J. Agric. Food Chem. 1980, 28, 250–255

Metabolism in Rats of the Potent Knockdown Pyrethroid Kadethrin

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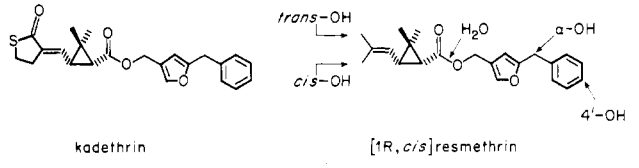
Kadethrin [5-benzyl-3-furylmethyl (1*R*,*cis*(*E*))-2,2-dimethyl-3-(2′-oxo-3′-thiocyclopentylidenemethyl)-1-cyclopropanecarboxylate] is almost completely metabolized and the metabolites excreted within a few days following oral administration to male rats at 0.8 mg/kg. Seventeen identified metabolites account for 79 and 32% of the dose of ^{14}C-acid- and ^{14}C-alcohol-labeled kadethrin, respectively. Excreted esters (14%) are kadethrin, its α-hydroxy and 4′-hydroxy derivatives, and the thiolcarboxylic acid from opening the thiolactone ring. Cleavage of the cyclopropanecarboxylate (>67%) and thiolactone (≥45%) esters probably results primarily from esterase and oxidase action, respectively. The liberated cyclopropanecarboxylic acid (25%) is excreted free and as conjugates with glucuronic acid and glycine. The thioldicarboxylic acid, from both cyclopropanecarboxylate and thiolactone cleavage, is either oxidized to the sulfonic acid (19%) or methylated and subsequently oxidized (23%), ultimately forming the methyl sulfone. The alcohol moiety yields 0.2–7% each of 5-benzyl-3-furylmethyl alcohol and 5-benzyl-3-furancarboxylic acid and its metabolites (glucuronide and glycine conjugates; α-hydroxy, α-keto, 4′-hydroxy, and 4′-sulfate derivatives).

Potent insecticides are obtained on esterifying 5-benzyl-3-furylmethyl alcohol with (1*R*,*trans*)-chrysanthemic acid (bioresmethrin; Elliott et al., 1967), (1*R*,*cis*)-chrysanthemic acid [(1*R*,*cis*)-resmethrin; Barlow et al., 1971], and a related (1*R*,*cis*) acid in which the isobutenyl group is replaced by a 2-oxo-3-thiocyclopentylidenemethyl substitutent (kadethrin; Martel and Buendia, 1974).



kadethrin [1R,*cis*]resmethrin

Kadethrin is exceptionally effective as a knockdown agent (Lhoste and Rauch, 1976; Roussel-Uclaf-Procida, 1976).

Metabolism of (1*R*,*cis*)-resmethrin in rats and/or rat and mouse microsomal enzyme systems involves not only hydrolysis but also hydroxylation at the methyl groups of the isobutenyl moiety and the 4′- and α-methylene positions of the alcohol moiety (Ueda et al., 1975a,b). With kadethrin, the thiolactone group provides possible sites for additional reactions, e.g., hydrolysis and oxidation at the sulfur with or without methylation. Kadethrin is much more toxic when administered intravenously or intraperitoneally than orally, i.e., acute LD_{50} values for male rats of 0.5, 18 and 1324 mg/kg, respectively, with poly(ethylene glycol) 200 as the carrier (Roussel-Uclaf-Procida, 1976). In mouse microsomal systems, kadethrin and (1*R*,*cis*)-resmethrin are oxidized >10-fold faster than they are hydrolyzed and kadethrin is oxidized 1.8-fold faster than (1*R*,*cis*)-resmethrin (Soderlund and Casida, 1977). Thus, the sulfur is important for knockdown activity and apparently also for rapid biodegradation.

The present investigation considers the metabolic fate of kadethrin and its acid moiety in orally administered rats.

MATERIALS AND METHODS

Chromatography and Radiocarbon Analyses. Thin-layer chromatography (TLC) utilized silica gel 60 F-254 chromatoplates (EM Laboratories, Inc., Elmsford, NY) with 0.25- and 0.5-mm gel thickness for analysis and preparative isolations, respectively. The solvent systems used and R_f values for kadethrin derivatives are given in Table I. In referring to solvent systems for two-dimensional development, AXB indicates development in the first direction with A and in the second with B. In general, the AXB system was used for esters including mono- and dicarboxylic acids after methylation and for apolar compounds, the CXD system for ester cleavage products, and the EXF system for polar metabolites. Unlabeled compounds for cochromatography were detected with UV visualization, phosphomolybdic acid reagent (Ueda et al., 1974), or $KMnO_4$ solution (0.5% w/v in water). Procedures for radioautography and liquid scintillation counting (LSC) were generally those indicated by Ueda et al. (1975b).

