Comparative Metabolism of the Pyrethroids Bifenthrin and Deltamethrin in the Bulb Mite *Rhizoglyphus robini*

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The fate of ^{14}C-radiolabeled bifenthrin and deltamethrin was studied in the mite, *Rhizoglyphus robini*. Administered either by ingestion or by contact, both pyrethroids were efficiently metabolized, but deltamethrin was degraded to a much greater extent. The identified metabolites arise from a combination of ester cleavage, oxidation, and conjugation reactions. With ^{14}C-acid- and ^{14}C-alcohol-labeled bifenthrin, the free metabolites detected were the 4′-hydroxy derivative of the ester, the primary ester cleavage products, the acid, and its 4′-hydroxy derivative from the alcohol moiety, as well as several unidentified metabolites. Using ^{14}C-alcohol-labeled deltamethrin, 3-phenoxybenzoic acid and its 4′-hydroxylated product and several unknown metabolites were detected. Conjugates comprised the bulk of total pyrethroid metabolites. In addition to ester cleavage products, the 4′-hydroxylated bifenthrin was also identified. For the first time in invertebrates, a conjugated pyrethroid ester was observed.

Photostable pyrethroids with favorable toxicological and environmental properties (Elliott et al., 1978) have been introduced for the control of a wide spectrum of insect pests. Only recently have pyrethroids with miticidal activity such as fluvalinate (Henrick et al., 1980), fenpropathrin (Matsuo et al., 1976), and bifenthrin (Plummer and Pincus, 1981; Plummer et al., 1983) been reported. Among the insecticidal compounds that are highly toxic to beneficial predatory mites (Aliniazee and Cranham, 1980; Hull and Starner, 1983), many have also been implicated in severe outbreaks of pestiferous species such as spider mites (Gerson and Cohen, 1988).

Mites are known to develop resistance rapidly to most acaricides, but information concerning the biochemical basis for this phenomenon is limited. Enhanced enzymatic activities of esterases (Kuwahara et al., 1981) and glutathione-*S*-transferases (Motoyama et al., 1971; Mullin et al., 1982; Cohen and Gerson, 1986) may be implicated in resistance to various pesticides.

Previous reports on the effects of pyrethroids on mite populations deal largely with their direct toxicity and repellency response to residues (Penman et al., 1986). McKee and Knowles (1984, 1985) have studied penetration, degradation, and effect on respiration of various pyrethroids in the spider mite, *Tetranychus urticae*. Extensive metabolism studies of pesticides, including pyrethroids, in arthropods have been restricted mainly to insects (Shono et al., 1978, 1979; Ruzo et al., 1981). Metabolism studies in mites are limited because of the great difficulties that arise in obtaining sufficient quantities of biological material.

The bulb mite, *Rhizoglyphus robini*, used in this study, which is a pest of onion and garlic, can be mass-reared and obtained in gram quantities suitable for pyrethroid metabolism studies. This report examines the in vivo and in vitro metabolism of bifenthrin and deltamethrin as representatives of acaricidal and insecticidal pyrethroids, respectively.

MATERIALS AND METHODS

Chemicals. The structures of relevant compounds are shown in Figure 1. Bifenthrin (bif) [(2-methyl-1,1′-biphenyl-3-yl)methyl *cis,trans*-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate] and the ^{14}C-labeled material (see Figure 1) were provided by FMC Corp. (Princeton, NJ). The pyrethroids with radiocarbon in the acid and alcohol moieties had specific activities of 11.9 and 33.5 mCi/mmol, respectively. The following standards were also obtained from FMC: *cis,trans*-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylic acid (CA); 2-methyl-3-phenylbenzyl alcohol (bif-alc); 2-methyl-3-phenylbenzoic acid (bif-acid); 3-(4′-hydroxyphenyl)-2-methylbenzyl *cis,trans*-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate (4′-OH-bif). 3-(4′-Hydroxyphenyl)-2-methylbenzyl alcohol (4′-OH-bif-alc) was prepared from 4′-OH-bif by hydrolysis of 2 mg in 1 mL of saturated sodium carbonate solution for 24 h at 85–90 °C. The product was extracted with ether after acidification of the mixture, the solvent evaporated, and 4′-OH-bif-alc then purified by TLC (Table I). The compound was characterized by chemical ionization mass spectrometry using methane as reagent gas (0.8 Torr): *m/e* 243 (M + 29, 6%), 215 (M + 1, 12%), 197 ([M + 1] − H_2O, 100%).

