31.8)
PBald-cyanohydrin	0.4 (0.4)	0.5 (0.6)	0.5 (0.5)
4'-HO-PBacid	1.7 (3.7)	0.9 (2.3)	0.6 (5.0)
5-HO-PBacid	0.0 (0.0)	0.6 (1.3)	0.4 (1.2)
unknowns			
organosoluble			
origin	1.2 (0.1)	3.0 (4.8)	10.3 (9.1)
higher R_f	3.6 (4.8)	2.6 (0.4)	0.0 (8.2)
water-soluble	0.9 (0.6)	0.8 (0.5)	1.9 (1.6)
total	15.3 (89.2)	19.9 (46.8)	33.2 (71.5)

[a] No racemization in alcohol moiety since only (*S*)-α epimer recovered. [b] Both *t*-CHO- and *c*-CHO-esters are present since $NaBH_4$ reduction yields both *t*-CH_2OH- and *c*-CH_2OH-esters or the corresponding *c*-CH_2OH-X_2CA-lactone. [c] These metabolites, if present, are included in unknown organosoluble products since, at the time of these experiments, authentic standards were not available. [d] Assignments based on chromatographic position not on cochromatography.

degree.

Cleavage of the α-cyanophenoxybenzyl derivatives to the cyanohydrin may result from both esteratic and oxidative mechanisms, at least with *cis*-cypermethrin and decamethrin, since large amounts of the cleavage products normally analyzed (PBalc, PBald, PBacid, and HO-PBacid derivatives) are evident in the oxidase system which lacks esterase activity.

Previous studies on metabolism of these pyrethroids by mouse liver microsomes used a ~35-fold higher substrate level than in the present investigation (Soderlund and Casida, 1977a,b). At the higher substrate level, the metabolism rate of the cypermethrin isomers is much less than that of the permethrin isomers under all assay conditions and decamethrin is not detectably metabolized. Some stereospecificity is encountered with mouse and rat microsomal oxidases in the preferred methyl group for hydroxylation in the 1*R*- vs. 1*S*-permethrin isomers (Soderlund and Casida, 1977c). The present study utilized 1*RS* isomer mixtures and is therefore not appropriate to detect differences in metabolism of the 1*R* and 1*S* optical antipodes.

The low toxicity of *trans*-permethrin and -cypermethrin to mice relative to the corresponding cis isomers (Miyamoto, 1976; Soderlund and Casida, 1977b) is consistent with their greater ease of biodegradation in the mouse microsomal systems.

ACKNOWLEDGMENT

The authors thank Loretta Gaughan, Roy Holmstead, David Hutson, Luis Ruzo, and Tadaaki Unai, current or former colleagues in this laboratory, for assistance and helpful suggestions.

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Received for review May 22, 1978. Accepted October 23, 1978. Study supported in part by the National Institutes of Health (Grant P01 ES00049) and grants from: FMC Corp., Middleport, NY; Roussel-Uclaf-Procida, Paris, France; McLaughlin Gormley King Co., Minneapolis, MN; Mobil Foundation Inc., Edison, NJ.

Fate of Potassium 3,4-Dichloro-5-isothiazolecarboxylate in Cotton Plants and White Rats

Ted N. Shaver,* Don L. Bull, and G. Wayne Ivie

^{14}C-labeled potassium 3,4-dichloro-5-isothiazolecarboxylate (PDIC) was applied to individual leaves (100 μg/leaf) of field-grown cotton plants for studies of absorption, photodecomposition, and metabolism and to whole plants in a small plot (1121 g of AI/ha) for studies of radioactive residues in different parts of the cotton plant. Also, ^{14}C-labeled PDIC was administered orally to white rats to determine metabolism, accumulation in tissues, and excretion. There was some photodecomposition (<1% of dose) of PDIC on leaf surfaces. The chemical was rapidly absorbed (55% after 24 h) from leaf surfaces and then readily translocated throughout the plant. Appreciable residues of radiocarbon (>200 ppm) accumulated in cottonseed; most of this was the parent compound. PDIC was rapidly excreted (ca. 95% in 24 h) in the urine of white rats; only minimum concentrations (0.01–0.17 ppm) remained in any tissues after 24 h.

There is considerable interest at present in the use of plant growth regulating chemicals either to terminate the fruiting cycle of cotton at a certain time during the latter part of the growing season (Kittock et al., 1973; Kittock et al., 1978) or to precondition the plant so that eventual application of conventional defoliants results in a more efficient removal of foliage. Potential advantages of such procedures include (1) a reduction of latter season infestations of insect pests such as the pink bollworm (*Pectinophora gossypiella* Saunders) and the boll weevil (*Anthonomus grandis* Boheman) by eliminating squares and small bolls essential to the development of these pests, (2) improvements in overall lint quality through the removal of some bolls that might have incompletely developed lint at harvest, and (3) potential improvement in dust problems at cotton gins through a reduction of foliage contaminants of machine-harvested seed cotton.

One of the more promising of these plant growth regulators is potassium 3,4-dichloro-5-isothiazolecarboxylate (herein referred to as PDIC). This experimental chemical, under evaluation by Pennwalt Corp., is a water-soluble (48.5 g/100 mL), white crystalline powder that is essentially nontoxic to mammals (acute oral LD_{50} to rats is ca. 1.2 g/kg). The present report describes the fate of PDIC in cotton plants and in white rats.

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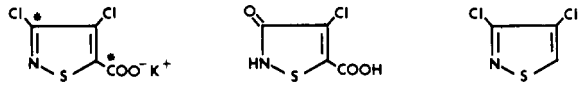
EXPERIMENTAL SECTION

Chemicals. The Pennwalt Corp., Tacoma, WA, provided pure samples of PDIC radiolabeled with ^{14}C at the 3- and carboxyl-carbon positions of the molecule (sp act., 19.32 mCi/mmol). Also supplied were nonradioactive samples of technical PDIC and two potential metabolites: I (4-chloro-3-oxo-5-isothiazolidinecarboxylic acid) and II (3-4-dichloroisothiazole).



PDIC I II

Fate on Field-Grown Cotton. *Foliar Application to Individual Leaves.* The ^{14}C-labeled PDIC was diluted with 6.25 parts of the nonradiolabeled material and then dissolved in water to form a solution having a concentration of 1000 ppm active ingredient (AI). This solution was applied in situ to fully expanded leaves of field-grown 'SP-37' cotton by spreading a 100-μL aliquot (100 μg of AI) uniformly over the upper surface of each leaf with a micropipet.

At 0, 1, 3, 7, and 14 days posttreatment, three treated leaves were collected at random from different plants and processed immediately. Unabsorbed (external) radioactive material was recovered by rinsing the leaves thoroughly with methanol, and absorbed (internal) radioactive material was extracted by homogenizing the rinsed leaves with