Chemicals. *Abbreviations.* Chemicals are referred to by abbreviations given in Figure 1. Kadethrin hydrolyzed at the thiolactone (SL) group gives SH,COOH-kad and hydroxylated in the alcohol moiety gives α-OH- and 4′-OH-kad. The acid moiety, SL-CA, yields SH,COOH-CA on hydrolysis of the thiolactone group. Oxidation of the latter compound gives SO_3H,COOH-CA, whereas methylation and then oxidation yields SO_2CH_3,COOH-CA as the terminal product. Benzylfurylmethyl alcohol (BFA) forms benzylfurancarboxylic acid (BFCA) on oxidation. Glucuronides are abbreviated as gluc. Isokad and SL-isoCA refer to isomerized kadethrin and SL-CA, i.e., the 1*RS*,*trans*(*E*) isomers formed primarily by photochemical processes (Ohsawa and Casida, 1979).

^{14}C Compounds. Roussel-Uclaf-Procida (Paris, France) provided kadethrin with ^{14}C labeling in the acid and alcohol moieties (Figure 1) and ^{14}C-SL-CA with a comparable labeling site. The specific activities were 54, 44, and 53 mCi/mmol, respectively, and the radiochemical purities were >98.5% after TLC cleanup (AXB for kadethrin and CXD for SL-CA).

Unlabeled Standards. Kadethrin, SL-CA, and SL-isoCA (1*RS* configuration) were provided by Roussel-Uclaf-Procida. Preparation of SH,COOH-kad and SH,COOH-CA was reported earlier (Ohsawa and Casida, 1979). BFA, 4′-OCH_3-BFA, BFCA, α-OH-BFCA, α-keto-BFCA, and 4′-OCH_3-BFCA were available from a previous study (Ueda et al., 1975a). Other compounds required were synthesized

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Table I. Thin-Layer Chromatographic Properties of Kadethrin and Its Metabolites and Derivatives

compound	R_f value with indicated solvent system[a]					
	A	B	C	D	E	F
			Esters			
kadethrin	*0.58*[b]	*0.45*	*0.81*	*0.67*		0.80
SH,COOH-kad	(*0.57*)[c]	(*0.67*)	*0.58*	*0.50*		0.75
α-OH-kad			0.50	0.48		
4'-OH-kad	(*0.50*)	(*0.35*)	0.65 (*0.74*)	0.57		0.81
			Acid Moiety and Related Compounds			
SL-CA	0.08 (*0.31*)	(*0.47*)	*0.42*	*0.45*	0.72	0.66
SL,CA-gluc					0.29	0.09
SL,CA-glycine			(*0.08*)	(*0.21*)	*0.53*	*0.17*
SL-isoCA[d]	0.11 (*0.36*)	*0.58*	*0.48*	*0.49*		0.79
SH,COOH-CA	0.02 (*0.46*)	(*0.63*)	*0.19*	*0.19*		0.66
SCH_3,COOH-CA	0.02 (0.48)		0.27	0.18		0.68
$SOCH_3$,COOH-CA	(0.03)		(*0.06*)	(*0.05*)	*0.58*	*0.41* (0.54)
SO_2CH_3,COOH-CA	(0.09)		(*0.29*)	(*0.25*)	0.61	0.47 (0.71)
SO_3H,COOH-CA			(0.66)	(0.57)	*0.36*	*0.19*
			Alcohol Moiety and Related Compounds			
BFA	*0.24*	*0.25*	*0.43*	*0.44*		0.75
α-OH-BFA			*0.09* (0.25)	*0.18* (0.44)		
4'-OH-BFA	(0.10)	(0.16)	(*0.34*)	(*0.32*)		
BFCA	0.18 (*0.68*)	(*0.85*)	*0.55* (0.75)	*0.43* (0.80)		0.65
BFCA-gluc					0.39	0.13
BFCA-glycine	(0.06)		0.04 (*0.21*)	(*0.45*)	*0.62*	*0.26*
α-OH-BFCA	0.04 (*0.30*)	(*0.35*)	*0.20* (0.46)	*0.24* (0.61)		0.55
α-keto-BFCA	0.04 (*0.44*)	(*0.57*)	*0.30* (0.67)	*0.31* (0.77)		0.31
4'-OH-BFCA	(*0.59*)	(*0.78*)	0.39	0.36		(0.80)
4'-OH-BFCA-sulfate					0.51	0.25

[a] TLC solvent systems are as follows: A = carbon tetrachloride-ether (3:1), developed twice; B = hexane-ether (2:1), developed twice; C = benzene saturated with formic acid-ether (10:3), developed twice; D = benzene-ethyl acetate-methanol (15:5:1); E = butanol-glacial acetic acid-water (6:1:1); F = ethyl acetate-methanol-water (13:3:1). [b] Italics designate combinations of compounds and solvent systems used for tentative product identification by cochromatography. [c] Parentheses designate compounds analyzed as methyl esters or ethers. [d] 1*RS*, *trans*(E) isomer.

