acids, terpene carboxylic acids and their glucuronides, and terpene alcohols as their glucuronides or sulfates. About 2% of the ^{14}C appears as expired products, probably $^{14}CO_2$, and this portion arises from methyl, chloromethyl, or dichloromethyl substituents in the original toxaphene components. On the assumption that most of the toxaphene components are polychlorobornanes related to the identified 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane and in light of the extensive or even complete metabolic dechlorination of some components, a portion of the terminal metabolites may be monocyclic or acyclic compounds.

Even though most of the toxaphene components undergo rapid metabolism in mammals, there are probably large rate differences for metabolism of the various components. The components of highest toxicity are presumed to be those that combine appropriate configurations for disruption of nerve activity with some degree of resistance to metabolism. However, limited evidence from studies with toxicants A and B indicates that they are no more persistent in rats than most of the other toxaphene components. A portion of the dose of these compounds is excreted in feces without metabolism. Some of the metabolites have gc properties similar to those of other toxaphene components. Thus, it is likely that tissue residues will include metabolites formed by dechlorination that fall within the normal gc range of the toxaphene components, a complication in gc residue analyses. Tissue analyses for total radioactivity indicate that toxaphene components are not persistent materials in rats. However, further studies are needed to define the chemical nature of the tissue residues resulting from administration of toxaphene and of individual toxaphene components, such as toxicants A and B. The gc–CI–ms technique appears to be an appropriate method for these investigations. There is also a need for information on the biological activity of the toxaphene metabolites.

Toxaphene is a complex mixture of related C_{10} polychloro compounds but most if not all of these components undergo extensive metabolic dechlorination in rats. Toxaphene differs in this respect from many other chlorinated hydrocarbon insecticides and environmental pollutants.

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Metabolism of (+)-*trans*- and (+)-*cis*-Resmethrin in Rats

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Rats treated orally at about 1 mg/kg with either 5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate [(+)-*trans*-resmethrin] or the corresponding (+)-cis compound excrete the following metabolites: no esters with (+)-*trans*-resmethrin; two unidentified esters with (+)-*cis*-resmethrin; 5-benzyl-3-furoic, 5-(α-hydroxybenzyl)-3-furoic, and 5-(4′-hydroxybenzyl)-3-furoic acids; chrysanthemic acid, chrysanthemumdicarboxylic acid, and the intermediate alcohol and aldehyde oxidation products; conjugates of each of these acids. The metabolite(s) persisting longest in the body is derived from the alcohol moiety of (+)-*trans*-resmethrin. The isobutenyl moiety is oxidized at either the cis or trans methyl group with (+)-*cis*-resmethrin but only at the trans methyl group with (+)-*trans*-resmethrin. An unanticipated metabolic pathway involves epimerization at C-3 of the cyclopropane group leading to excretion of isomerized forms of chrysanthemumdicarboxylic and hydroxylated chrysanthemic acids. Three of the (+)-*trans*-resmethrin metabolites are much more toxic than the parent compound.

Resmethrin consists of two highly insecticidal components, the (+)-trans isomer (bioresmethrin) and the (+)-cis isomer, and two noninsecticidal components, the corresponding (−) isomers, in an approximate 40:10:40:10 ratio, respectively (Elliott, 1971; Elliott *et al.*, 1967; Jao and Casida, 1975). The acute oral toxicity to rats is more than 48-fold greater for (+)-*cis*-resmethrin than for the (+)-trans compound (Verschoyle and Barnes, 1972). (±)-*trans*-Resmethrin undergoes rapid hydrolysis in orally treated rats followed by oxidation of the alcohol moiety to yield benzylfuroic acid and several oxidized derivatives of this acid which are excreted in free or conjugated form (Miyamoto *et al.*, 1971). These studies did not define the fate of the alcohol moiety from *cis*-resmethrin or the chrysanthemate moiety from either *trans*- or *cis*-resmethrin. Mouse liver microsomal esterases hydrolyze (+)-*trans*-resmethrin 8- to 14-fold more rapidly than (+)-*cis*-

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resmethrin when acetone powder preparations are used or 15- to >2500-fold more rapidly with fresh microsomes, whereas the microsomal oxidases metabolize (+)-*trans*-resmethrin more slowly, only 0.7- to 0.8-fold the rate of the (+)-cis isomer (Abernathy *et al.*, 1973; Casida *et al.*, 1975; Jao and Casida, 1975). It appears likely that the liver microsomal esterase specificity for the *trans*- relative to the *cis*-chrysanthemate contributes to the differences in their mammalian toxicity.

The present investigation considers the metabolism in rats of both the acid and alcohol moieties of each of (+)-*trans*- and (+)-*cis*-resmethrin. Knowledge of the metabolic fate of the individual (+)-resmethrin isomers is important in evaluating the safety of this insecticide and understanding the basis for selective toxicity with pyrethroids in general.

MATERIALS AND METHODS

Chemicals. Unlabeled (+)-*trans*-resmethrin from Roussel-Uclaf-Procida (Paris, France) was purified as previously described (Abernathy *et al.*, 1973). Unlabeled (−)-*cis*-resmethrin (mp 46.5–48°) was prepared from (−)-*cis*-chrysanthemic acid (CA) provided by Sumitomo Chemical Co. (Takarazuka, Japan). These resmethrin samples were greater than 99.5% pure relative to their trans–cis isomer content as determined by gas–liquid chromatography (glc) under conditions described below. Sumitomo Chemical Co. also supplied (+)-*cis*-CA which, according to their analyses, contained no trans isomer and 0.2% (−)-*cis*-CA and gave an optical rotation value, $[\alpha]^{21}D$ ($CHCl_3$), of +85.6°.

(+)-*trans*- and (+)-*cis*-resmethrin were individually labeled with ^{14}C at the carbonyl group of the acid moiety (^{14}C-labeled acid preparation) and the phenyl group of the alcohol moiety (^{14}C-labeled alcohol preparation) as previously described (Ueda *et al.*, 1974a). The specific activities of the acid- and alcohol-labeled resmethrin preparations were 2.3 and 1.9 mCi/mmol, respectively, each with a radiochemical purity of >99%. The purity relative to the trans–cis isomers was determined by glc to be >99.5% for each labeled resmethrin preparation. The [^{14}C]CA used in the preparation of [^{14}C]resmethrin gave optical rotation values, $[\alpha]^{21}D$ ($CHCl_3$), of +27.0° (*c* 0.118) and +84.5° (*c* 0.806) for the (+)-trans and (+)-cis isomers, respectively; under comparable conditions the unlabeled CA isomers resolved in larger amounts gave values of $[\alpha]^{24}D$ +25.8° (*c* 8.60) and +85.6° (*c* 1.00), respectively (Abernathy *et al.*, 1973). The carbonyl-labeled and unlabeled preparations of each of (+)-*cis*-CA and (−)-*cis*-resmethrin (Ueda *et al.*, 1974a) were mixed to obtain samples with specific activities of 0.012 mCi/mmol and 0.16 μCi/mmol, respectively.

Authentic standards of unlabeled CA and 5-benzyl-3-furylmethanol (BFA) derivatives were used for tentative identification of resmethrin metabolites. These standards are given code designations as described below and shown later in Figure 1. The suffix Me indicates that a compound is derivatized as the methyl ester.

The following six compounds were prepared as previously described (Miyamoto *et al.*, 1971): 5-benzyl-3-furoic acid (BFCA) and its methyl ester (BFCAMe); 5-benzoyl-3-furoic acid (α-keto-BFCA) and its methyl ester (α-keto-BFCAMe); 5-(α-hydroxybenzyl)-3-furoic acid (α-OH-BFCA) and its methyl ester (α-OH-BFCAMe). 5-(4′-Methoxybenzyl)-3-furylmethanol (4′-MeO-BFA) and the corresponding furoic acid (4′-MeO-BFCA) and its methyl ester (4′-MeO-BFCAMe) were synthesized by described procedures (Elliott *et al.*, 1971).

