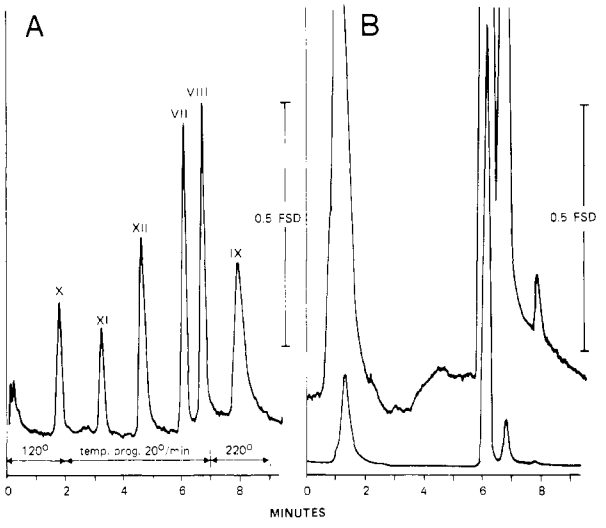

Figure 2. Gas chromatograms of the TFA derivatives of Mesurol and its metabolites and a derivatized extract of field-treated blueberries: (A) Mesurol phenol TFA (X) 3 ng, Mesurol sulfoxide phenol TFA (XI) 8 ng, Mesurol sulfone phenol TFA (XII) 6 ng, Mesurol TFA (VII) 5 ng, Mesurol sulfoxide di-TFA (VIII) 10 ng, and Mesurol sulfone TFA (IX) 12 ng; (B) acetone extract of field-treated blueberries after partition and derivatization.

removal of the solvent was derivatized with trifluoroacetic anhydride. A GC chromatogram of the derivatized crude extract is shown in Figure 2B. It indicates the presence of Mesurol, Mesurol sulfoxide, and a small amount of sulfone at an estimated level of 5, 0.9, and 0.03 ppm, respectively. It is interesting to note the absence of phenol metabolites in the sample.

Trifluoroacetylation, together with the use of a selective sulfur GC detector, thus enables the determination of Mesurol and its metabolites to be carried out with virtually crude extracts of blueberries. This eliminates the oxidation, hydrolysis, and column cleanup steps present in the reported methods for the analysis of Mesurol. The trifluoroacetyl derivatives can also be detected by GC–electron capture detector, although cleanup may be required for the extracts in this case.

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Radiosynthesis and Metabolism in Rats of the 1*R* Isomers of the Insecticide Permethrin

Michael Elliott,* Norman F. Janes, David A. Pulman, Loretta C. Gaughan, Tadaaki Unai, and John E. Casida

Permethrin [3-phenoxybenzyl (±)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] is more stable in air and light than previous pyrethroids, and therefore has a greater potential for controlling a wide range of insect pests. Metabolism of the [1*R*,*trans*]- and [1*R*,*cis*]-esters, the active isomers of permethrin, following oral administration to rats at about 1 mg/kg, was examined using compounds labeled with ^{14}C in the acid or alcohol moieties. These were synthesized either from [1*R*,*trans*]- or [1*R*,*cis*]-acid labeled in the side chain [$Cl_2{}^{14}C{=}CH-$] or from alcohol labeled at α-CH_2 or in the phenoxy substituent. The [1*R*,*trans*]- and [1*R*,*cis*]-esters are readily metabolized by ester cleavage, by hydroxylation of the geminal dimethyl group in the acid, or the phenoxy group of the alcohol, and by conjugation of the resulting carboxylic acids and phenols. The metabolites are quickly excreted and do not persist significantly in tissues.

Although natural and synthetic pyrethroids are excellent insecticides with low mammalian toxicity (Elliott, 1976), most are too unstable in air and light to protect agricultural crops effectively. Thus, in chrysanthemates such as pyrethrin I (Ia, Figure 1) and *S*-bioallethrin (Ib) the isobutenyl side chain is a site for photosensitized oxidative attack (Chen and Casida, 1969) and in bioresmethrin (IIa) a second sensitive center is the furan ring (Ueda et al., 1974). However, recent work (Elliott et al., 1973a) has shown that the photolabile groups in pyrethroids can be replaced by others giving much greater stability, and equal or increased insecticidal activity. Table I indicates the insecticidal potency of some important pyrethroids, their

Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Herts., England AL5 2JQ (M.E., N.F.J., D.A.P.), and the Division of Entomology and Parasitology, University of California, Berkeley, California 94720 (L.C.G., T.U., J.E.C.).

Table I. Insecticidal Activity, Mammalian Toxicity, and Photostability of Some Pyrethroids

Compound	Formula	LD_{50}, mg/kg: Mustard beetles[a]	LD_{50}, mg/kg: Rats[b]	Photostability,[c] days
Pyrethrin I	Ia	0.3	260-420	<1
Bioresmethrin	IIa	0.5	>8 000	<1
Dichloro analogue of bioresmethrin	IIb	0.2	>400[d]	<1
Biophenothrin	IIIa	0.7	>10 000	6
Biopermethrin	IIIb	0.2	>2 000[d]	30

[a] *Phaedon cochleariae* Fab. By topical application of measured drops in acetone. Data from Elliott et al. (1974a). [b] By oral administration to males. Data from Elliott et al. (1972), Verschoyle and Barnes (1972, 1974), and Miyamoto et al. (1974). [c] For thin films on glass exposed to indoor daylight. Data from Elliott et al. (1973b). [d] For (±)-*trans*-ester.

toxicity to rats, and their photostability, and shows that biopermethrin (IIIb), in which the photosensitive centers have been replaced, is relatively stable in light and retains high insecticidal activity. Both the (±)-trans isomer (Table I) and the (±)-cis isomer (Verschoyle and Barnes, 1974) have low oral toxicities to rats and their selectivity between insects and mammals is very favorable. Permethrin (IIIc) thus appears very promising for controlling a wide range of insects. In connection with this potential widespread practical use, it was therefore important to investigate its metabolism in mammals.