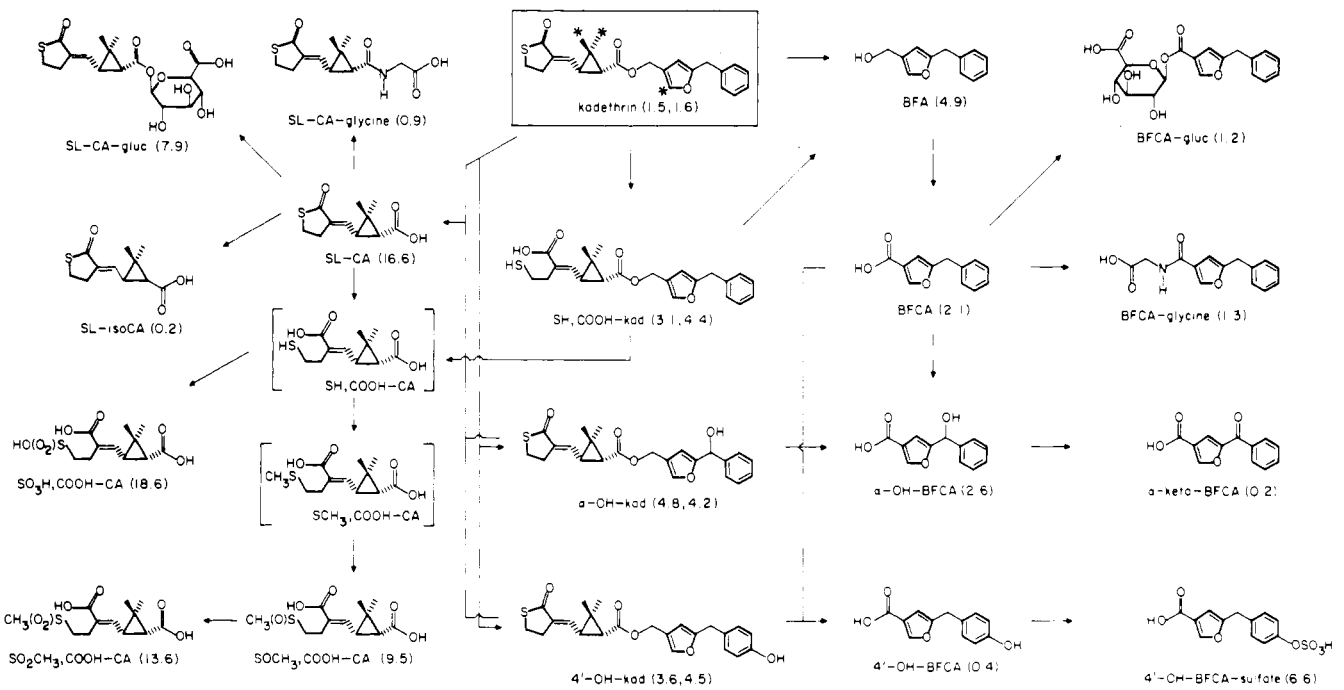

Figure 1. Partial metabolic pathways for kadethrin in rats. Asterisks with the kadethrin structure designate positions of ^{14}C labeling in the acid and alcohol moieties. Abbreviations used for various metabolites are indicated. Numbers in parentheses are the percentage amounts in the excreta relative to the administered dose given, in the case of esters, first for the ^{14}C-acid and then for the ^{14}C-alcohol preparations. Several unidentified metabolites are formed by additional pathways.

as described below. They each gave appropriate spectral data (nuclear magnetic resonance, chemical ionization–mass spectrometry, and infrared) determined under conditions previously reported (Ohsawa and Casida, 1979).

4'-OCH_3-Kadethrin. 4'-OCH_3-BFA (30 mg, 0.14 mM) and equimolar dry pyridine in dry benzene (0.5 mL) were added to the acid chloride of SL-CA, prepared by stirring SL-CA (32 mg, 0.14 mM) and $SOCl_2$ (33 mg, 0.28 mM) in dichloromethane (1 mL) for 2 h at 25 °C and distillation of excess $SoCl_2$. After 15 h at 25 °C and suitable washing with HCl, aqueous $NaHCO_3$, and water, 4'-OCH_3-kad was isolated (35% yield) by preparative TLC (B).

Dimethyl Ester of SCH_3,COOH-CA. A solution of SH,COOH-CA (1.8 g, 7.4 mM) in methanol (5 mL) containing concentrated H_2SO_4 (1 mL) was refluxed for 2 h at 60 °C. The dimethyl ester of SH,COOH-CA (90% yield) was recovered by extraction into ether, washing the ether with water, and silica gel column chromatography using hexane–ether (2:1). This thiol diester (1006 mg, 3.7 mM) in methanol (5 mL) was mixed with sodium (85 mg, 3.7 mM) and then methyl iodide (525 mg, 3.7 mM). After stirring for 8 h at 50 °C, the mixture was poured into water and extracted with ether, and the dimethyl ester of SCH_3,COOH-CA (mp 52–53 °C) was recovered in 36% yield by preparative TLC (A).