The oxidized derivatives of CA and resmethrin were obtained from J. Martel (Roussel-Uclaf-Procida) or K. Yamashita (Tohoku University, Sendai, Japan) or they were synthesized by procedures similar to those reported for analogous compounds (Matsui *et al.*, 1957; Ueda and Matsui, 1970; Sugiyama *et al.*, 1972a,b). These derivatives are: four isomers of the BFA ester of 2,2-dimethyl-3-(2′-methoxycarbonyl-1′-propenyl)cyclopropanecarboxylic acid referred to as BFA-*tE*-CDAMe, BFA-*tZ*-CDAMe, BFA-*cE*-CDAMe, and BFA-*cZ*-CDAMe; the 4′-MeO-BFA ester of 2,2-dimethyl-3-(2′-(*E*)-methoxycarbonyl-1′-propenyl)cyclopropane-1-*cis*-carboxylate (4′-MeO-BFA-*cE*-CDAMe); four isomers of chrysanthemumdicarboxylic acid (CDA) referred to as *tE*-CDA, *tZ*-CDA, *cE*-CDA and *cZ*-CDA, and their dimethyl esters; two isomers of 2,2-dimethyl-3-(2′-formyl-1′-propenyl)cyclopropanecarboxylic acid (CAA) referred to as *tE*-CAA and *cE*-CAA, and their methyl esters; four isomers of 2,2-dimethyl-3-(2′-hydroxymethyl-1′-propenyl)cyclopropanecarboxylic acid (CHA) referred to as *tE*-CHA, *tZ*-CHA, *cE*-CHA, and *cZ*-CHA, and their methyl esters. Samples of *tZ*-CHA and *cZ*-CHA were only available for use in one series of experiments described later. The epoxy-*trans*-CA isomers and the corresponding diols were prepared by reaction of *trans*-CA with *m*-chloroperoxybenzoic acid by the general procedure of Ueda *et al.* (1974a) followed by epoxide hydrolysis under acid conditions (Hammock *et al.*, 1974).

Chromatography. Thin-layer chromatography (tlc) utilized precoated 20 × 20 cm chromatoplates as follows: silica gel 60 F-254 with 0.25 mm gel thickness (Merck) for analytical studies; silica gel F-254 with 0.5 mm gel thickness (Merck) and silica gel GF with 1.0 mm gel thickness (Analtech, Inc., Newark, Del.) for preparative isolations. Seven tlc solvent systems were used: (A) benzene–ethyl acetate–methanol (15:5:1); (B) benzene saturated with formic acid–ether (10:3); (C) hexane–ether (2:1); (D) carbon tetrachloride–hexane–ether (8:1:1); (E) hexane–isopropyl ether (2:1); (F) ether–hexane (2:1); (G) chloroform–hexane–ether (8:1:1). The tlc R_f values for resmethrin, its hydrolysis products, and various derivatives of these hydrolysis products are given in Table I. For cochromatography the zones detected under ultraviolet light (254 nm) and with phosphomolybdic acid spray reagent were compared with the radioactive spots detected by autoradiography (Ueda *et al.*, 1974a). The radioactive gel regions were quantitated by liquid scintillation counting (lsc).

Metabolites of (+)-*cis*-[^{14}C]CA were purified on a column (27.5 cm × 4 cm i.d.) prepared from a mixture of 100 g of silicic acid (100 mesh, Mallinckrodt) and 50 g of Celite slurried in hexane and developed, in sequence, with ether–hexane (1:4) (200 ml), ether–hexane (2:3) (200 ml), ether–hexane (4:1) (200 ml), ether (600 ml), and methanol (400 ml), monitoring the fractions by lsc and tlc and combining fractions of similar composition.

Gas–liquid chromatography for routine analyses utilized glass columns (1.8 m × 2 mm i.d.) containing 3% OV-17 on Chromosorb W-HP (80–100 mesh), 3% SE-30 on Gas-Chrom Q (80–100 mesh), or 6% QF-1 on Chromosorb W-AW (HMDS, 60–80 mesh), in each case with a N_2 flow rate of 15 ml/min and a flame ionization detector. R_t values with the OV-17 column are given in Table I for resmethrin, its hydrolysis products, and certain derivatives. The OV-17 column generally provided better resolution than the SE-30 column. The QF-1 column at 200° was used for determination of the isomeric purity of resmethrin, resulting in R_t values of 14.3 and 12.6 min for *trans*- and *cis*-resmethrin, respectively. When a packed column provided inadequate resolution for analytical purposes, a capillary column (300 m × 0.75 mm i.d.) was used with a coating consisting of a mixture of 95% SF-96-50 and 5% Igepal, a He inlet pressure of 1.75 kg/cm², and a flame ionization detector. Preparative glc utilized a glass column (1.5 m × 4 mm i.d.) containing 3% OV-17 as above maintained at 150° with a He flow rate of 50 ml/min and a thermal conductivity detector.

Spectroscopy. Chemical ionization mass spectra were obtained with the Finnigan 1015D mass spectrometer cou-

Table I. Chromatographic Properties of (+)-*trans*- and (+)-*cis*-Resmethrin and of Various Derivatives of the Resmethrin Hydrolysis Products

Compound	Glc R_t, min, and temp, °C, for methylated derivatives on OV-17 column	Tlc R_f with indicated solvent systems				
		Carboxylic acids, B×2	Methylated derivatives			
			A	C×2	D×2	E×3
		Resmethrin				
(+)-*trans*-Resmethrin	6.4 (240)	0.82	0.71	0.73	0.68	
(+)-*cis*-Resmethrin	6.3 (240)	0.83	0.71	0.73	0.67	
		5-Benzyl-3-furylmethanol and Derivatives				
BFA	10.5 (160)	0.43	0.39	0.15	0.08	
BFCA	12.9 (160)	0.57	0.64	0.60	0.61	
4′-OH-BFCA		0.37	0.65	0.61	0.51	
α-OH-BFCA	7.1 (200)	0.21	0.53	0.19	0.09	
α-keto-BFCA	7.5 (200)	0.31	0.57	0.35	0.25	
		Chrysanthemic Acid and Derivatives				
t-CA	3.6 (120)	0.69	0.66	0.74	0.73	
c-CA	3.6 (120)	0.71	0.66	0.74	0.74	
tE-CHA	3.8 (160)	0.20	0.45	0.15	0.07	
cE-CHA	3.5 (160)	0.16	0.46	0.12	0.05	
tE-CAA	3.2 (160)	0.41	0.57	0.39	0.25	
cE-CAA	3.2 (160)	0.38	0.58	0.47	0.31	
tE-CDA	4.7 (160)	0.29	0.62	0.54	0.49	0.61
tZ-CDA	4.0 (160)	0.23	0.62	0.52	0.41	0.57
cE-CDA	5.3 (160)	0.19	0.62	0.49	0.39	0.54
cZ-CDA	3.5 (160)	0.39	0.65	0.63	0.59	0.74

pled to a System Industries Model 150 control system utilizing methane at about 1000 μ pressure as the reactant gas. Prior to mass spectrometry, the components were separated by glc on the Finnigan 9500 gas chromatograph utilizing either a glass column (2.7 m × 2 mm i.d.) containing 3% Dexsil 300 on Varaport-30 or a glass column (1.8 m × 2 mm i.d.) containing 3% OV-17 on Chromosorb W-HP, in each case with a methane flow rate of 25 ml/min. Optical rotations were measured with the ETL-NPL Automatic Polarimeter Type 143A. The Perkin-Elmer R12B spectrometer was used to obtain nuclear magnetic resonance (nmr) spectra for which chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and coupling constants (*J*) are expressed in hertz. Radioactivity measurements were made with the Beckman LS-150 liquid scintillation system unless indicated otherwise.

Metabolism Studies. The studies utilized male, albino Sprague-Dawley rats (160–180 g) from Horton Laboratories Inc., Oakland, Calif. All excreta and tissue samples were stored at −50° until analyzed and the quantitative data in ^{14}C investigations are corrected for quench.