Knowledge of the pathways by which natural and synthetic pyrethroids are metabolized in mammals has developed rapidly in the past 5 years (Abernathy et al., 1973; Casida, 1973; Casida et al., 1976; Elliott et al., 1972; Miyamoto et al., 1971, 1974; Suzuki and Miyamoto, 1974; Ueda et al., 1975a,b) but only compounds with 3-isobutenyl or 3-(2-methoxycarbonylpropenyl) substituents (chrysanthemates and pyrethrates) have been studied in detail. Permethrin (IIIc) is closely related to phenothrin (IIIa, all stereoisomers) (Fujimoto et al., 1973), the compounds differing only in the side chain (X = Cl or CH_3 in formula III). When [1*R*,*trans*]-phenothrin (IIIa) is administered orally to male rats at 200 mg/kg, 3-phenoxybenzyl alcohol is rapidly liberated and oxidized, 4'-hydroxy-3-phenoxybenzoic acid and smaller amounts of 3-phenoxybenzoic acid and 3-phenoxybenzoylglycine being excreted (Miyamoto et al., 1974). (The system used here to designate stereochemistry is discussed by Elliott et al., 1974b.) When incubated with liver enzyme preparations of several mammalian species, [1*R*,*trans*]- and [1*S*,*trans*]-phenothrin are hydrolyzed much more rapidly than the [1*R*,*cis*] and [1*S*,*cis*] isomers (Abernathy et al., 1973; Casida et al., 1976; Miyamoto et al., 1974). The [1*R*,trans] but not the [1*R*,cis] isomer of permethrin is also rapidly hydrolyzed when incubated with preparations of mouse liver microsomes and both isomers are rapidly oxidized when this system is fortified with reduced nicotinamide adenine dinucleotide phosphate (Casida et al., 1976).

In this paper, the syntheses of [1*R*,*trans*]- and [1*R*,*cis*]-permethrin labeled in acidic and alcoholic components with ^{14}C (IVa–c and Va–c) are described. These compounds are used in a preliminary examination of the metabolism of IVa, IVb, Va, Vb, and some of their hydrolysis products in rats which had been dosed orally at 0.5–2.9 mg/kg.

MATERIALS AND METHODS

General Procedures. Nuclear magnetic resonance

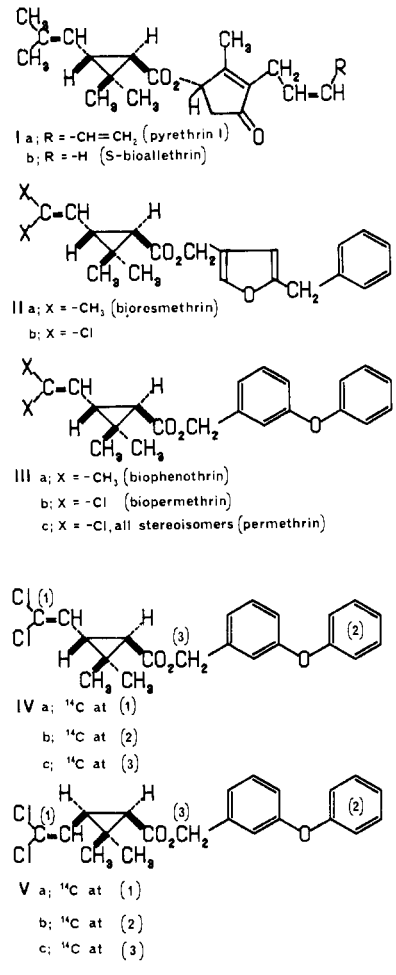

Figure 1. Structures of various pyrethroids (I-III) and positions of ^{14}C labeling for [1*R*,*trans*]- and [1*R*,*cis*]-permethrin (IV and V).

(NMR) spectra were determined for dilute solutions in carbon tetrachloride using tetramethylsilane as internal standard, on a Perkin-Elmer R10 spectrometer at 60 MHz. For thin-layer chromatography (TLC), silica gel 60 F-254 chromatoplates (0.25 mm gel thickness) were developed with the following solvent systems as indicated: solvent system A, 1-butanol–acetic acid–water (6:1:1); solvent system B, benzene saturated with formic acid–ether (10:3).

Unlabeled Compounds. The following compounds were used as standards for thin-layer cochromatography: 3-phenoxybenzyl alcohol, 3-phenoxybenzoic acid, [1*R*,*trans*]- and [1*R*,*cis*]-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acids (referred to in this paper as the dichlorovinyl acids) (Burt et al., 1974), (±)-*trans*- and (±)-*cis*-3-(2,2-dichlorovinyl)-2-*cis*-hydroxymethyl-2-*trans*-methylcyclopropanecarboxylic acids and their derived lactones, (±)-*trans*- and (±)-*cis*-3-(2,2-dichlorovinyl)-2-*trans*-hydroxymethyl-2-*cis*-methylcyclopropanecarboxylic acids (Unai and Casida, 1975), 3-phenoxybenzoylglycine, and methyl 4'-methoxy-3-phenoxybenzoate (Miyamoto et al., 1974). Methyl esters were prepared with diazomethane.