Dimethyl Esters of $SOCH_3$,COOH-CA and *SO_2CH_3,COOH-CA.* A chloroform solution (5 mL) of SCH_3,COOH-CA COOH-CA dimethyl ester (300 mg, 1.05 mM) and *m*-chloroperbenzoic acid (181 mg, 1.05 mM) was stirred for 1 h at 10 °C. The sulfoxide (50 mg) and sulfone (20 mg) were isolated by direct preparative TLC (D).

SO_3H,COOH-CA and Its Sodium Salt and Trimethyl Ester. SH,COOH-CA (1 g, 4 mM) was introduced into a stirred solution prepared by adding Br_2 (2.03 g, 12.7 mM) to NaOH (1.4 g, 35 mM) in 12 mL of water at −10 °C. After additional stirring for 8 h at 5 °C and 16 h at 25 °C, the mixture was acidified with HCl and evaporated to dryness, and SO_3Na,COOH-CA was recrystallized from ethanol–water mixture (yield 44%). The sulfonic acid, obtained by passage through a column of acid-washed cation-exchange resin (Dowex 50W-X), was converted almost quantitatively to its trimethyl ester on treatment with CH_2N_2 and preparative TLC (D).

SL-CA-Glycine. The acid chloride of SL-CA (prepared as above from 4.4 mM SL-CA) was dissolved is dioxane (3 mL) and added to a solution of glycine hydrochloride (554 mg, 5.0 mM) in 3 mL of water at 10 °C and maintained at pH ~8 by addition of aqueous $NaHCO_3$. After stirring for 1.5 h at 10 °C, the reaction mixture was poured into saturated NaCl solution which was acidified with HCl and extracted with ether. SL-CA-glycine was recovered (21% yield) by preparative TLC (chloroform–methanol, 8:1; R_f 0.35). Treatment with CH_2N_2 gave the methyl ester.

The taurine conjugate of SL-CA was prepared by a method used previously to make 3-phenoxybenzoyltaurine (Hutson and Casida, 1978) but with $NaHCO_3$ instead of NaOH to prevent thiolactone hydrolysis.

BFCA-Glycine. A solution of glycine hydrochloride (111 mg, 1 mM) and NaOH (40 mg, 1 mM) in 3 mL of water was mixed with a benzene solution (5 mL) of the acid chloride of BFCA (1 mM). After vigorous stirring for 2 h at 25 °C, BFCA-glycine was recovered as with SL-CA-glycine, then recrystallized from ethyl acetate (mp 177–178 °C, yield 33%). Treatment with CH_2N_2 gave the methyl ester. The glutamate conjugate was made by the same procedure.

Distribution and Metabolism in Rats. Male, albino Sprague-Dawley rats (160–180 g, Simonsen Labs, Inc., Gilroy, CA) were individually treated orally by stomach tube with [^{14}C]kadethrin and [^{14}C]SL-CA in methoxytriglycol (MTG) (25 μL), followed by a MTG rinse (100 μL) of the stomach tube. Urine and feces were collected for 7 days and $^{14}CO_2$ for 2 days [method of Gaughan et al. (1977)]. Urine was analyzed directly by TLC (CXD; EXF) and LSC. Feces were extracted with methanol (10 mL/g of feces; Polytron homogenizer, Kinematica, GmbH, Lucerne, Switzerland) for analysis of the soluble portion by TLC (CXD; EXF) and LSC and of the unextractable portion by combustion and LSC. Tissue residues, determined by combustion, are given as parts per billion equivalents of the administered ^{14}C-labeled compound based upon tissue wet weights. Although all tabulated results are from individual rats, they are supported by studies on at least one additional animal in each case.

Microsomal Metabolism. A described procedure was used to prepare mouse liver microsomes, some of which were incubated with tetraethyl pyrophosphate (TEPP) to inhibit esterase activity (Shono et al., 1979). Each reaction mixture in a 25-mL Erlenmeyer flask contained 2.0 mL of microsomal fraction (10% fresh wt/vol) in phosphate buffer (pH 7.4, 100 mM), NADPH (0 or 2.2 μM in 0.5 mL of buffer) and [^{14}C-acid]kadethrin (1.3 μg in 3 μL of acetone). Following 25 min of incubation at 37 °C, the reaction mixtures were acidified with HCl, supplemented with 1 g of $(NH_4)_2SO_4$, and extracted with ether (10 mL × 3). The aqueous fraction was analyzed for total ^{14}C content by direct LSC. The organosoluble fraction was analyzed by TLC (CXD) and LSC.