Low Doses of [^{14}C]*Resmethrin.* The (+)-[^{14}C]resmethrin preparations in 100 μl of dimethyl sulfoxide (Me_2SO) were individually administered by stomach tube followed by a Me_2SO rinse (150 μl) of the stomach tube. Two experiments were made, involving the following resmethrin dosages (milligrams/kilogram) for the trans and cis acid and trans and cis alcohol labeled preparations, respectively: 0.95, 0.96, 1.32, and 1.22 in experiment I; 0.79, 0.79, 0.96, and 0.96 in experiment II. The animals were held in metabolism cages for 6 days during which time the urine and feces were collected. A trap for $^{14}CO_2$ (Krishna and Casida, 1966) was also used for rats administered acid-labeled resmethrin in experiment I. Daily samples were analyzed for ^{14}C in the urine, a methanol extract of the feces, and as expired $^{14}CO_2$. After the sixth day, the animals were anesthetized with ether, blood was obtained by cardiac puncture, the animals were sacrificed, and the desired tissues were dissected.

The procedure for extraction of urine and feces varied somewhat between experiments I and II. In the first experiment, the 0–24-hr urine samples were extracted with peroxide-free diethyl ether (referred to hereafter as ether) (3 × 3 vol) after addition of $(NH_4)_2SO_4$ (0.2 g/ml). The pH was then adjusted to 2 by addition of concentrated HCl followed by extraction with ether–ethanol (2:1) (3 × 3 vol). The ether and the ether–ethanol extracts were individually dried (Na_2SO_4), concentrated with a stream of dry N_2 or with a rotary evaporator, and examined by tlc. The primary purpose of this first experiment was to provide cochromatographic evidence on the identity of the resmethrin metabolites.

In the second experiment, one-third of the 0–144-hr composite urine sample was treated by addition of sodium acetate to obtain 0.1 *M* sodium acetate, pH adjustment to 5.0 with concentrated HCl, extraction with ether (4 × 3 vol), and then analysis of the ether extract as before. After evaporation of residual ether from the aqueous phase, one-half of the aqueous portion (3–7 ml) was treated with glusulase (50,000 Fishman Units of β-glucuronidase and 5000 Whitehead Units of aryl sulfatase; Calbiochem, San Diego, Calif.) while the other half was not; then both portions were incubated at 37° for 12 hr followed by extraction with ether (4 × 3 vol) and tlc examination of the ether-soluble products. The remaining aqueous fraction was then adjusted to pH 2 and extracted with ether–ethanol (2:1) (2 × 2 vol), aliquots from both phases were subjected to lsc, and the ether–ethanol soluble products were examined by tlc.

The 0–24-hr feces in the first experiment were homogenized in methanol (25 ml/6 g of feces) with a Virtis Homogenizer (Virtis Research Equipment, Gardner, N. Y.) for 2 min and then filtered through a Büchner funnel. The insoluble portion was reextracted with methanol (25 ml) in the same manner and the combined filtrate was con-

Table II. Radiocarbon in the Urine and Feces of Rats after Oral Administration of ^{14}C-Labeled Acid and ^{14}C-Labeled Alcohol Preparations of Each of (+)-*trans*- and (+)-*cis*-Resmethrin at 0.79–1.32 mg/kg

Resmethrin isomer and position of labeling	% of administered radiocarbon								
	Urine			Feces			Total		
	Expt I	Expt II	Av	Expt I	Expt II	Av	Expt I	Expt II	Av
	One Day after Administration								
Trans acid	45	28	36	20	28	24	65	56	60
Cis acid	22	13	17	26	36	31	48	49	48
Trans alcohol	22	14	18	11	8	9	33	22	27
Cis alcohol	15	7	11	22	44	33	37	51	44
	Six Days after Administration								
Trans acid	50	31	41	30	33	32	80	64	73
Cis acid	25	17	21	43	54	49	68	71	70
Trans alcohol	40	35	38	16	14	15	56	49	53
Cis alcohol	23	11	17	40	63	52	63	74	69

centrated to dryness (rotary evaporator) and the residue extracted twice with ether for tlc examination of the organosoluble ^{14}C components. The insoluble portion was checked for additional radioactive components by maceration in 5 vol of water, addition of NaOH to a weighed aliquot, and heating of this sample in 1 *N* NaOH at 100° for 1.5 hr prior to lsc of the aqueous portion. The daily feces samples in the second experiment were homogenized in methanol (25 ml/g of feces) and filtered, and then the residue was held 18 hr at 25° with a second portion of methanol prior to filtration. This procedure is better than that used in experiment I for extraction of the fecal radioactivity. One-third of the composite 0–144-hr sample from each rat was evaporated to dryness and sodium acetate–acetic acid buffer (pH 4.5, 15 ml) was added; then the pH was adjusted to 5.0 with concentrated NaOH solution and the mixture was extracted with ether (4 × 3 vol). This ether extract and the aqueous portion were analyzed by the same procedure used for urine analysis, including incubation with glusulase. The insoluble portion from the feces was then treated as before with NaOH for extraction of additional ^{14}C-labeled components.

High Doses of Resmethrin and Chrysanthemic Acid. Unlabeled (+)-*trans*-resmethrin, (−)-*cis*-[^{14}C]resmethrin, and (+)-*cis*-[^{14}C]CA were administered twice daily by the intraperitoneal (ip) route to each of two rats using 200 μl of methoxy triglycol (Union Carbide Corp., New York, N. Y.) and 350 and 250 μl of Me_2SO as the administration vehicles, respectively. The cumulative amounts of each chemical administered to the two rats were 6.0 g for (+)-*trans*-resmethrin, 4.3 g for (−)-*cis*-resmethrin, and 2.4 g for (+)-*cis*-CA; the number of doses per rat was 6, 6, and 5, respectively. The daily urine samples [(+)-*trans*-resmethrin, each sample diluted with water to 20 ml] and the 6- and 7-day composite urine samples [(−)-*cis*-resmethrin and (+)-*cis*-CA, respectively] were adjusted to pH 2 with HCl and extracted with ether (2 vol × 4) and the combined ether extract was dried (Na_2SO_4) and concentrated for analysis by tlc or glc or both. The feces were also examined in two cases, using the ether-extractable fraction from (+)-*trans*-resmethrin administration for analysis similar to the procedure used in experiment II with low doses of [^{14}C]resmethrin and using combustion for determination of total fecal radiocarbon with (−)-*cis*-resmethrin.

Identification of Metabolites. In experiments involving low doses of [^{14}C]resmethrin, radioactive components in the ether and ether–ethanol extracts of urine and feces were tentatively identified by tlc cochromatography. The extracts were evaporated to dryness and the residual material was dissolved in acetone for transferral to tlc plates as narrow bands. The chromatoplates were developed twice with solvent system B, a procedure usually providing good separation of the metabolites. When the separation was not satisfactory, the gel from regions of the unresolved metabolites was extracted with acetone and the tlc separation was repeated. Each resolved metabolite, recovered by acetone extraction of the appropriate gel region, was treated with diazomethane for 10 min at 25° (for carboxylic acids) or for 18 hr at 5° (for phenols). Two-dimensional tlc with appropriate solvent systems was used for tentative identification of components in the esterified fractions and lsc served to quantitate the amounts of metabolites in gel portions or their extracts.

When high doses of resmethrin and CA were administered, the urinary metabolites were isolated as described later for examination by tlc, glc, glc–mass spectrometry, nmr, and optical rotation.

Tissue Analysis. Tissues dried over anhydrous $CaCl_2$ in a vacuum desiccator were ground to a fine powder with a mortar and pestle; then aliquots were combusted with a Packard Tri-Carb sample oxidizer for lsc with the Packard Tri-Carb liquid scintillation spectrometer Model 3375. The heparinized blood was diluted with an equal volume of water and an aliquot was analyzed as above. Fat samples were digested in methanolic 1 *N* KOH plus 10% chloroform and then an aliquot was subjected to lsc. The tissue residues are given as parts per million resmethrin equivalents relative to fresh tissue weights.

Bioassays. The mouse ip LD_{50} values were determined according to Ueda *et al.* (1974a).