Deltamethrin (del), radiocarbon labeled at the benzylic methine (58 mCi/mmol), was a gift of Roussel-Uclaf (Paris, France). The unlabeled standards, 3-phenoxybenzoic acid (PBA) and 3-(4′-hydroxyphenoxy)benzoic acid (4′-OH-PBA), have been previously described (Ruzo et al., 1978).

Chromatography and Analysis. Thin-layer chromtography (TLC) utilized silica gel 60 F254 chromatoplates (0.25-mm thickness) (EM Laboratories, Elmsford, NY) and development with solvent systems A and B as indicated in Table II. Radiolabeled pyrethroids and their metabolites were chromatographed as previously described (Ruzo et al., 1978; Shono et al., 1978, 1979). Products were detected either by autoradiography, in the case of radiolabeled material, or by fluorescence quenching using cochromatography with unlabeled standards added to the preparation before application to the chromatoplates. The

Table I. Recovery of Bifenthrin and Deltamethrin after Exposure to the Mite, *R. robini*, by Contact and by Ingestion

pyrethroid	recovery,[a] % contact	ingestion
bifenthrin		
^{14}C-acid label	53.7	19.5
^{14}C-alcohol label	58.0	22.1
deltamethrin		
^{14}C-alcohol label	0.7	0.1

[a] Results are the average of two experiments.

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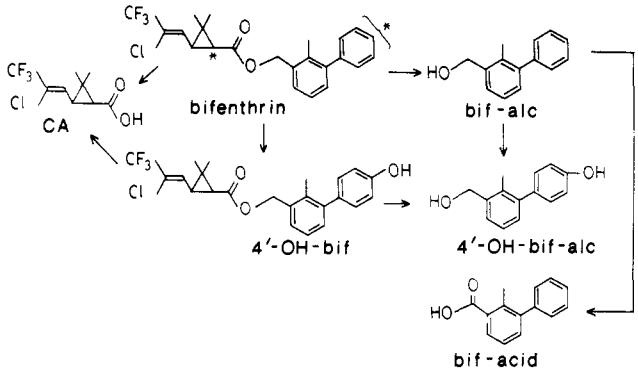

Figure 1. Metabolic pathway for bifenthrin in *R. robini*. Asterisks indicate sites of radiocarbon label.

Table II. Metabolites of Bifenthrin and Deltamethrin in *R. robini*

compound	yield,[a] % contact	yield,[a] % ingestion	R_f[b] A	R_f[b] B
bifenthrin, ^{14}C-acid			0.62	0.77
4'-OH-bif	1.8	2.3	0.23	0.56
1	0.4	0.2	0.27	0.59
2	0.4	0.2	0.25	0.54
3	0.1	0.1	0.21	0.52
CA	8.8	17.5	0.29	0.50
4	6.4	13.6	0.10	0.18
conjugates	82.1	66.1	0.00	0.00
bifenthrin, ^{14}C-alcohol			0.62	0.77
4'-OH-bif	2.2	3.0	0.23	0.56
1	0.5	0.9	0.27	0.56
2	0.3	0.3	0.25	0.54
3	0.1	0.0	0.21	0.52
bif-acid	1.8	2.6	0.14	0.45
bif-alc	15.4	9.9	0.13	0.36
4'-OH-bif-alc	19.4	35.1	0.05	0.23
conjugates	60.1	48.2	0.00	0.00
deltamethrin, ^{14}C-alcohol			0.54	0.81
1	0.7	0.6	0.22	0.50
2	1.8	0.9	0.20	0.46
PBA	28.9	23.0	0.15	0.40
4'-OH-PBA	14.0	7.3	0.08	0.17
conjugates	54.6	68.2	0.00	0.00

[a] Metabolite yield as percentage of metabolized pyrethroid. [b] A and B: developing systems consisting of carbon tetrachloride–ether (3:1) and toluene saturated with acetic acid–ether (10:3), respectively.

relevant zones on TLC were scraped, extracted with chloroform–methanol (3:1), and quantitated by liquid scintillation counting.