RESULTS

Radiocarbon Distribution. Radiocarbon from orally administered [^{14}C]SL-CA and [^{14}C-acid]kadethrin is almost completely excreted within 3 days with little additional excretion between 3 and 7 days and almost no retention in tissues at 7 days (Table II). The radiolabel from [^{14}C-alcohol]kadethrin is excreted somewhat more slowly so that at 7 days there are 14–87 ppb equivalent levels in fat, kidney, liver, and testes (Table II). Radiocarbon from ^{14}C-SL-CA appears almost equally in the urine and feces, whereas that from ^{14}C-acid and ^{14}C-alcohol labeled kadethrin is mostly in the feces. Metabolic fragmentation is not sufficient to liberate $^{14}CO_2$. About 90% of the dose appears in soluble form in the urine and feces, and most of the remainder (~8%) is in the unextractable portion of the feces.

Excreted Metabolites. Quantitative data on individual excreted metabolites are given in Figure 1. The metabolites are tentatively identified by two-dimensional TLC cochromatography as indicated in Table I and below.

Each labeled preparation gives several excreted metabolites which are resolved by TLC in two different two-dimensional solvent systems and quantitated separately for the feces and urine as shown in Figure 2. The feces contains kadethrin and three ester metabolites detected with both the ^{14}C-acid and ^{14}C-alcohol preparations. All other metabolites are ester cleavage products detected with one label only. Although care was taken to minimize metabolite photodecomposition during analysis, there was some photoisomerization of the acid portion (Ohsawa and Casida, 1979) evident by trace levels of isokadethrin and SL-isoCA.

Kadethrin and Ester Metabolites. A small amount (~1.5%) of kadethrin is excreted along with larger amounts (~4% each) of one hydrolysis product (SH,COOH-kad) and two hydroxylated derivatives (α-OH- and 4'-OH-kad). Direct cochromatography served to identify kadethrin (AXB; CXB) and SH,COOH-kad (CXD). Methylation with CH_2N_2 and cochromatography supported the identification of SH,COOH-kad (AXB) and indicated the structure of 4'-OH-kad (AXB and C). Structural assignments for the three ester metabolites were confirmed by hydrolysis (0.1 N KOH in 0.1 mL of methanol, 30 min, 65 °C) and cochromatography of the cleavage products with or without methylation. Metabolites designated SH,COOH-kad, α-OH-kad, and 4'-OH-kad each hydrolyzed to products cochromatographing with SH,COOH-CA (CXD) and its dimethyl ester (CH_2N_2 then AXB). The liberated alcohol moieties chromatographed respectively with BFA (AXB; CXD), α-OH-BFA (CXD), and 4'-

Table II. Radiocarbon in the Urine, Feces, and Tissues of Rats up to 7 Days after Oral Administration of ^{14}C-Acid and ^{14}C-Alcohol Preparations of Kadethrin and Its ^{14}C-Acid Moiety

sample analyzed	^{14}C-SL-CA	kadethrin ^{14}C-acid	kadethrin ^{14}C-alc
Administered Dose, mg/kg	0.83	0.81	0.74
% of Administered Radiocarbon[a]			
urine			
0-1 day	44.0	17.8	10.9
1-3 days	6.4	2.4	6.8
3-7 days	0.9	0.2	5.3
feces			
methanol extract			
0-1 day	26.6	59.9	61.7
1-3 days	14.2	11.5	4.2
3-7 days	0.2	0.4	0.7
unextractable, 0-7 days	7.4	7.5	7.6
$^{14}CO_2$, 0-2 days	0.0	0.0	0.0
carcass and tissues, 7 days	0.3	0.3	2.8
Tissue Residue at 7 Days, ppb of SL-CA or Kadethrin Equiv[b]			
fat	0.3	0.7	87
kidney	5.4	7.5	28
liver	1.5	5.3	20
testes	0.6	0.2	14

[a] Corrected for total ^{14}C recovery values of 104.8% for ^{14}C-SL-CA, 101.0% for [^{14}C-acid]kadethrin, and 97.2% for [^{14}C-alcohol]kadethrin. [b] Additional tissues analyzed showing <9 ppb of SL-CA or kadethrin equivalents are blood, bone, brain, heart, lung, muscle, spleen, and stomach.

OCH_3-BFA (CH_2N_2 then CXD).

Acid Moiety Metabolites. Five metabolites of both [^{14}C-acid]kadethrin and [^{14}C]SL-CA are identified by cochromatography as follows: SL-CA (CH_2N_2 then AXB; CXD); SL-CA-glycine and $SOCH_3$,COOH-CA (CH_2N_2 then CXD; EXF); SO_2CH_3,COOH-CA (CH_2N_2 then CXD); SO_3H,COOH-CA (EXF). The glycine conjugate of SL-CA is hydrolyzed (1 N KOH in 0.2 mL of methanol, 30 min, 65 °C) to SH,COOH-CA identified as above with and without methylation. The metabolite designated SL-CA-gluc is quantitatively cleaved to SL-CA (CH_2N_2 then AXB; CXD) on treatment with β-glucuronidase but not with the enzyme plus saccharic acid 1,4-lactone or with sulfatase [method of Gaughan et al. (1977)]. A small amount of SL-isoCA is detected as an artifact (see above). There are three unidentified metabolites in the feces but none in the urine. Unk 1 is a minor product from kadethrin only and unks 2 and 3 are in larger amounts from both kadethrin and SL-CA. Unk 3 chromatographs in the region of SO_3H,COOH-CA as anticipated (Miaullis et al., 1977) for the corresponding sulfinic acid, SO_2H,COOH-CA, which is an intermediate in sulfonic acid formation. The unidentified metabolites do not include the taurine conjugate of SL-CA.