RESULTS

Low Doses of [^{14}C]Resmethrin. *Rate of Excretion and Tissue Residues.* The radioactivity from orally administered [^{14}C]resmethrin at a dose of about 1 mg/kg is slowly eliminated from rats, only 53–73% being accounted for in the urine and feces after 6 days (Table II). The slowest and least extensive elimination occurs with alcohol-labeled *trans*-resmethrin. Radioactivity in urine is the same as or greater than that in feces with acid- and alcohol-labeled (+)-*trans*-resmethrin, while feces has a larger amount of radioactivity than urine following administration of (+)-*cis*-resmethrin. No detectable $^{14}CO_2$ is expired by rats given acid-labeled (+)-*trans*- and (+)-*cis*-resmethrin and none is expected from the phenyl-labeled compounds.

The tissue ^{14}C levels 6 days after treatment are highest with alcohol-labeled (+)-*trans*-resmethrin (Table III) which also gives the lowest excretion of radioactivity from the animal (Table II). The acid-labeled resmethrin preparations yield much lower ^{14}C levels than the alcohol-la-

Table III. Radiocarbon in Various Tissues of Rats Six Days after Oral Administration of ^{14}C-Labeled Acid and ^{14}C-Labeled Alcohol Preparations of Each of (+)-*trans*- and (+)-*cis*-Resmethrin at 0.79–1.32 mg/kg

	^{14}C-Labeled alcohol – resmethrin, ppm of resmethrin equiv[a]			
	Trans		Cis	
Tissue	Expt I	Expt II	Expt I	Expt II
Blood	0.34	0.58	0.03[b]	0.06
Brain	<0.01	0.01	0.01	<0.01
Fat	1.56[c]		0.51[c]	
Heart	0.08	0.11	0.02	0.02
Kidney	0.11	0.21	0.03	0.03
Liver	0.09	0.14	0.06	0.05
Lung	0.08	0.61	0.02	0.06
Muscle	0.06	0.08	0.01	0.01
Pancreas		0.12		0.01
Spleen	0.04	0.06	<0.01	0.01
Testis		0.10		0.01

[a] The results with ^{14}C-labeled acid–resmethrin are <0.01 ppm of resmethrin equivalents in every case except as noted below. [b] ^{14}C-Labeled acid–resmethrin equivalents = 0.02 ppm. [c] ^{14}C-Labeled acid–resmethrin equivalents = 0.25 ppm.

beled preparations. Tissues with the highest radiocarbon content are fat and blood, whereas the brain retains almost no resmethrin-derived compounds.

Nature of Excreted Metabolites. The distribution of radioactivity in experiment II among various fractions of urine and feces is dependent both on the position of labeling and the isomer involved (Table IV). The urinary and fecal metabolites are only partially recovered on extraction from an aqueous solution into ether. Tlc examination with solvent system B of components in the remaining aqueous phase shows that almost all of the radioactivity remains at the origin. However, when the aqueous phase is incubated at pH 5, in buffer with or without the addition of glusulase, many components are recovered on extraction with ether and most of these organosoluble materials released on incubation move free from the origin on tlc. These results are interpreted as indicating that the urine but not the feces contain almost equal parts of unconjugated metabolites (recovered in the initial ether extract) and conjugated metabolites (recovered on ether extraction after incubation). They further indicate that the conjugates are unstable at pH 5 since in most cases they appear to be cleaved as rapidly by incubation in pH 5 buffer alone as they are in the buffer containing glusulase. An additional portion of the metabolites is recovered on acidification of the aqueous phase to pH 2 and extraction with ether–ethanol. Metabolites in the ether–ethanol extract are not converted to ether-extractable compounds by evaporation of the organic solvents, addition of a pH 5 buffer containing glusulase, and further incubation. The polar products in the ether–ethanol extracts are in particularly large amount with alcohol-labeled (+)-*trans*-resmethrin where they account for more than half of the total urinary and fecal radiocarbon. Thus, it appears that the excreta contain unconjugated metabolites, glucuronide and/or sulfate conjugates which are mostly in the urine, and polar metabolites which are not glucuronides or sulfates. No attempt was made to define the nature of the conjugating moiety involved in these metabolites. The unconjugated metabolites and those conjugates cleaved on incubation with glusulase were subjected to tlc analyses as described below.

Most of the identified metabolites found in the original ether extract of urine are also found in ether extracts prepared after glusulase or buffer treatment (Table V). Comparison of the metabolites detected from alcohol- and acid-labeled resmethrin samples using tlc solvent systems B, D, and G indicates that the administered compound and ester metabolites are not excreted in the urine and feces of rats given (+)-*trans*-resmethrin but two ester metabolites are detected in the feces following (+)-*cis*-resmethrin administration. The less polar ester metabolite of (+)-*cis*-resmethrin (7% of the administered radiocarbon), after treatment with diazomethane, gives a spot at slightly higher tlc position than BFA-*cE*-CDAMe on development with solvent system F (R_f 0.60 and 0.57, respective-

Table IV. Fractionation of Radiocarbon in the Urine and Feces of Rats Six Days after Oral Administration of ^{14}C-Labeled Acid and ^{14}C-Labeled Alcohol Preparations of Each of (+)-*trans*- and (+)-*cis*-Resmethrin by Solvent Partitioning before and after Incubation with Glusulase[a]

	% of administered radiocarbon				
		Ether-extracted aqueous phase after incubation with glusulase			
Resmethrin isomer and position of labeling	Initial ether extract	Ether extract	Ether–ethanol extract	Aqueous phase	Total for all fractions
			Urine		
Trans acid	14.6	10.9 (11.8)[b]	1.6 (2.8)	4.0 (1.6)	31.1
Cis acid	7.1	6.1 (4.6)	2.9 (4.9)	0.9 (0.3)	17.0
Trans alcohol	5.6	6.0 (2.8)	22.1 (26.6)	1.0 (0.0)	34.7
Cis alcohol	3.4	3.1 (2.2)	2.9 (4.7)	1.7 (0.7)	11.0
			Feces Extract		
Trans acid	18.0	0.9 (0.6)	9.9 (10.8)	1.2 (0.6)	30.0[c]
Cis acid	38.8	2.0 (2.0)	8.7 (10.7)	1.5 (0.0)	51.0[c]
Trans alcohol	4.8	0.4 (0.9)	7.5 (6.6)	0.3 (0.8)	13.0[c]
Cis alcohol	29.1	2.2 (1.1)	17.9 (19.6)	7.8 (6.2)	57.0[c]

[a] Experiment II involving a resmethrin dose of 0.79–0.96 mg/kg. [b] Numbers in parentheses refer to comparable incubations of the ether-extracted aqueous phase without glusulase addition. [c] Additional radiocarbon released on alkali digestion of the extracted feces is 3, 3, 1, and 6%, respectively, of the administered amount with the trans acid, cis acid, trans alcohol, and cis alcohol preparations.

Table V. Identified Metabolites in the Urine and Feces of Rats Six Days after Oral Administration of ^{14}C-Labeled Acid and ^{14}C-Labeled Alcohol Preparations of Each of (+)-*trans*- and (+)-*cis*-Resmethrin[a]

	% of administered radiocarbon					
	Trans			Cis		
Metabolite[b]	Urine	Feces	Total	Urine	Feces	Total
			Alcohol-Derived Metabolites			
BFCA	2.5 (0.8)[c]		3.3	0.4 (0.4)		0.8
4′-OH-BFCA	1.1 (1.3)		2.4	(0.2)		0.2
α-OH-BFCA	1.1		1.0	(0.3)		0.3
Total	4.6 (2.1)		6.7	0.4 (0.9)		1.3
			Acid-Derived Metabolites			
t-CA	1.5 (0.6)	2.0	4.1			
c-CA				1.0 (1.2)	4.3	6.5
CHA[d]	1.7 (1.5)	1.3	4.5	0.9		0.9
cE-CHA	1.9 (1.6)	0.4	3.9	1.6 (1.4)	3.9	6.9
tE-CDA	5.3 (2.3)	6.3	13.9	0.4 (0.2)		0.6
cE-CDA	2.9	4.7	7.6	0.9 (0.7)	10.5	12.1
cZ-CDA				0.4		0.4
Total	13.3 (6.0)	14.7	34.0	5.2 (3.5)	18.7	27.4