(−)-Menthyl Esters for NMR Examination of Trans → Cis Epimerization. *Preparation.* (±)-*trans*-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (0.3 g) was treated with thionyl chloride (2 ml) for 1 h (20°C) and 10 min (50°C). Excess thionyl chloride was then evaporated and two successive portions of benzene (5 ml) were added and again evaporated to remove traces of thionyl chloride. (−)-Menthol (0.3 g) in benzene (10 ml)

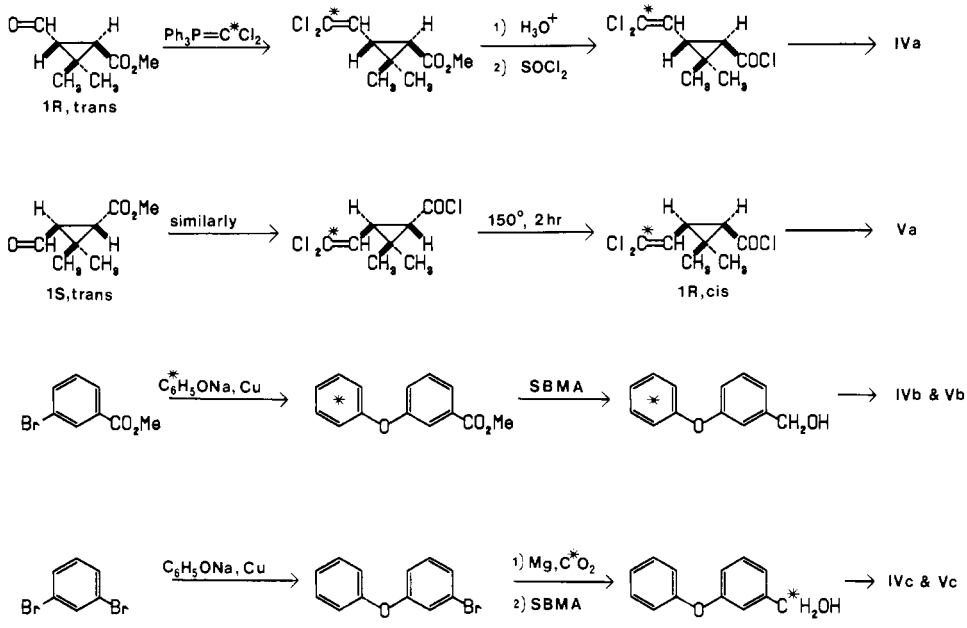

Figure 2. Reactions used to prepare [1*R*,*trans*]- and [1*R*,*cis*]-[^{14}C]permethrin.

and pyridine (0.12 ml) were then added. After 3 h at 0–20°C the mixture was added to alumina (ca. 3 g) and eluted with benzene. Evaporation of the benzene gave a semisolid residue of (–)-menthyl (±)-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate: NMR peaks at τ 4.43 and 4.44 (2 × d, each 8 Hz, =CH), 7.7–8.0 (2 × dd, 3-H), 8.63 (d, 5 Hz, 1-H), 8.77 and 8.81 (2 × s, CMe_2) from acidic constituent and at 5.0–5.6 (m, C*H*O), 8.0–9.3 (m, 18 protons) from menthyl residue. Similarly prepared were (–)-menthyl (±)-*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate [mp 69–70°C; NMR peaks at 3.73 and 3.81 (2 × d, each 8 Hz, =CH), 7.8–8.4 (m, 3-H and 1-H), 8.7–8.8 (m, CMe_2) from acidic constituent and menthyl peaks as above]; (–)-menthyl [1*R*,*cis*]-ester [mp 45–46°C; NMR peaks at 3.73 (d, 8 Hz, =CH), 7.8–8.4 (m, 3-H and 1-H), 8.71 (s, CMe_2), and menthyl peaks as above]; (–)-menthyl [1*S*,*cis*]-ester [mp 94–95°C; NMR peaks at 3.81 (d, 8 Hz, =CH), 7.8–8.4 (m, 3-H and 1-H), 8.75 and 8.80 (2 × s, CMe_2), and menthyl peaks as above].

Epimerization. By a procedure similar to that used for the (±)-*trans*-dichlorovinyl acid, [1*S*,*trans*]-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (0.3 g) was converted to the acid chloride with thionyl chloride (2 ml). This acid chloride was then heated at 150°C for 2 h; after cooling, the NMR spectrum indicated that ca. 20% cis isomer was present. The chloride was dissolved in benzene, (–)-menthol (0.25 g) and pyridine (0.2 ml) were added, and the ester (0.46 g, semicrystalline) was purified by chromatography on alumina. The NMR spectrum (see Results section) showed by comparison with authentic samples (above) that [1*R*,*cis*]- was present and [1*S*,*cis*]-ester was not present within the limits of detection (± 10%).

Labeled Compounds. These were detected on chromatoplates by radioautography with x-ray film at 10°C and characterized by cochromatography with authentic unlabeled standards detected by uv visualization and/or phosphomolybdic acid reagent (Ueda et al., 1974). The radioactive products revealed by such radioautography were often complex mixtures. However, by scraping and extracting appropriate zones with acetone or methanol, products were obtained that appeared pure when examined by radioautography following cochromatography in solvent systems A and B and others referred to later in this paper and elsewhere (Unai and Casida, 1975).

The glucuronide esters of the [1*R*,*trans*]-[^{14}C]- and [1*R*,*cis*]-[^{14}C]dichlorovinyl acids and of 3-[^{14}C]phenoxybenzoic acid and the ether glucuronide of 3-[^{14}C]phenoxybenzyl alcohol were obtained enzymatically by incubating the appropriate [^{14}C]aglucone (see below) and uridine 5'-diphosphoglucuronic acid with rat liver microsomes (Mehendale and Dorough, 1971).