Materials remaining at the origin of the chromatoplates (conjugates, Table II) after developing (in A and B) were rechromatographed with butanol–acetic acid–water (4:1:1). The major bands were extracted and cleaved by treatment with 6 N HCl for 8 h at 85 °C. After hydrolysis, samples were extracted with chloroform–methanol (3:1) and cochromatographed with appropriate standards (Table III).

Mite Cultures. The bulb mite, *R. robini* Clamparede, was reared in plastic Petri dishes (9 cm in diameter) in the dark at 27 °C on water-soaked ground peanuts. The mites were mass-reared in stock cultures to yield the quantities required for the metabolism studies.

In Vivo Experiments. For contact exposure, radiolabeled bifenthrin and deltamethrin (5 μg) were each dissolved in 1 mL of 50% aqueous solution of acetone containing 1% Triton X-100. The mites (500 mg) were soaked in the pyrethroid solution for 2 min, and excess solution was withdrawn by suction. A considerable amount of the radioactivity (between 10 and 20%) adhered to the mites, which were placed in Petri dishes and held under

Table III. Partial Identification and Quantitation of Bifenthrin and Deltamethrin Conjugates

source[a]	% yield	R_f	cleavage products	% yield[b]
bifenthrin, ^{14}C-acid				
conj 1	22.0	0.54	4'-OH-bif	3.9
			CA	4.0
conj 2	18.1	0.31	4'-OH-bif	2.3
			CA	1.5
bifenthrin, ^{14}C-alcohol				
conj 1	12.0	0.54	4'-OH-bif	0.8
			bif-acid	1.3
			4'-OH-bif-alc	8.5
conj 2	8.6	0.50	bif-alc	4.8
conj 3	8.9	0.31	4'-OH-bif	0.6
			bif-acid	1.4
			4'-OH-bif-alc	6.0
conj 4	7.0	0.24	bif-alc	4.7
deltamethrin, ^{14}C-alcohol				
conj 1	42.8	0.44	PBA	2.7

[a] Major conjugates obtained from TLC separation of polar material in Table II, utilizing butanol–acetic acid–water (4:1:1) as the development system. [b] Radiocarbon remaining at the origin on TLC (A, B) accounts for the difference in yield of cleaved and intact conjugates. The percent cleavage is as follows. Bifenthrin, ^{14}C-acid: 36 (conj 1), 21 (conj 2). Bifenthrin, ^{14}C-alcohol: 88 (conj 1), 60 (conj 2), 90 (conj 3), 67 (conj 4). Deltamethrin: 6 (conj 1).

humid condition for 72 h at 27 °C. In the ingestion experiment the radiolabeled pyrethroids (5 μg) dissolved in chloroform (100 μL) were applied on black filter paper (4 cm in diameter) and the solvent was evaporated. The filters were placed in glass Petri dishes, mites (750 mg) were introduced, and humidity was provided by adding water (750 μL) every 24 h. After exposure (72 h), the whole filter paper was essentially consumed and the experiment was terminated. The mites were routinely examined under a stereoscopic microscope and revealed no adverse effects by either the contact or the ingestion methods. After exposure to the toxicants the mites were homogenized in a glass homogenizer with 6 mL of chloroform–methanol (3:1) solution. The homogenate was filtered through glass wool and the filtrate concentrated (500 μL) under nitrogen. Aliquots were removed for scintillation counting and TLC.

In Vitro Experiment. Mites (3 g) removed from stock cultures were homogenized in 8 vol of 50 mM sodium phosphate buffer, pH 7.4, with a Teflon glass homogenizer. The homogenate was filtered through glass wool and the filtrate subjected to low-speed centrifugation (3000*g*) for 10 min. The supernatant served as the enzyme source, and aliquots of the extract were subjected to 90 °C for 10 min and served as controls. The radiolabeled pyrethroids (1 μg) in chloroform were added to the incubation vial, and the solvent was removed by evaporation. A mixture containing 15 μL of dimethyl sulfoxide, 685 μL of sodium phosphate buffer, and 300 μL of the mite homogenate supernatant in a total volume of 1 mL was added to the pyrethroid. Incubation was carried out at 35 °C for 3 h in a shaking bath, and the reaction was stopped by addition of chloroform (1 mL). The organic phase was removed, and the remaining aqueous phase was extracted again with chloroform. Further handling of the extract was carried out as described for the in vivo experiments.