[a] Experiment II involving a resmethrin dose of 0.79–0.96 mg/kg. [b] Metabolites are tentatively identified by methylation with diazomethane and then two-dimensional tlc cochromatography with the corresponding authentic unlabeled compound, with the exception of CA where methylation was not involved. The tlc solvent systems used were as follows: BFCA, C, D, and F; 4′-OH-BFCA, A and D; α-OH-BFCA, A and B; CA isomers, A, B, C, and D; CHA isomers, A, B, C, and D; CDA isomers, C, D, and E. [c] Values in parentheses are metabolites released on incubation with glusulase. [d] Isomers other than *cE*-CHA probably consisting mostly of *tE*-CHA from (+)-*trans*-resmethrin and a mixture of *tE*-CHA and *cZ*-CHA from (+)-*cis*-resmethrin.

ly), and a spot at slightly lower tlc position on development with solvent system D (R_f 0.22 and 0.26, respectively). Since the authentic samples of the other three isomers of BFA-CDAMe consistently give the same or higher R_f values than the *cE* isomer under these conditions, this metabolite is not one of the BFA-CDAMe isomers. The other ester metabolite (2% of the administered radiocarbon), after diazomethane treatment, appears at a R_f position in solvent system F lower than 4′-MeO-BFA-*cE*-CDAMe (R_f 0.30 and 0.45, respectively). Attempts to identify these two ester metabolites were unsuccessful, in part because of their instability.

Three metabolites derived from the alcohol moieties of (+)-*trans*- and (+)-*cis*-resmethrin are identified by tlc cochromatography and quantitated as shown in Table V. Alcohol-labeled resmethrin gives urinary metabolites of BFCA, 4′-OH-BFCA, and α-OH-BFCA from the (+)-trans isomer but only BFCA from the (+)-cis isomer in the original ether extracts; however, glusulase treatment of the (+)-*cis*-resmethrin urinary metabolites releases small amounts of these three alcohol-derived products (Table V) as does incubation with buffer only. The major identified urinary metabolite from the alcohol moiety of both resmethrin isomers is BFCA. BFA and α-keto-BFCA are not detected in urine or feces after administration of the ^{14}C-labeled alcohol-resmethrin isomers. In addition, the feces does not contain any BFCA, 4′-OH-BFCA, or α-OH-BFCA. Only a portion of the alcohol-derived metabolites is identified, each resmethrin isomer giving several metabolites other than those indicated above in both the urine and feces. The failure to identify a larger portion of these metabolites is attributed, in part, to their instability under the isolation conditions used (Ueda *et al.*, 1974b).

Several metabolites are derived from the acid moieties of the resmethrin isomers (Table V). A small amount of the corresponding CA isomer is excreted in the urine and feces in each case. Isomers of CHA, formed on hydroxylation of a methyl group in the isobutenyl side chain of CA before or after cleavage of the ester bond, appear in quite large amounts, only the *cE* isomer being separately analyzed in this investigation. It is interesting to note that a significant level of *cE*-CHA is formed from (+)-*trans*-resmethrin as well as from (+)-*cis*-resmethrin. The CAA isomers are not found as excreted metabolites of (+)-*trans*- or (+)-*cis*-resmethrin in these tracer studies; however, as noted below, *tE*-CAA appears as a (+)-*trans*-resmethrin metabolite in studies with high ip doses of the unlabeled compound. The most detailed information is available on the isomeric relationships of the terminal oxidation products, the CDA isomers, since all four of these isomers are separable by methylation and tlc (Table I). Two CDA isomers are found as metabolites of (+)-*trans*-resmethrin and three as metabolites of (+)-*cis*-resmethrin. The major CDA metabolites are the *tE* isomer from (+)-*trans*-resmethrin and the *cE* isomer from (+)-*cis*-resmethrin. *cE*-CDA is the only CDA isomer present in the feces following (+)-*cis*-resmethrin administration and this isomer appears in very large amounts in the feces relative to the urine in contrast to the CDA isomers recovered from other sources. Although the trans methyl group is predominantly oxidized with each resmethrin isomer, the cis methyl group of (+)-*cis*-resmethrin is also oxidized so *cZ*-CDA is found as a metabolite. The most unanticipated finding was that of isomerized compounds at the cyclopropane moiety, as indicated by *cE*-CHA from (+)-*trans*-resmethrin and confirmed by the detection of *cE*-CDA from (+)-*trans*-resmethrin and *tE*-CDA from (+)-*cis*-resmethrin. As shown in Table VI, the degree of isomerization in formation of the excreted CHA and CDA isomers is much greater starting with (+)-*trans*- than with (+)-*cis*-resmethrin; also, the isomerization is far in excess of any isomeric contaminant present in the original resmethrin samples used for treatment of the rats. The acid-derived metabolites of (+)-*trans*-resmethrin include two ether-extractable unknowns (1.5% of the administered radiocarbon), neither of which is epoxy-*trans*-CA or the corresponding diols.

High Doses of Resmethrin and Chrysanthemic Acid. The aforementioned ^{14}C investigations provide tlc evidence for tentative identification of many resmethrin metabolites including some formed by hydrolysis, oxidation,

Table VI. Amounts of Unisomerized and Isomerized Hydroxylated Chrysanthemic Acid and Chrysanthemumdicarboxylic Acid Excreted by Rats Administered (+)-*trans*-, (+)-*cis*-, and (−)-*cis*-Resmethrin and (+)-*cis*-Chrysanthemic Acid

Compound administered[a]	% administered dose recovered as indicated metabolites[b]			
	Unisomerized		Isomerized	
	CHA	CDA	CHA	CDA
(+)-*trans*-Resmethrin, 0.79 mg/kg oral, single dose, urine and feces	4.5[c]	13.9	3.9	7.6
(+)-*cis*-Resmethrin, 0.79 mg/kg oral, single dose, urine and feces	6.9	12.5	0.9[c]	0.6
(−)-*cis*-Resmethrin, 2110 mg/kg ip, 6 doses, urine only[d]	0.008	0.2	0.005	0.05
(+)-*cis*-Chrysanthemic acid, 1410 mg/kg ip, 5 doses, urine only		3.9		0.7

[a] The per cent of the other geometrical isomer present as a contaminant in the administered compound was less than 0.5% for (+)-*trans*-, (+)-*cis*-, and (−)-*cis*-resmethrin and no *trans*-CA was detected in the (+)-*cis*-CA. [b] Values based on radiocarbon content with (+)-[^{14}C]resmethrin, radiocarbon content and glc with (−)-*cis*-[^{14}C]resmethrin, and weight of isolated product with (+)-*cis*-CA administration. The excreta samples analyzed were from 6 days collection except with (+)-*cis*-CA where samples for 7 days were used. [c] This is a maximal value since the indicated CHA consisted of one or more of *tE*-, *tZ*-, and *cZ*-CHA which were not individually analyzed. [d] The low recovery values result primarily from the small amount of urinary metabolites in this study.

and isomerization of the acid moiety. This latter sequence of reactions is sufficiently unexpected so that other types of chromatographic evidence and nmr, mass spectral, and optical rotation data were sought to confirm the tentative identifications. Three compounds were selected to administer at high dose in unlabeled form or with a low ^{14}C specific activity for the following reasons: (+)-*trans*-resmethrin because it is the major insecticidal component of resmethrin; (−)-*cis*-resmethrin because the corresponding (+) isomer is relatively toxic to rats and *in vitro* investigations (Ueda *et al.*, 1974b) indicated a greater specificity for oxidation of the cis methyl group of the isobutenyl moiety with (−)-*cis*-resmethrin than with the other resmethrin isomers; (+)-*cis*-CA in order to obtain greater amounts of the CDA metabolites than recovered following resmethrin administration for more detailed characterization of their isomeric relationships.

Unlabeled (+)-trans-Resmethrin. Resmethrin administered ip at 2940 mg/kg twice daily for a total of six doses yields no detectable parent compound (glc, SE-30, 245°) in the first and second day urine samples but resmethrin is detected in the third through fifth day urine samples (tlc, solvent system B) and in the sixth and seventh day urine samples (glc). The feces samples from days 6 and 7 after (+)-*trans*-resmethrin administration contain three and six times larger total amounts of resmethrin, respectively, than the corresponding urine samples based on glc analyses. Three compounds appearing in the ether extracts from each daily urine sample 1-7 days after resmethrin administration are identified by methylation and glc (OV-17) as follows: *tE*-CDAMe and BFCAMe as major components; α-OH-BFCAMe as a minor component.