[1*R*,*trans*]-3-(2,2-Dichloro[2-^{14}C]vinyl)-2,2-dimethylcyclopropanecarboxylic Acid and Its 3-Phenoxybenzyl Ester (IVa) (Figure 2). Carbon tetrachloride ($^{14}CCl_4$; 90 mg; 6.4 mCi/mmol) was transferred in benzene (1.3 ml) to a Pyrex tube containing triphenylphosphine (302 mg) and methyl-(+)-*trans*-caronaldehyde (Elliott et al., 1974b) (185 mg). After cooling to –180°C (liquid N_2), the tube was sealed in vacuo and heated for 46 h at 80°C (oil bath). The reaction mixture was then transferred to a round-bottomed flask with methylene chloride, concentrated (N_2 stream), and heated with acetic acid (6 ml), hydrobromic acid (48%, 4 ml), and water (2 ml) for 4 h. After adding water (50 ml) the product was extracted into methylene chloride (4 × 5 ml), taken into sodium hydroxide (10%; 4 × 5 ml), washed with methylene chloride (5 ml), and then acidified with concentrated hydrochloric acid (6 ml). The acid liberated was extracted with methylene chloride (2 × 5 ml) and then again (2 × 5 ml) after adding more hydrochloric acid (4 ml). The combined organic layers were evaporated (N_2 stream, then in vacuo) after washing with saturated sodium chloride and drying (Na_2SO_4) to give dichlorovinyl acid (60 mg) (NMR spectrum closely similar to that of unlabeled acid (Elliott et al., 1974b)). This ^{14}C-labeled acid was 88% pure, estimated by TLC (two developments with solvent system B (R_f 0.64) and elution from the gel with acetone; no cis isomer (R_f 0.70) was detected in the reaction mixture). Portions (0.1 mg) of this ^{14}C-labeled acid were purified as needed for investigating metabolism.

The 3-phenoxybenzyl ester (IVa) was prepared by adding thionyl chloride (5.2 μl) and pyridine (5.8 μl) in benzene (1 ml) to the acid product from the Wittig reaction above (15 mg; 88% by radioautography). After 2 h 3-phenoxybenzyl alcohol (17.3 mg) and pyridine (10 μl) in benzene (1 ml) were added; then 10 h later the reaction

mixture was eluted through alumina (ca. 1.0 g) with benzene (15 ml) to remove excess alcohol and pyridine. Evaporation of the solvent gave the crude ester (10.3 mg) which was stored in benzene and purified in 0.4-mg portions on a TLC plate developed in carbon tetrachloride–ether–hexane (8:1:1). The pure ester was stored in acetone.

[1*R*,*cis*]-3-(2,2-Dichloro[2-^{14}C]vinyl)-2,2-dimethylcyclopropanecarboxylic Acid and Its 3-Phenoxybenzyl Ester (Va) (Figure 2). Methyl (−)-*trans*-caronaldehyde was prepared from methyl (−)-*trans*-chrysanthemate by an ozonolysis procedure similar to that used for the (+)-ester (Elliott et al., 1974b). $^{14}CCl_4$ (41.5 mg, 3.7 mCi/mmol) diluted with unlabeled carbon tetrachloride (31 μl, 49.5 mg), triphenylphosphine (302 mg), and methyl (−)-*trans*-caronaldehyde (185 mg) gave crude acidic product (84.1 mg) by a procedure similar to that used for methyl (+)-*trans*-caronaldehyde. This product (10 mg) was transferred in benzene (100 μl) to the bottom of a Pyrex tube (23 cm × 3 mm) and purified thionyl chloride (Fieser and Fieser, 1967) (100 μl) was added. The tube was lightly stoppered and heated at 100°C (water bath) for 50 min. After cooling, benzene and excess thionyl chloride were evaporated by gentle suction (water pump) with agitation; then the tube was sealed in vacuo and heated (total immersion) in an oil bath at 140–150°C for 2 h to achieve epimerization. 3-Phenoxybenzyl alcohol (20 μl) and pyridine (10 μl) in benzene (70 μl) were added and, after 10 h, the reaction mixture was applied as bands to four TLC plates which were developed twice in benzene–carbon tetrachloride (1:1). The areas detected by radioautography containing [1*R*,*cis*]-ester (R_f 0.63) (18% of applied ^{14}C, 23% of recovered ^{14}C) and [1*S*,*trans*]-ester (R_f 0.54) (59% of applied ^{14}C, 77% of recovered ^{14}C) were each eluted with acetone. This procedure was repeated with further aliquots of the original acid to give adequate [1*R*,*cis*]-ester for metabolic studies. These samples were finally purified by rechromatography, and then cochromatographic comparison with [C_6H_5O-U-^{14}C]permethrin (below) and unlabeled esters showed the product (Va) to be radiochemically pure and identical with authentic ester.

3-[C_6H_5O-U-^{14}C]Phenoxybenzyl Alcohol and Its [1*R*,*trans*]- and [1*R*,*cis*]-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanecarboxylates (IVb,Vb) (Figure 2). Methyl 3-[C_6H_5O-U-^{14}C]phenoxybenzoate was prepared by adding [C_6H_5O-U-^{14}C]phenol (ca. 7 mg; 4.6 mCi/mmol) in ether (8 ml) to a mixture of methyl *m*-bromobenzoate (70 mg) and sodium hydride dispersion in oil (80%; 3.7 mg). When the initial reaction had subsided, the ether was evaporated (N_2 stream), copper powder (50 mg) was added, and the reaction mixture was heated (open flame) for 10 min. After cooling, the product was extracted with methylene chloride (2 ml), applied to a TLC plate (2 mm), and developed twice in benzene–petroleum ether, bp 40–60°C (1:2), to separate the required methyl 3-phenoxybenzoate, located by cochromatography with an authentic sample (R_f 0.12). The ester was obtained by extracting the appropriate region with ether, evaporating the solvent, and drying in vacuo. This radioactive ester (5.2 mg) was treated with sodium bis(2-methoxyethoxy)aluminium hydride (SBMA) (23% solution in benzene; 0.5 ml). After 2 h, dilute hydrochloric acid (0.5 ml) was added and the benzene layer was separated, washed with saturated sodium chloride, dried (Na_2SO_4), and evaporated, finally at 0.01 mm (2 h), to give the 3-[^{14}C]phenoxybenzyl alcohol (4.0 mg).