Alcohol Moiety Metabolites. A portion of the alcohol moiety is excreted as BFA (AXB; CXD) and four furancarboxylic acids [BFCA and its α-OH and α-keto derivatives (CH_2N_2 then AXB; CXD); 4'-OH-BFCA (CH_2N_2 then AXB)]. BFCA-gluc is identified by enzyme cleavage and cochromatography as with SL-CA-gluc (see above). BFCA-glycine cochromatographs with the standard (CH_2N_2 then CXD; EXF). 4'-OH-BFCA-sulfate is the major identified metabolite of the alcohol moiety. Using hydrolysis conditions previously described (Gaughan et al., 1977), it is readily cleaved to 4'-OH-BFCA (CH_2N_2 then AXB) by 2.5 N HCl (30 min, 70 °C) or aryl sulfatase and is partially cleaved by aryl sulfatase/glucuronidase mixture; as expected, it is not hydrolyzed by β-glucosidase. The unknowns include six metabolites in the urine (unks 1, 2, and 6–9) and three in the feces (unks 3–5). Unk 1 is possibly a ring-hydroxylated BFCA derivative based on its chromatographic properties without and with methylation (CH_2N_2). An unidentified oxidation product of BFCA appears in the urine both free (unk 2) and as a sulfate conjugate (unk 6). Although a major metabolite, little is known about unk 3 except that it decomposes on treatment with NaOH or HCl and is converted by CH_2N_2 to a less polar derivative. Unk 9 liberates BFCA on hydrolysis (KOH and HCl but not glucuronidase); the very polar conjugating moiety is not defined. BFCA-glutamate does not cochromatograph with any unidentified metabolite.

Microsomal Metabolism. Kadethrin is hydrolyzed to SL-CA by a TEPP-sensitive esterase(s) in mouse liver microsomes (Table III). Addition of NADPH increases the SL-CA yield with TEPP and decreases it without TEPP, indicating oxidase involvement in both ester cleavage and further metabolism of SL-CA. These oxidases form predominantly polar products retained at the origin on TLC (CXD) or remaining in the aqueous phase on ether extraction. They are likely to include SO_3H,-COOH-CA and other acidic compounds. The apolar organosoluble products, which are in larger amount with than without NADPH, include compounds chromatographing (CXD) in the position of α-OH- and 4'-OH-kad.

DISCUSSION

This is the first detailed report on metabolism of a sulfur-containing pyrethroid. Kadethrin metabolism involves hydroxylation at the methylene and 4-position of the benzyl substituent, cleavage of the cyclopropanecarboxylate and thiolactone ester groups, conjugation of the carboxylic acids as glucuronide and glycine conjugates and of a phenolic metabolite as a sulfate, and conjugation and/or oxidation of the liberated mercaptan to give methyl sulfoxide, methyl sulfone and sulfonic acid derivatives (Figure 1). The latter reactions of the sulfur are analogous to those involved in metabolism of certain phosphorothiolates and thioethers (Menn et al., 1976; Miaullis et al., 1977). Esterase(s) are probably more important than oxidase(s) for cyclopropanecarboxylate cleavage and vice versa for thiolactone cleavage.

Several metabolites form two or more types of conjugates that appear in urine or feces or both. The glucuronides, glycine conjugates, and sulfate are only urinary metabolites. The glucuronide/glycine ratio is 9:1 for conjugation of SL-CA and ~1:1 for BFCA. SH,COOH-CA is almost equally oxidized and methylated based on the identified metabolites. It was surprising to find that $SOCH_3$,COOH-CA, SO_2CH_3,COOH-CA, and SO_3H,-COOH-CA are prominent metabolites in feces but not urine since their molecular weights are relatively low for extensive biliary excretion (Smith, 1973).

A higher proportion of the rat metabolites of kadethrin is identified than those derived from resmethrin, i.e., 79 and 32% for the acid and alcohol moieties of kadethrin, respectively, and 27–34% for the acid moieties and 1–17% for the alcohol moiety of *cis*- and *trans*-resmethrin (Miyamoto et al., 1971; Ueda et al., 1975a). Greater care was taken in the kadethrin studies to identify conjugates and minimize metabolite photodecomposition.