The composite ether extract of 3-7-day urine was subjected to preparative tlc (1.0 mm gel thickness, solvent system B). Gel bands detected by uv visualization corresponding in R_f values to authentic samples of resmethrin, BFCA, *tE*-CDA, and α-OH-BFCA were recovered for spectral analysis as were certain of the other bands. Resmethrin recovered from near the solvent front in solvent system B was repurified with solvent system D, giving 8 mg of material with the same nmr spectrum as an authentic sample. Each tlc band higher than those for the CDA isomers was examined by methylation and glc (SE-30, 255°) seeking any one of the four BFA-CDAMe isomers since, if present, they would appear in this tlc region; none were found. Each metabolite region was recovered by ether or methanol extraction of the gel. The components were then esterified with diazomethane and examined by glc. The compounds identified were those previously noted in the ether extracts of urine, *i.e.*, BFCAMe, α-OH-BFCAMe, and *tE*-CDAMe; compounds not detected were BFA, α-keto-BFCAMe, any of the CHAMe isomers, and CDAMe isomers other than *tE*-CDAMe (see Table I for the chromatographic characteristics of the standard compounds).

The band corresponding to BFCA gave a white crystalline material which showed substantially the same nmr spectrum in CCl_4 solution as that of authentic BFCA. Treatment of this metabolite with diazomethane and further purification by preparative tlc (solvent system D) gave 15 mg of BFCAMe with a nmr spectrum identical with that of an authentic standard. The metabolite band corresponding to α-OH-BFCA was esterified with diazomethane and repurified as above to yield a product identical with authentic α-OH-BFCAMe based on glc (OV-17) and glc-mass spectra (Dexsil): mass spectra *m/e* (rel intensity, first for authentic compound and then for metabolite) 261 (5-4) (M + 29), 234 (9-9), 233 (64-53) (M + 1), 232 (5-9), 216 (12-11), 215 (78-58), 201 (11-12), 156 (8-9), 155 (100-100), 127 (7-9), 119 (6-7), 117 (7-7), 107 (17-19), 105 (11-33). A similar work-up and isolation procedure yielded 12 mg of pale yellow oil with the same nmr spectrum as an authentic sample of *tE*-CDAMe: nmr (CCl_4) δ 1.25 (3 H, s), 1.29 (3 H, s), 1.65 (1 H, d, J = 4.5 Hz), 1.93 (3 H, d, J = 0.5 Hz), 2.13 (1 H, d of d, J = 10 Hz, 4.5 decoupled to a doublet by irradiation at δ 4.39), 3.64 (3 H, s), 3.66 (3 H, s) and 4.39 ppm (1 H, d, J = 10 Hz).

Another metabolite from the gel region between BFCA and *tE*-CDA was isolated by methylation and repurification as above. Analyses by glc (OV-17) indicated that the methylated metabolite was *tE*-CAAMe and that this material was present in about 2% of the amount of *tE*-CDAMe. This compound chromatographs on tlc (solvent system D, two developments) identical with authentic *tE*-CAAMe but not with *cE*-CAAMe (see Table I). The aldehyde character of the metabolite was verified by detecting an orange spot at the appropriate R_f on spraying the developed chromatoplate with 2,4-dinitrophenylhydrazine-H_2SO_4 solution. The structure of *tE*-CAAMe was confirmed by glc-mass spectrometry (Dexsil) in comparison with an authentic sample: mass spectra *m/e* (rel intensity, first for authentic compound and then for metabolite) 225 (5-5) (M + 29), 197 (22-18) (M + 1), 179 (6-6), 165 (27-26), 147 (5-5), 139 (17-17), 138 (14-13), 137 (100-100), 136 (27-18), 121 (8-9), 109 (54-55), 108 (18-14).

(−)-cis-[^{14}C]Resmethrin. Only a small amount of the administered radioactivity was excreted, 3.0 and 3.2% appearing in the urine and 13.0 and 13.7% in the feces after 6 and 8 days, respectively. Despite the slow excretion rate, the 6-day urine provided a convenient source of metabolites from the acid moiety for use in structure determination. Relative to the administered dose, this urine sample contained the following components: 1.5% of the radioactivity appears in the ether and 1.5% in the aqueous phase

on extraction of the urine with ether; no resmethrin is present based on tlc (solvent system B) and radioassay; 0.25% of the radioactivity consists of CDA isomers; 0.013% CHA isomers and 0.027% CA are detected by glc (OV-17) (with correction for change in molecular weight). Preparative tlc (0.5 mm gel thickness, solvent system B) with monitoring by glc (methylated derivatives, OV-17) allowed separation of the fractions containing CA, CHA, and CDA.

The CA fraction was methylated with diazomethane, yielding an ester identical with authentic CAMe in glc characteristics (Table I) and glc–mass spectra (OV-17): mass spectra *m/e* (rel intensity, first for authentic compound and then for methylated metabolite) 183 (22–22) (M + 1), 182 (6–8), 181 (8–8), 152 (4–4), 151 (33–37) (M − 31) (M − OCH_3), 127 (10–16), 124 (10–9), 123 (100–100) (M − 59) (M − $COOCH_3$), 122 (3–5), 121 (12–15), 115 (10–13), 109 (8–8), 107 (5–5). The trans and cis isomers of CAMe are not differentiated by the glc or glc–mass spectral conditions used but they are separated on the capillary column at 152°, yielding R_t values of 34 and 33 min, respectively. Analysis with the capillary column revealed that the methylated metabolite was >98% *c*-CAMe.

The CHA fraction consisted of a mixture of isomers that are difficult to separate. Accordingly, authentic samples of all four isomers of CHAMe were prepared especially for this phase of the study. The authentic CHAMe isomers give glc R_t values (OV-17, 150°) as follows: *cZ*-CHAMe 4.1, *tZ*-CHAMe 4.3, *cE*-CHAMe 4.8, and *tE*-CHAMe 5.3 min. The methylated CHA metabolite fraction gave identical peaks. Glc–mass spectroscopy (OV-17) of the methylated metabolites confirmed the identification of three products as *cZ*-, *cE*-, and *tE*-CHAMe but did not firmly establish that the shoulder on the *cZ*-CHAMe peak was due to *tZ*-CHAMe because of the poor separation. The mass spectral data are as follows: *m/e* (rel intensity, first for authentic compound and then for methylated metabolite) *cZ*-CHAMe, 182 (5–2), 181 (48–23) (M − 17) (M − OH), 167 (11–7), 149 (11–8), 139 (14–11), 125 (12–11), 122 (11–10), 121 (100–100) (M − 77) (M − H_2O − $COOCH_3$), 115 (5–6), 107 (6–8); *cE*-CHAMe, 181 (52–26), 167 (8–10), 149 (10–10), 139 (12–14), 122 (10–10), 121 (100–100), 115 (3–6), 107 (4–8); *tE*-CHAMe, 181 (34–24), 167 (9–9), 149 (10–9), 139 (11–9), 122 (11–11), 121 (100–100), 115 (3–4), 109 (4–6), 107 (4–7). The *cZ*, *cE*, *tZ*, and *tE* isomers of CHAMe are separated on a capillary column at 135°, giving R_t values of 141, 153, 156, and 179 min, respectively. The CHAMe metabolite fraction, purified by preparative glc, gave a CHAMe isomer ratio of 10:3:2:6 for *cZ*:*cE*:*tZ*:*tE* when analyzed on the capillary column.