The [1*R*,*trans*]- and [1*R*,*cis*]-esters (IVb and Vb) were prepared by first treating the respective acids (1.3 mg) with thionyl chloride (0.71 μl) and pyridine (1.0 μl) in benzene (10 μl) for 5 min. More pyridine (3.0 μl) was then added followed, after 30 min, by 3-[C_6H_5O-U-^{14}C]phenoxybenzyl alcohol (1.0 mg) in benzene (125 μl). After 15 h the reaction mixture was applied (15 cm band) to a TLC plate which was developed twice in benzene–carbon tetrachloride (1:1) mixture. The [1*R*,*trans*]-ester (R_f 0.63; 28% yield) and the [1*R*,*cis*]-ester (R_f 0.70; 21% yield) were obtained by extracting appropriate bands with acetone. The identities of the products were confirmed by cochromatography; the remaining radioactivity was recovered as unreacted 3-[C_6H_5O-U-^{14}C]phenoxybenzyl alcohol.

3-Phenoxy[α-^{14}C]benzyl Alcohol and Its [1*R*,*trans*]- and [1*R*,*cis*]-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanecarboxylates (IVc and Vc) (Figure 2). *m*-Dibromobenzene (from *m*-bromoaniline; Hartwell, 1955) was converted to *m*-bromodiphenyl ether by reaction with sodium phenolate (Gilman and Marrs, 1960). Dry ether (2 ml) was added to magnesium (35.2 mg), *m*-bromodiphenyl ether (1.0 g), and a crystal of iodine under N_2. The mixture was warmed and when reaction had started, additional ether (8 ml) was added and refluxing was continued until all the magnesium had been consumed (1 h). A portion (4 ml) of this Grignard solution was transferred to the aperture above the break seal of an ampoule containing $^{14}CO_2$ (8.0 mg; 58 mCi/mmol) which was then closed and cooled (liquid N_2) and the break seal was opened (magnetic hammer). After 16 h at room temperature, concentrated hydrochloric acid (1 ml) was added gradually, with agitation. The aqueous phase was removed, the ether solution was extracted with sodium hydroxide solution (10%; 3 × 1 ml), and both aqueous phases were acidified (concentrated hydrochloric acid). The product was extracted into methylene chloride and dried (Na_2SO_4) and the solvent was evaporated to give 3-phenoxy[α-^{14}C]benzoic acid (32 mg) which was then dissolved in benzene (1 ml).

A portion of the 3-phenoxy[α-$^{14}CO_2$H]benzoic acid solution (170 μl, about 5 mg of acid) was added to SBMA (23% solution in benzene, 150 μl). After 1 h dilute hydrochloric acid (10%; 0.5 ml) was added and the aqueous layer was removed. The benzene solution was washed with sodium carbonate solution (10%) and with saturated sodium chloride solution and then dried (Na_2SO_4). Evaporating the solvent gave a residue which was dried in vacuo over phosphoric anhydride and dissolved in benzene (1 ml).

3-Phenoxy[α-^{14}C]benzyl esters were prepared as follows. A portion (20 μl) of a solution prepared by diluting thionyl chloride (71.3 μl) and pyridine (100 μl) to 1 ml with benzene was added to [1*R*,*trans*]- or [1*R*,*cis*]-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (3 mg) in benzene (300 μl). After 5 min pyridine (10 μl) and a portion (300 μl) of the total solution (1 ml) of the reduction product from the 3-phenoxy[α-$^{14}CO_2$H]benzoic acid were added. The reaction mixture was evaporated (N_2 stream) after 16 h, then applied in hexane (5 portions) and ether (2 portions) to a TLC plate. The chromatogram was developed twice in the same direction with a hexane–ether (5:1) mixture and the required products were located (R_f 0.66 and 0.78 for [1*R*,*trans*]- and [1*R*,*cis*]-esters, respectively) by comparison with unlabeled ester and with ^{14}C-labeled (acid) ester (from $^{14}CCl_4$). The radiochemically pure products recovered by acetone extraction of the gel were obtained in 86 and 58% yields for the [1*R*,*trans*]- and [1*R*,*cis*]-esters, respectively. The remaining radioactivity was recovered as unreacted 3-phenoxy[α-^{14}C]benzyl alcohol (R_f 0.10).

Metabolism. The following ^{14}C-labeled compounds in dimethyl sulfoxide were administered orally to male albino Sprague-Dawley rats (160 to 180 g) at 0.5 to 2.9 mg/kg: acid-^{14}C and C_6H_5O-U-^{14}C preparations of [1*R*,*trans*]- and [1*R*,*cis*]-permethrin (IVa, IVb, Va, and Vb); [1*R*,*trans*]-[^{14}C]dichlorovinyl acid; and 3-[C_6H_5O-U-^{14}C]phenoxybenzyl alcohol. Urine, feces, and carbon dioxide were collected over 4 days for total radiocarbon assays; then the animals were sacrificed to determine by combustion the total radiocarbon levels in various tissues. The 0–1 day samples of urine and feces were used to identify tentatively and to estimate the ^{14}C-labeled metabolites by a combination of TLC, radioautography, and cochromatography with suitable authentic labeled and unlabeled standards and their derivatives.