RESULTS AND DISCUSSION

The objective of the study was to determine the extent of metabolic breakdown and differences in metabolic products of bifenthrin and deltamethrin by the mite, *R. robini*. It was not physically possible to apply the pyrethroids to individual mites nor to count a fixed number of subjects. Thus, we relied on weight to provide comparative data. Overall recovery of radiolabel was good, yet

it is not possible to determine how much pyrethroid penetrated into the mites on contact or how much was ingested. Further, in dealing with this species it is not feasible or practical to separate excreted radiocarbon from material remaining on the dish. Therefore, we were restricted in conducting our study using two routes of administration.

The mite, *R. robini*, efficiently metabolized both bifenthrin and deltamethrin in vivo (Table I), but much greater conversion of deltamethrin was observed. As previously mentioned, it was not possible to accurately determine the extent of pyrethroid penetration in the experiments involving contact application, yet a chloroform rinse of the mites before homogenization recovered up to 20% of radiocarbon from bifenthrin, consisting largely of starting material. Radiocarbon recovered from rinses of deltamethrin-treated mites consisted almost entirely of metabolites. By the ingestion method of application both pyrethroids were degraded to a greater extent as compared to the contact method. This might be related to a prolonged exposure time combined with more efficient penetration of the pesticides. Because of practical reason, a 72-h period of exposure was necessary in the ingestion experiments. This period allowed complete consumption of the treated filter paper. Otherwise, it is difficult to collect and separate mites from the uningested paper prior to extraction of pyrethroids and metabolites.

The identities of pyrethroid metabolites from mites exposed by contact or by ingestion were the same, but the relative percentage varied (Table II). Metabolites of bifenthrin (Figure 1) and deltamethrin arise from a combination of ester cleavage and oxidation reactions followed by conjugation. ^{14}C-Acid-labeled bifenthrin yielded the acid (CA) as well as an unknown (4), probably arising from hydroxylation at either *gem*-dimethyl group, in analogy to other cyclopropanecarboxylates (Casida and Ruzo, 1980). The major metabolites characterized from both pyrethroids arise from ester cleavage, probably subsequent to aryl hydroxylation. Thus, formation of 4′-OH-bif, detected from both ^{14}C-acid- and ^{14}C-alcohol-labeled bifenthrin, may be followed by cleavage to yield 4′-OH-bif-alc, which is then conjugated. Minor unknowns 1–3 (Table II) may be positional isomers of hydroxylation. In a manner analogous to the 3-phenoxybenzyl-substituted pyrethroids (Ruzo et al., 1978; Shono et al., 1979), hydroxylation occurs predominantly at the least sterically hindered and most electron-rich para position. The same hydroxylation position was reported for permethrin, cypermethrin, and deltamethrin in several insect species (Shono et al., 1978, 1979). Deltamethrin was degraded to two major metabolites, PBA and 4′-OH-PBA, and to two minor unidentified products (Table II).

In the in vivo studies conjugated materials comprised the bulk of the recovered radiocarbon from bifenthrin and deltamethrin (Table II). After TLC separation the ^{14}C-acid-labeled bifenthrin extract revealed two major conjugates (Table III), while four major conjugates were evident from the ^{14}C-alcohol label (Table III). Upon acid hydrolysis both radiolabels released significant amounts of 4′-OH-bif, in addition to the corresponding ester cleavage products previously described as free metabolites. To our knowledge, this is the first instance in invertebrates in which a pyrethroid metabolite is conjugated without previous cleavage of the ester bond. A sulfate conjugate of 4′-hydroxypermethrin has been previously reported (Gaughan et al., 1978). In no case was acid hydrolysis completely efficient in cleaving bifenthrin or deltamethrin conjugates (Table III). However, the former products were cleaved to a much greater extent, in particular those arising from the alcohol moiety. Cleavage of the major deltamethrin conjugate was very inefficient, liberating only small amounts of PBA (Table III). Pyrethroids are conjugated in insects with glucose or with a number of amino acids (Shono et al., 1978). The nature of the conjugating moieties in *R. robini* was not investigated.