The CDA isomer fraction was further purified by esterification with diazomethane and then tlc (solvent system C) yielding isomerically pure (glc monitoring, OV-17, Table I) *cZ*-CDAMe and *tE*-CDAMe, but the *cE*-CDAMe portion would also contain unresolved *tZ*-CDAMe, if present. The isomer ratio of *cZ*-, *cE*-, *tZ*-, and *tE*-CDA was found to be 3:1:<0.1:1 based on radioactivity and glc analysis of their methyl esters. The structures of *cZ*-, *cE*-, and *tE*-CDAMe were verified by glc–mass spectroscopy (OV-17). The mass spectral data alone, in the absence of glc data, are not sufficient for identification of the four isomers since they give similar fragmentation patterns. Their mass spectral data are as follows: *m/e* (rel intensity, first for authentic compound and then for methylated metabolite) *cZ*-CDAMe, 255 (3–3) (M + 29), 227 (4–3) (M + 1), 225 (5–5), 196 (12–11), 195 (100–100) (M − 31) (M − OCH_3), 167 (21–19), 163 (25–20), 143 (14–12), 135 (28–22), 125 (12–9), 113 (7–5), 112 (47–35), 107 (21–16); *cE*-CDAMe, 255 (5–11), 227 (4–3), 196 (13–13), 195 (100–100), 167 (17–16), 164 (4–4), 163 (35–32), 135 (30–20), 125 (19–<3), 112 (17–12), 107 (21–13); *tE*-CDAMe, 255 (18–18), 228 (14–15), 227 (97–100), 196 (11–9), 195 (100–76), 167 (41–26), 164 (11–8), 163 (92–69), 135 (43–9), 125 (34–19), 112 (20–11), 107 (35–21); *tZ*-CDAMe, 255 (3–5), 227 (2–12), 225 (4–9), 196 (15–11), 195 (100–100), 194 (4–8), 167 (16–50), 163 (32–<3), 143 (12–<3), 136 (3–<3), 135 (24–<3), 125 (11–<3), 112 (19–<3), 107 (13–53). The last compound tabulated, *tZ*-CDAMe, shows a relatively poor agreement between the metabolite and the authentic compound.

(+)-*cis*-[^{14}C]*Chrysanthemic Acid.* Isomerization of the acid moiety was detected in the CHA and CDA metabolites derived from (+)-*trans*- and (+)-*cis*-resmethrin administered at tracer levels and from (−)-*cis*-[^{14}C]resmethrin administered at high levels but not with unlabeled (+)-*trans*-resmethrin given in large amounts. These studies did not yield sufficient CDA metabolites for determination of their optical rotation, a measurement essential in assigning the carbon at which epimerization occurs. Accordingly, (+)-*cis*-CA was administered in large amounts to rats for isolation and optical rotation measurements of the CDA isomers formed. The 0–7-day cumulative urine sample contained 55% of the administered radioactivity. Analysis of the ether-extractable fraction of the urine by tlc (solvent system B prior to and E after methylation) revealed at least two isomers of CDA, *cE*-CDA as the major and *tE*-CDA as a minor component. These CDA isomers were isolated by subjecting the residue (600 mg) from evaporation of the ether extract to column chromatography as described before and then preparative tlc (1 mm gel thickness, solvent system B). A portion of the CDA isomer fraction was esterified with diazomethane and analyzed by glc (OV-17) yielding peaks corresponding to *cE*-CDAMe and *tE*-CDAMe with an area ratio of 5:1. Extensive examination of the methylated CDA material (tlc, glc, glc–mass spectroscopy) failed to reveal any *cZ*-CDAMe or *tZ*-CDAMe.

Samples of *cE*-CDA (63 mg) and *tE*-CDA (12 mg) formed metabolically were isolated by preparative tlc (0.5 mm gel thickness, solvent system B). The cis isomer, *cE*-CDA, crystallized and the trans isomer, *tE*-CDA, became partially crystalline after several days at 5°. The identities of the two compounds were confirmed by nmr. Optical rotation measurements of *cE*-CDA and *tE*-CDA showed the values of $[\alpha]^{22}D$ +12.8 and +52.1°, respectively, in methanol; the reported value for (+)-*tE*-CDA is $[\alpha]^{17}D$ +72.8° (CH_3OH) (Staudinger and Ruzicka, 1924). Both metabolites were further purified by esterification with diazomethane and preparative tlc (0.5 mm gel thickness, solvent system D). Neither of these samples showed any impurities on glc (OV-17). Their properties were as follows: (+)-*cE*-CDAMe, nmr (CCl_4) δ 1.27 (3 H, s), 1.31 (3 H, s), 1.65–2.0 (2 H, m), 1.88 (3 H, d, J = 0.7 Hz), 3.63 (3 H, s), 3.70 (3 H, s), and 6.94 ppm (1 H, d, J = 8 Hz), the methylated metabolite being identical with an authentic sample; $[\alpha]^{22}D$ +18.5° (C_2H_5OH, *c* 2.59); (+)-*tE*-CDAMe, nmr spectrum for the methylated metabolite same as that described above; $[\alpha]^{22}D$ +90.8° (C_2H_5OH, *c* 0.413), which can be compared with an authentic sample which showed $[\alpha]^{22}D$ +105.4° (C_2H_5OH, *c* 2.21). The observed optical rotation value for *tE*-CDAMe establishes that the *tE*-CDA metabolite was predominantly (93%) the (+) isomer, *i.e.* the epimerization occurred at C-3 of the cyclopropane ring. The yield of isomerized CDA, although relatively low (Table VI), was well above that attributable to any impurities in the administered (+)-*cis*-CA.

Toxicity of Metabolites. The reported mouse ip LD_{50} values (milligrams/kilogram) for compounds of interest here are >1500 for (+)-*trans*-resmethrin, 320 for (+)-*cis*-resmethrin, 98 for (+)-*trans*-CA, and 600 for (+)-*cis*-CA (Abernathy *et al.*, 1973; Jao and Casida, 1975; Ueda *et al.*, 1974a). Other resmethrin metabolites were found to have the following LD_{50} values: 408 for (+)-*tE*-CDA, 75 for BFA, and 46 for BFCA. The carrier vehicle for administering the BFCA was a 4:1 ratio of methoxy triglycol and Me_2SO.

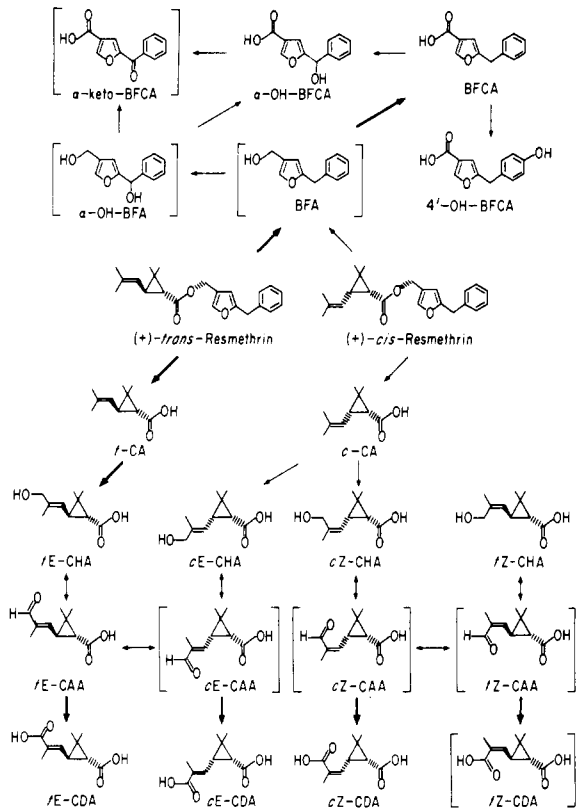

Figure 1. Partial metabolic pathways for the acid and alcohol moieties of (+)-*trans*- and (+)-*cis*-resmethrin based on the metabolites excreted by rats treated orally. A comparable series of reactions appears to take place for the acid moiety derived from (−)-*cis*-resmethrin. The pathways illustrated involve ester hydrolysis and then oxidation of the liberated fragments. An alternative possibility, *i.e.* oxidation of the ester followed by hydrolysis, is known to be more important with (+)-*cis*- than with (+)-*trans*-resmethrin.