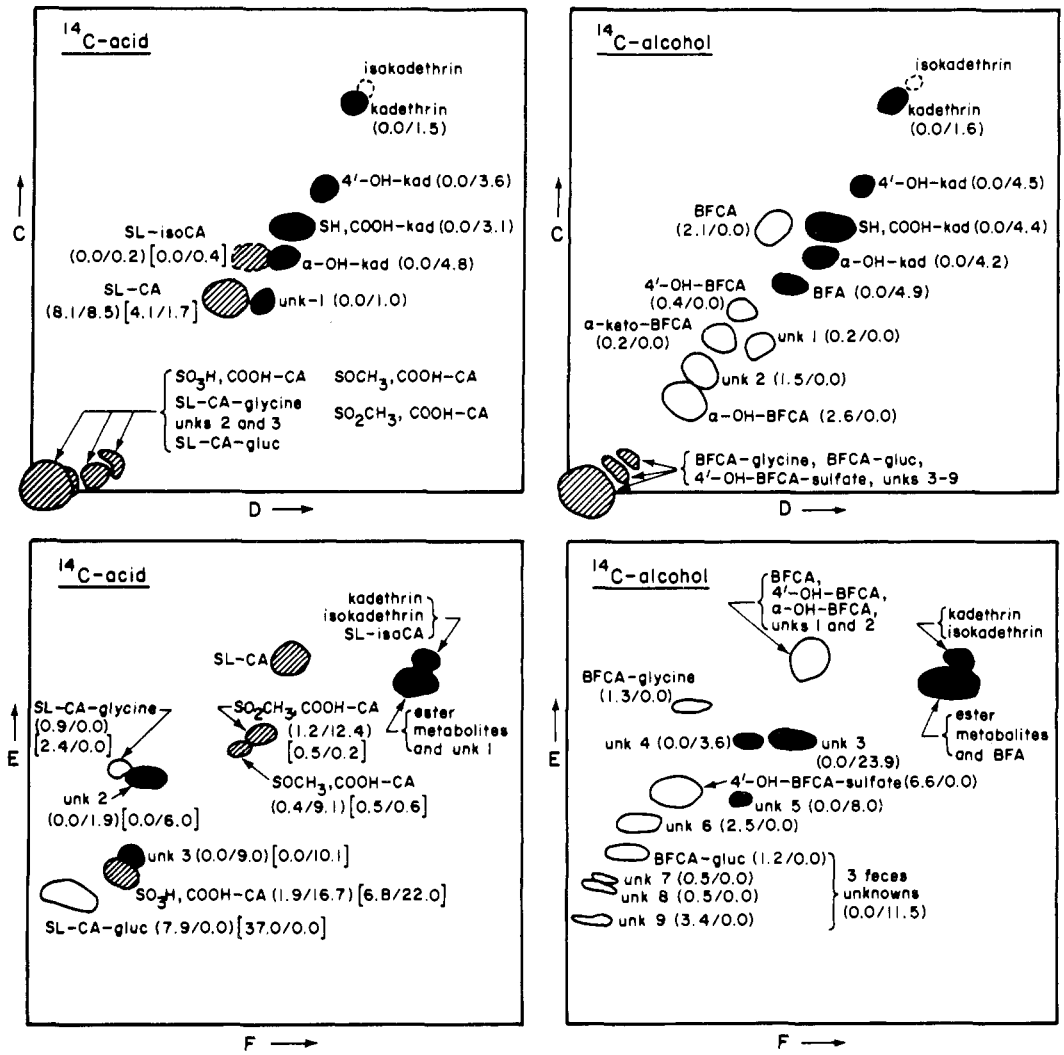

Figure 2. ^{14}C-Labeled compounds in the urine and in the methanol extract of feces of rats after oral administration of ^{14}C-acid and ^{14}C-alcohol labeled preparations of kadethrin and its ^{14}C-acid moiety as resolved by two-dimensional TLC. Figure 1 gives the structures of the compounds. Metabolites appear in the feces only (solid circles), urine only (open circles), or both urine and feces (shaded circles). Numbers in parentheses are the percentage amounts in the excreta relative to the administered [^{14}C]kadethrin given first for the urine and then for the feces. Numbers in brackets are given on the same basis for [^{14}C]SL-CA. Metabolites designated as "unk" are unidentified. A dotted perimeter (isokadethrin and SL-isoCA) indicates a photoisomer not a metabolite. The origin is at the lower left and the solvent fronts are the appropriate outlines of the figures.

Table III. Metabolites of [^{14}C-Acid]Kadethrin in the Mouse Liver Microsome System with or without TEPP and NADPH

kadethrin, metabolite or fraction	amount, %, with various incubation media			
	microsomes only		microsomes + NADPH	
	+TEPP	−TEPP	+TEPP	−TEPP
kadethrin	81.7	51.7	10.8	9.8
SH,COOH-kad	0.1	0.2	0.3	0.3
SL-CA	2.4	38.7	19.5	11.6
organosoluble[a]				
apolar	6.8	6.0	11.2	10.5
polar (origin)	0.4	0.3	32.6	16.4
water-soluble	8.6	3.1	25.6	51.4

[a] Separated by TLC using CXD (see Figure 2).