The urine (40 to 100 μl) or a methanol extract of the feces (equivalent to 20 to 90 mg of feces) (Ueda et al., 1975a) was chromatographed (TLC) first with solvent system A, which displaced all ^{14}C products from the origin and resolved conjugates, then twice in the second direction with solvent system B, which separated unconjugated products. The patterns of metabolites from [*acid*-^{14}C]- and [*alcohol*-^{14}C]permethrin preparations were compared with each other and with metabolites from [1*R*,*trans*]-dichloro[^{14}C]vinyl acid and 3-[^{14}C]phenoxybenzyl alcohol to identify those metabolites retaining the ester linkage. To confirm structures, individual metabolites, recovered from chromatoplates by extracting appropriate regions with methanol, were cochromatographed in two dimensions with unlabeled or labeled standards, either directly or as derivatives, in solvent systems A and B or others. Derivatives were prepared by: methylation (diazomethane; Miyamoto et al., 1974; Ueda et al., 1975b); incubation with conjugate-cleaving enzymes (glusulase, cf. Ueda et al., 1975a; β-glucuronidase; aryl sulfatase) with or without saccharo-1,4-lactone (a β-glucuronidase inhibitor; Capel et al., 1974); acid hydrolysis (3 N hydrochloric acid, 100°C, 30 min); alkaline hydrolysis (10% methanolic sodium hydroxide, 25°C, 18 hr). In addition, the glucuronides and the dichlorovinyl-2-*cis*-hydroxymethyl-2-*trans*-methyl acids (see below) were partially or completely lactonized with hydrochloric acid at pH 2 for 5 min at 25°C or during TLC in solvent system A and subsequent rechromatography. Some lactonization also occurred with the *cis*-hydroxymethyl acids during TLC in solvent system B and rechromatography. Such lactonization was possibly induced by the high concentrations of acetic and formic acids when solvents were evaporated from the TLC plate.

RESULTS

Radiosynthesis. [1*R*,*trans*]- and [1*R*,*cis*]-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acids were previously isolated from the racemic mixture of ethyl esters by fractional distillation, followed by hydrolysis and optical resolution (Burt et al., 1974), but this route could not be conveniently adapted for radioactive intermediates. Therefore, for the present work, the dichlorovinyl side chain was attached to appropriate optically pure *methyl* caronaldehydes with $^{14}CCl_4$ in Wittig reactions (see Figure 2), a preliminary attempt to use *3-phenoxybenzyl* caronaldehyde to give the required esters directly having failed. Even under the best conditions (see Materials and Methods section), with an excess of all nonradioactive reagents, the reaction product from methyl [1*R*,*trans*]-caronaldehyde contained an impurity (the compound with a 3-$CHCl_2$ substituent). This was selectively destroyed by refluxing with hydrobromic acid in aqueous acetic acid. Despite this complication, the acid obtained gave an ester with 3-phenoxybenzyl alcohol (IVa) which had a specific activity of 6.4 mCi/mmol and was pure by chromatographic and NMR criteria.

To prepare the corresponding [1*R*,*cis*]-ester, an analogous procedure with methyl [1*R*,*cis*]-caronaldehyde was examined, but only products from cleavage of the cyclopropane ring were obtained under conditions appropriate for radiosynthesis. The required [1*R*,*cis*] system was therefore generated by thermally epimerizing a [1*S*,*trans*] intermediate (see Figure 2). Ozonolysis of methyl (–)-*trans*-chrysanthemate gave methyl [1*S*,*trans*]-caronaldehyde; the [1*S*,*trans*]-dichlorovinyl acid chloride derived from it was heated at 150°C for 2 h to give a 3:1 mixture of [1*S*,*trans*] and [1*R*,*cis*] compounds, and thence a mixture of esters which were separated by TLC. The [1*R*,*cis*]-3-phenoxybenzyl ester (Va) (1.7 mCi/mmol) was radiochemically pure by TLC criteria. The optical purity of the acid chloride intermediate, and therefore of the 3-phenoxybenzyl ester, was established from the NMR spectra of (–)-menthyl esters. The olefinic doublets of the (–)-menthyl esters from the (+)- and (–)-trans acids were barely resolved, but the corresponding doublets for esters from the (+)- and (–)-cis acids were well separated and were identified from authentic materials, the (+)-cis [=1*R*,cis] form resonating downfield at τ 3.73 (see Materials and Methods section). The NMR spectrum of the (–)-menthyl esters prepared from an acid chloride mixture obtained by thermal epimerization of the [1*S*,*trans*]-acid chloride showed only one doublet from cis isomers, that from the 1*R* form, as anticipated.

Radiolabeled 3-phenoxybenzyl alcohol was prepared by two methods (Figure 2), the first involving an Ullman reaction, and the second following the method of Miyamoto et al. (1974) and using the less expensive intermediate $^{14}CO_2$ at 58 mCi/mmol. After reducing to 3-phenoxybenzyl alcohol, esterification gave alcohol-labeled [1*R*,*trans*]- and [1*R*,*cis*]-esters (IVc and Vc) of high specific activity.