DISCUSSION

No unmetabolized resmethrin is excreted by rats orally administered low levels of either the (+)-trans or (+)-cis isomer but some unmetabolized compound appears in the excreta following high level ip treatments, that portion in the urine probably resulting from contamination of the urine by feces during collection of the excreta. There are large differences between (+)-*trans*- and (+)-*cis*-resmethrin in the excretion patterns. The acid and alcohol moieties from (+)-*trans*-resmethrin appear to a greater extent in urine than in feces whereas those from (+)-*cis*-resmethrin appear predominantly in the feces. This suggests that different types of metabolites are formed from (+)-*cis*-resmethrin than from (+)-*trans*-resmethrin, at least for a portion of the dose. In a previous study (Miyamoto *et al.*, 1971), oral administration of [^{14}C]furan-(±)-*trans*-resmethrin at 500 mg/kg yielded more radioactivity in feces than in urine, this variance in the major elimination route as compared with the current studies possibly resulting from the large differences in the administered dose. The metabolites most slowly excreted and persisting the longest in the body arise from the alcohol moiety of (+)-*trans*-resmethrin. Although the mechanism for preferential retention in the liver of products derived from the alcohol moiety is not defined, it may be related to a phenomenon observed with mouse liver microsomes under *in vitro* conditions, *i.e.*, the alcohol but not the acid fragment of the resmethrin isomers is bound to particulate material, probably protein, when the incubations are made in the presence but not in the absence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Ueda *et al.*, 1974b).

Thirteen metabolites formed by cleavage of the ester group and usually oxidation of the liberated alcohol or acid moiety are identified from analyses of the excreta of rats receiving low or high doses of the resmethrin isomers. The metabolic pathways shown in Figure 1 are based on these identified metabolites.

Ester metabolites are not detected in the excreta following (+)-*trans*-resmethrin administration at low levels but the feces of rats treated with (+)-*cis*-resmethrin contain significant amounts of two unidentified and relatively unstable ester metabolites. It appears likely that metabolism of the major portion of the (+)-*trans*-resmethrin dose involves hydrolysis and then oxidation of the liberated fragments, as proposed before for the alcohol moiety of (±)-*trans*-resmethrin (Miyamoto *et al.*, 1971). However, (+)-*cis*-resmethrin may undergo initial metabolic attack by either oxidative or hydrolytic mechanisms so a portion of the dose is excreted as ester metabolites. This speculation is supported by two findings from *in vitro* studies: (+)-*cis*-resmethrin undergoes relatively slow enzymatic hydrolysis (Abernathy *et al.*, 1973; Casida *et al.*, 1975); it gives major ester metabolites on incubation with the mouse liver microsome–NADPH system (Ueda *et al.*, 1974b).

The pathways for metabolism of the alcohol moiety of (+)-*trans*- and (+)-*cis*-resmethrin are similar to those proposed for (±)-*trans*-resmethrin (Miyamoto *et al.*, 1971). Two postulated intermediates, BFA and α-OH-BFA, are included since they appear as metabolites of (+)-*trans*-resmethrin in the microsome–NADPH system (Ueda *et al.*, 1974b). Although not found in the present study, α-keto-BFCA is known to be a metabolite of (±)-*trans*-resmethrin (Miyamoto *et al.*, 1971).

Metabolism of the chrysanthemate moiety derived from the resmethrin isomers involves both expected and unexpected features. With (+)-*trans*-resmethrin only the methyl group of the isobutenyl side chain trans (*E*) to the cyclopropane undergoes oxidation, confirming previous studies on allethrin and pyrethrin I in this respect (Elliott *et al.*, 1972). However, both the geometrical and optical configurations of the chrysanthemate moiety influence the preferred site of oxidation. Thus, (+)-*cis*-resmethrin is also oxidized at the isobutenyl methyl group cis (*Z*) to the cyclopropane. Further, (−)-*cis*-resmethrin undergoes preferential oxidation at the cis (*Z*) relative to the trans (*E*) methyl group. Another novel finding is that resmethrin yields isomerized CHA and CDA in urine and feces when the (+)-trans isomer is administered at low levels and in urine when the (+)-cis isomer is given; at high doses, (−)-*cis*-resmethrin and (+)-*cis*-CA also give isomerized metabolites. Quantitative studies establish that the isomerized metabolites are not derived from isomeric contaminants in the resmethrin samples used. The epimerization occurs predominantly at the cyclopropane carbon (C-3) bearing the oxidized isobutenyl side chain as indicated by the appropriate optical rotation measurements. It appears possible that the isomerized metabolites are formed by abstraction of the hydrogen cation or radical at cyclopropane C-3 to form the corresponding carbanion or radical which undergoes isomerization. There are two observations that point to the aldehyde (CAA) as the most likely compound for isomerization in the metabolic sequence: CAA undergoes facile cis–trans isomerization under strong alkaline conditions (Ueda *et al.*, 1974b); the isomerized metabolites observed do not include CA but only ones oxidized at a methyl group of the isobutenyl side chain, *i.e.* CHA and CDA, which might form on reduction and oxidation, respectively, of the isomerized CAA.

Verschoyle and Barnes (1972) suggested that although (+)-*trans*-resmethrin has a low toxicity following intravenous administration to rats it may be converted to a toxic metabolite since there is an appreciable delay in the poisoning signs even when a lethal dose is injected. Their in-

terpretation may be correct since some of the resmethrin metabolites [(+)-*trans*-CA, BFA, and BFCA] are much more toxic than resmethrin. However, it is obvious that these metabolites do not accumulate to toxic levels except when exceedingly high doses of (+)-*trans*-resmethrin are administered.

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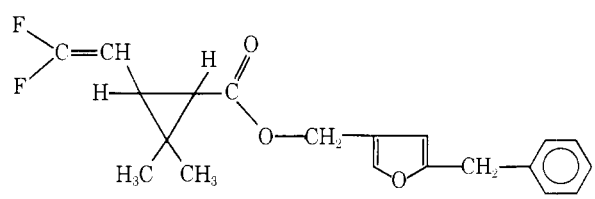
Halopyrethroids. II. A Difluoropyrethroid

Dale G. Brown, Otelia F. Bodenstein, and Scott J. Norton*

The fluorine-containing pyrethroid, (±)-*trans*-(5-benzyl-3-furyl)methyl 3-(2,2-difluorovinyl)-2,2-dimethylcyclopropanecarboxylate, has been synthesized and its insecticidal potency studied in DDT-susceptible house flies, yellow-fever mosquitoes, and chlordane resistant German cockroaches. This pyrethroid, called fluorethrin, is superior to both bromethrin and resmethrin in insecticidal quality. Additional insect toxicity studies of bromethrin are also included.

Studies in this and other laboratories have involved the synthesis of pyrethroids having increased insecticidal toxicity. In an earlier paper we reported the synthesis of a potent pyrethroid, bromethrin, and preliminary toxicity data were given (Brown *et al.*, 1973). This pyrethroid, which has also been synthesized and reported by others (Elliott *et al.*, 1973), resulted from the replacement of the isobutenyl methyl groups of chrysanthemic acid with bromine atoms followed by esterification of this acid moiety with (5-benzyl-3-furyl)methyl alcohol. Comparative toxicity studies of this compound were made with the known pyrethroid of analogous structure, resmethrin (Elliott, 1967). Due to the previous successes with both bromine and chlorine replacements of the isobutenyl methyl groups of the chrysanthemic acid moiety (Elliott *et al.*, 1973; Farkas *et al.*, 1958), we wished to investigate the insecticidal activity of analogous pyrethroids having fluorine atom replacements. We wish to report the synthesis and certain toxicity data for the difluoro analog (±)-*trans*-(5-benzyl-3-furyl)methyl 3-(2,2-difluorovinyl)-2,2-dimethylcyclopropanecarboxylate (I), which we call fluorethrin. Additionally, we wish to report more extensive insect toxicity data for the previously reported pyrethroid, bromethrin.



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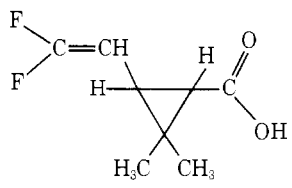

II

The acid moiety (II) was synthesized starting from commercial ethyl chrysanthemate as the starting material. This material, after initial ester hydrolysis and isolation of

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