With such a variety of sites and mechanisms for metabolic attack, it is not surprising that there is rapid metabolism of kadethrin and excretion of its metabolites.

Supplementary Material Available: Spectral data (NMR, CI-MS, and IR) for several kadethrin metabolites and derivatives (1 page). Ordering information is given on any current masthead page.

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Received for review July 2, 1979. Accepted October 22, 1979. This study was supported in part by the National Institutes of Health (Grant P01 ES00049), the Environmental Protection Agency (Grant R805999), and Roussel-Uclaf-Procida (Paris, France).

Methods for the Determination of Diphenylamine Residues in Apples

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Two methods for the determination of diphenylamine residues in apple peel were devised and used during a fruit storage experiment. One was based on extraction in a Soxhlet apparatus and formation of the fluorobutyryl derivative for electron-capture gas chromatographic determination; the second method used steam distillation and direct determination of diphenylamine with a nitrogen-sensitive thermionic gas chromatographic detector. The latter method, after correction for recovery losses, gave residue levels ca. one-third higher during the 118 days of the storage experiment.

The possible withdrawal (in the United Kingdom) of the chemical now used for scald control has prompted further investigation of the levels of diphenylamine on apples, following laboratory and commercial scale storage trials. A number of methods based on spectrophotometric procedures have been described for the determination of diphenylamine residues. Yatsu (1956) and Harvey (1958) utilized the blue oxidation product produced with vanadium pentaoxide in sulfuric acid while Bruce et al. (1958) coupled diphenylamine with diazotized 2,4-dinitroaniline. As these methods appeared to lack the selectivity and sensitivity required, a gas chromatographic technique was sought. The method described by Gutenmann and Lisk (1963) in which diphenylamine is converted to an electron-capturing bromo derivative was unsatisfactory because it gave significant blank values with the fruit used and was insufficiently sensitive.

A method using another diphenylamine derivative which had greater specificity and a lower limit of detection was therefore developed. Subsequently, during the analysis of the prestorage samples, other apparatus and equipment became known and available to us which allowed a more simple method to be devised. Both methods are given as their utility depends on the equipment available.

METHODS

(i) General. Treatment of amines with heptafluorobutyric anhydride yields derivatives with excellent electron-capture sensitivity; this reaction has been utilized, for example, in the determination of ethoxyquin (Winell, 1976) and carbofuran (Lawrence et al., 1977). Diphenylamine also reacts in this way, but when used with spiked apple extracts no derivative was obtained, presumably due to interfering compounds extracted from the apple.

A cleanup step was therefore essential prior to reaction with the anhydride. A number of conventional procedures were tried, including partition with an acidic aqueous phase and column chromatography on alumina or magnesium oxide/Celite, but none of these were successful. A suitable procedure is given below.

During the course of this work a GC with a heated bead nitrogen detector became available to us, making it possible to determine diphenylamine directly without cleanup and derivatization. In initial work with this detector, a Soxhlet extractor was used to extract the diphenylamine but the direct extracts caused some contamination of the GC column; however, a combined steam-distillation and solvent extraction apparatus was then used which gave clean extracts.

(ii) Extraction. (*a*) *Preparation of Fruit.* Diphenylamine was determined in apple peelings approximately 1 mm in thickness. For some poststorage samples the apple flesh (i.e., all the tissue remaining after removal of the peel) was also analyzed.

Each individual apple was weighed and its surface area obtained from tables (Turrell, 1946) after measurement of its major and minor axes. Peel from each apple was extracted either in a Soxhlet extractor or using a special steam-distillation and solvent extraction unit (Veith and Kiwus, 1977).

(*b*) *Soxhlet Extraction.* For Soxhlet extraction, 150 mL of petroleum ether (boiling range 60–80 °C) was placed in a 250-mL B24 round-bottom flask and a 100-mL capacity Soxhlet extractor containing the chopped sample was attached. The petroleum ether was allowed to boil under reflux for 3 h. The extract was dried over anhydrous sodium sulfate and then made up to 150 mL.

(*c*) *Steam Distillation/Solvent Extraction.* In this method the peelings or, in some investigations, the flesh was placed in a 500-mL B24 round-bottom flask, 200 mL of water was added, and the steam-distillation/solvent extraction apparatus was attached. The apparatus was filled with water to a depth of 10–20 mm and 20 mL of petroleum ether (boiling range 60–80 °C) added with a pipet to form an upper layer. The contents of the flask were allowed to boil vigorously for 60 min after which the petroleum ether layer was transferred. Three rinses of 5 mL of distilled water, 3 mL of petroleum ether, and 5 mL of distilled water were sufficient to transfer all the extract through a Whatman phase-separating paper into a 20-mL volumetric flask. The petroleum ether rinse was almost sufficient to replace solvent which was "lost" either as a

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0021-8561/80/1428-0255$01.00/0 © 1980 American Chemical Society