Metabolism of Permethrin Isomers in Rats. When administered orally to rats at 0.5 to 2.9 mg/kg, 76 to 95% of the radiocarbon from [1*R*,*trans*]-[*acid*-^{14}C]-, [1*R*,*trans*]-[*alcohol*-^{14}C]-, [1*R*,*cis*]-[*acid*-^{14}C]-, and [1*R*,*cis*]-[*alcohol*-^{14}C]permethrin, [1*R*,*trans*]-dichloro[^{14}C]vinyl acid, or 3-[^{14}C]phenoxybenzyl alcohol was recovered in the excreta after 4 days, most being eliminated during the first 24 h. Acid- and alcohol-labeled [1*R*,*cis*]-permethrin gave the lowest overall recoveries. An insignificant quantity (≤0.3%) of $^{14}CO_2$ was obtained from any of the administered compounds. Four days after treatment, the residues of permethrin and its metabolites in tissues, determined as the total radiocarbon by combustion, were below 0.01 ppm permethrin equivalents in blood, bone, brain, fat, heart, kidney, liver, lungs, muscle, spleen, and testes with all the ^{14}C-labeled preparations with the following exceptions: 0.12 to 0.40 ppm was found in the fat and 0.01 to 0.06 ppm in the kidney and liver with both the alcohol-^{14}C-labeled preparations; 0.02 to 0.03 ppm was found in the lungs with [1*R*,*trans*]- and [1*R*,*cis*]-[*alcohol*-^{14}C]permethrin, the blood, bone, and testes with [1*R*,*cis*]-[*alcohol*-^{14}C]permethrin, the liver with [1*R*,*trans*]-[*acid*-^{14}C]permethrin, and the fat with [1*R*,*cis*]-[*acid*-^{14}C]permethrin. These findings indicate that [1*R*,*trans*]- and [1*R*,*cis*]-permethrin, their hydrolysis products, and metabolites are excreted from the body in a short time and are not retained to an unusual extent in the tissues.

Identity of Excreted Metabolites. The use of alcohol-^{14}C- and acid-^{14}C-labeled preparations permitted direct recognition of metabolites retaining the ester linkage.

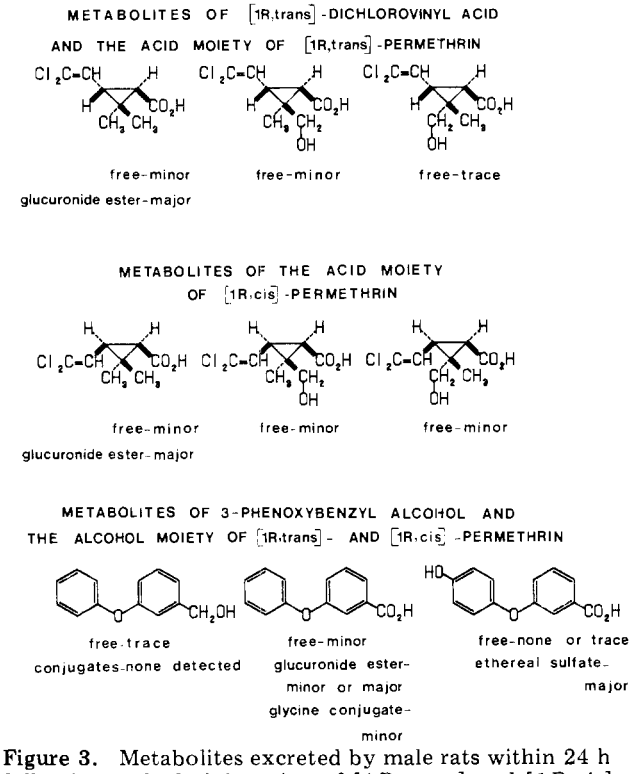

Figure 3. Metabolites excreted by male rats within 24 h following oral administration of [1*R*,*trans*]- and [1*R*,*cis*]-permethrin, [1*R*,*trans*]-dichlorovinyl acid, and 3-phenoxybenzyl alcohol. The amounts of ^{14}C-labeled metabolites are designated relative to the administered radiocarbon as follows: trace, < 2%; minor, 2–8%; major, 19–67%.

The ^{14}C-labeled metabolites excreted within 4 days after administering [1*R*,*trans*]-[*acid*-^{14}C]permethrin, [1*R*,*trans*]-[*alcohol*-^{14}C]permethrin, [1*R*,*trans*]-dichloro-[^{14}C]vinyl acid, and 3-[^{14}C]phenoxybenzyl alcohol were detected mainly in the urine (84 to 91% of excreted radiocarbon) rather than in the feces (9 to 16% of excreted radiocarbon). In contrast, the products from acid-^{14}C- and alcohol-^{14}C-labeled [1*R*,*cis*]-permethrin appeared to a greater extent in the feces than in the urine. This difference in metabolite distribution between urine and feces indicates that [1*R*,*trans*]-permethrin is more rapidly and completely converted to polar products than is [1*R*,*cis*]-permethrin, consistent with the finding by TLC cochromatography that 5% of the [1*R*,*cis*]-permethrin but only 2% of the [1*R*,*trans*]-permethrin dose was excreted unmetabolized in the feces. Furthermore, three unidentified ester metabolites appeared in the feces of the rats treated with [1*R*,*cis*]-[^{14}C]permethrin but ester metabolites were found in only trace amounts or were absent with [1*R*,*trans*]-[^{14}C]permethrin. 3-Phenoxybenzyl alcohol was identified by cochromatography as a metabolite of [1*R*,*trans*]-[*alcohol*-^{14}C]permethrin in the feces but not in the urine. None of the urinary metabolites of [1*R*,*trans*]- and [1*R*,*cis*]-[^{14}C]permethrin retained the ester linkage, and most of them were conjugates, found only in the urine and not in the feces.

Figure 3 shows the structures and relative proportions of the metabolites of [1*R*,*trans*]- and [1*R*,*cis*]-permethrin, of [1*R*,*trans*]-dichlorovinyl acid, and of 3-phenoxybenzyl alcohol tentatively identified by two-dimensional cochromatography in solvent systems A and B and by formation of derivatives.