In the in vitro studies the esteratic cleavage of bifenthrin was a mere 2% as compared to that of deltamethrin (ca. 15%). These results are consistent with that of the in vivo study indicating that the mite esterases can differentiate between the two pyrethroids. The major product observed with deltamethrin was PBA with minor amounts of 4′-OH-PBA, whereas hydroxylation of the bifenthrin cleavage products was not observed.

The bulb mite is highly tolerant to both bifenthrin and deltamethrin. Concentrations up to 10000 ppm did not affect the survival of the mites. Pyrethroids in general are very toxic to predatory mites, and the spider mite, *T. urticae*, was found more suceptible to deltamethrin than to the miticidal fenpropathrin (Kuwahara, 1986). Although bifenthrin has been reported to have strong acaricidal activity (Plummer et al., 1983), its extensive metabolism by *R. robini* in conjunction with possible target sites with reduced affinity to the pyrethroid are probably the reasons for the mite's remarkable insensitivity.

This study indicates a remarkable similarity between the metabolic processes of pyrethroids in *R. robini* and those previously reported in insects and mammals (Casida and Ruzo, 1980), although of significantly higher efficiency in the mite. It is also apparent that the species utilized in our studies is suitable for the examination of pyrethroid metabolism.

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Registry No. PBA, 3739-38-6; 4′-OH-PBA, 35065-12-4; *cis*-bif, 107538-32-9; *trans*-bif, 107538-34-1; *cis*-CA, 76647-97-7; *trans*-CA, 82796-20-1; bif-alc, 76350-90-8; bif-acid, 115363-11-6; *cis*-4′-OH-bif, 115340-45-9; *trans*-4′-OH-bif, 115404-73-4; 4′-OH-bif-alc, 115340-46-0; del, 52918-63-5.

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Properties of an Extract from Canada Thistle Roots That Stimulates Germination of Dormant Teliospores of Canada Thistle Rust (*Puccinia punctiformis*)

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A stimulator of teliospore germination was concentrated from thistle roots (*Cirsium arvense*) by steam distillation and extraction by hexane. Concentrations of 25 μL/L stimulated teliospore germination 50% in 7 days. Teliospores exposed to volatiles from crude extract at time intervals ranging from 1 min to 24 h reached a maximum germination plateau with 1 h or longer exposures when counted at 7 days. TLC, NMR, and GC–mass spectrometric analysis of the hexane extract indicated the presence of C_{17} unsaturated hydrocarbons with four, three, two, and one double bonds. 1-Pentadecene was also found. Most of the stimulatory activity occurred with the compound separated by TLC at R_f 0.26, shown to be (*Z,Z,Z*)-1,8,11,14-heptadecatetraene, or aplotaxene. The spot at R_f 0.60 contained 1-heptadecene and 1-pentadecene. These compounds were analyzed quantitatively by gas chromatography. Aplotaxene was found in thistle roots at concentrations of 0.032 μg/g of fresh roots. Synthetic aplotaxene was found to possess no stimulatory activity on Canada thistle rust teliospore germination.

Canada thistle [*Cirsium arvense* (L.) Scop.] is a widely dispersed noxious weed that is difficult and expensive to control by herbicides. This plant is susceptible to a rust, *Puccinia punctiformis*, that is particularly devastating in the aecial stage. Infection is initiated from basidiospores produced by germinating teliospores. Turner et al. (1982) reported a biologically active substance from Canada thistle roots that stimulated teliospore germination. French (1985) has reported stimulation of germination of urediniospores of a number of rust species by volatile aroma or flavor compounds. For example, urediniospores of *P. punctiformis* were stimulated by 5-methyl-2-hexanone (French, 1983). Stimulatory compounds such as nonanal and 6-methyl-5-hepten-2-one were identified in urediniospores of *Puccinia graminis* var. tritici, *Uromyces phaseoli*, and other species (French et al., 1977; Rines et al., 1974).

Teliospores are the dormant, overwintering stage of Canada thistle rust, and they germinate very slowly and irregularly. We are interested in inducing germination in these teliospores at will to facilitate Canada thistle infection with this systemic, highly damaging aecial phase of the fungus. Our ultimate goal is to develop an effective biocontrol procedure for this noxious, spiny, perennial weed that is becoming an increasingly important problem

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