The metabolites designated as free dichlorovinyl acids and their 2-*trans*- and 2-*cis*-hydroxymethyl derivatives were further characterized by methylation and cochromatography with appropriate unlabeled methyl esters and by converting the 2-*cis*-hydroxymethyl metabolites but not the 2-*trans*-hydroxymethyl metabolites to products of higher R_f values which cochromatographed with the unlabeled lactones. After methylation the ^{14}C-labeled metabolites designated as 3-phenoxybenzoic acid and 3-phenoxybenzoylglycine cochromatographed with the corresponding unlabeled methyl esters and the glycine conjugate was cleaved by acid, but not by alkaline digestion or by glusulase, to a ^{14}C-labeled product which cochromatographed with 3-phenoxybenzoic acid.

Chromatography indicated that one major metabolite from [1*R*,*trans*]- and [1*R*,*cis*]-[*alcohol*-^{14}C]permethrin was identical with that from 3-[^{14}C]phenoxybenzyl alcohol. This metabolite was tentatively identified as an ethereal sulfate conjugate because it was completely cleaved by acid, by alkali, or by aryl sulfatase but not by glucuronidase. The cleavage product, a carboxylic acid with R_f lower than that of 3-phenoxybenzoic acid in solvent systems A and B, was probably 4'-hydroxy-3-phenoxybenzoic acid, because methylation gave products tentatively identified as methyl 4'-hydroxy- and 4'-methoxy-3-phenoxybenzoates.

The remaining metabolites in urine were tentatively identified as glucuronide esters by two-dimensional chromatography in solvent systems A and B as follows: (a) when treated with acid, they were partly converted to products of higher R_f, assumed to be glucuronide lactones; (b) they were not changed by aryl sulfatase; (c) they were partially or completely cleaved by glusulase and completely cleaved by acid, alkali, or glucuronidase, the latter enzymatic reaction being inhibited by saccharo-1,4-lactone; (d) the aglucones released under each of these conditions were identified by two-dimensional cochromatography both before and after methylation. The glucuronides of the [1*R*,*trans*]- and [1*R*,*cis*]-dichlorovinyl acids and of 3-phenoxybenzoic acid were further identified by cochromatography in solvent system A with [^{14}C]ester glucuronide standards prepared enzymatically. The ether glucuronide of 3-phenoxybenzyl alcohol was not detected as a metabolite of the permethrin isomers or of 3-phenoxybenzyl alcohol itself.

DISCUSSION

The preliminary results with rats obtained in this study indicate that the organochlorine moiety of [1*R*,*trans*]- and [1*R*,*cis*]-permethrin and the [1*R*,*trans*]-dichlorovinyl acid is rapidly and almost completely eliminated from the body, and only traces remain in the fat and liver 4 days after oral administration. This ease of elimination is associated with the increased polarity of the products which results from rapid in vivo glucuronidation of the dichlorovinyl acids and, to a lesser extent, hydroxylation of one of the geminal dimethyl groups. It appears from preliminary experiments, not detailed here, that at least some of the hydroxylated acids undergo minor degrees of conjugation. Much less hydroxylated acid is formed from the [1*R*,*trans*]-dichlorovinyl acid itself than from [1*R*,*trans*]-permethrin indicating that permethrin is hydroxylated to some extent before hydrolysis. The predominant sites of hydroxylation in the dichlorovinyl acid appear to be the 2-cis position for [1*R*,*trans*]-permethrin and the 2-trans position for [1*R*,*cis*]-permethrin; presumably these methyl groups are sterically favored at the hydroxylation site of the microsomal oxidase system. Hydroxylation of the geminal dimethyl group has previously been noted in the metabolism of allethrin (Elliott et al., 1972) but the stereochemistry of the product was not determined.

Neither the parent isomer nor any metabolites of permethrin remained for an unusually long time or in an

unexpected location in the organs of the rats examined, the longest persistence being for products derived from 3-phenoxybenzyl alcohol in the fat, liver, and kidney. These findings are similar to those for 3-phenoxybenzyl alcohol from phenothrin (Miyamoto et al., 1974), except that in the present study the excreted metabolites are glucuronides and sulfates rather than the unconjugated benzoic acid derivatives observed with phenothrin. This difference may be related to the size of the administered dose, i.e. 0.5 to 2.9 mg/kg for permethrin and 200 mg/kg for phenothrin, or to variations in the processing and analytical procedures. Furthermore, the ratio of conjugated 4'-hydroxy-3-phenoxybenzoic acid to free and conjugated 3-phenoxybenzoic acid in the excreta is greater from [1*R*,*cis*]-permethrin than from either [1*R*,*trans*]-permethrin or 3-phenoxybenzyl alcohol. This suggests that hydroxylation of the phenoxy group in the 4' position is more important for detoxifying the less easily hydrolyzed permethrin isomer (i.e., 1*R*,cis) than for the more readily cleaved (i.e., 1*R*,trans) isomer or for 3-phenoxybenzyl alcohol. If there are other sites of hydroxylation in the phenoxybenzyl moiety (leading to some of the unidentified metabolites), they must constitute minor metabolic pathways compared with hydroxylation at the 4' position.

The use of chlorinated hydrocarbon insecticides such as aldrin, dieldrin, DDT, and the hexachlorocyclohexane isomers has been restricted, partly because they are only slowly metabolized in mammals and the original compounds or their metabolites are stored in fatty tissues to be eliminated over a long period. Like these insecticides, permethrin is highly lipophilic (Briggs et al., 1974) and contains chlorine. These features are important for the very high insecticidal activity of permethrin, but they do not appear to limit its biodegradability. Thus, the results discussed here indicate that metabolites are rapidly formed from permethrin by hydroxylation at methyl and phenoxy groups and by cleavage of the ester. The parent compound, these metabolites, their conjugates, or their further oxidation products are quickly eliminated from mammals.

A more detailed report on the permethrin metabolism studies at Berkeley will be published later.

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