Species-Specificity in Enzymatic Oxidation of Pyrethroid Insecticides: 3-Phenoxybenzyl and α -Cyano-3-phenoxybenzyl 3-(2,2-Dihalovinyl)-2,2-dimethylcyclopropanecarboxylates

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Species variations are noted in the relative importance of hydroxylation at the *trans*- and *cis*-methyl groups and the 2'-, 4'- and 6-positions of the phenoxybenzyl moiety on incubation of *trans*- and *cis*-permethrin with NADPH-fortified microsomal preparations of mouse, rat, trout and carp liver and housefly abdomens-plus-thoraces and microsome-plus-soluble fractions of cabbage looper gut. Housefly microsomes hydroxylate only the *trans*-methyl group of *trans*-permethrin and carp and looper enzymes preferentially hydroxylate the *trans*-methyl group over the *cis*-methyl group of *cis*-permethrin. The major site of attack in the alcohol moiety of the permethrin isomers is the 4'-position with all enzyme preparations; mouse and housefly microsomes also hydroxylate the 6-position of both *trans*- and *cis*-permethrin and mouse microsomes hydroxylate the 2'-position of *cis*-permethrin. The cyano-pyrethroids, *trans*- and *cis*-cypermethrin and decamethrin, are hydroxylated by mouse microsomes at the *trans*- and *cis*-methyl groups and the 4'- and 5-positions of the alcohol moiety, differing from the permethrin isomers in the significance of hydroxylation at the 5- versus the 6-position. With *trans*-permethrin, further oxidation of the *trans*- and *cis*-hydroxymethyl derivatives and their hydrolysis products yields the corresponding aldehydes and carboxylic acids.

INTRODUCTION

Insecticidal chrysanthemates are oxidized at a methyl group in the isobutenyl substituent by rats and houseflies and by rat liver and housefly microsomal oxidases.¹⁻⁸ This detoxification site is no longer present in highly potent pyrethroids with the isobutenyl group replaced by a dihalovinyl group (*e.g.*, permethrin, cypermethrin and decamethrin) (Fig. 1).⁹ Despite this type of structural modifica-

tion, mouse and rat microsomes rapidly hydroxylate the permethrin isomers,¹⁰⁻¹² rats quickly metabolize the permethrin isomers¹³ and decamethrin,¹⁴ and housefly (*Musca domestica* L.) adults and cabbage looper (*Trichoplusia ni* Hübner) larvae slowly degrade the permethrin isomers,¹⁵ in almost all cases by hydroxylation at either of the methyl groups and at the 4'-position.¹²⁻¹⁵ Additional sites of *in vivo* hydroxylation are the 6-position of the permethrin isomers in houseflies,¹⁵ the 2'-position of *cis*-permethrin in rats,¹³ and the 2'- and 5-positions of decamethrin in rats.¹⁴

The present study examines the sites of *in vitro* oxidation of the permethrin isomers by

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Fig. 1 Structures of pyrethroids examined and hydroxylation sites established.

[1RS, *trans*]- and [1RS, *cis*]-permethrin: X = Cl, Y = H.

[1RS, *trans*, α RS]- and [1RS, *cis*, α RS]-cypermethrin: X = Cl, Y = CN.

[1R, *cis*, α S]-decamethrin: X = Br, Y = CN.

enzymes of mouse, rat, trout and carp liver, housefly abdomens-plus-thoraces and cabbage looper guts and of the cypermethrin isomers and decamethrin by enzymes of mouse liver.

MATERIALS AND METHODS

Enzymes consisted of microsome preparations of mouse, rat, rainbow trout and carp liver, microsome preparations of housefly abdomens-plus-thoraces, and the microsome-plus-soluble fraction of cabbage looper guts. Substrate levels in the 2.5-ml reaction mixtures of pH 7.5 buffer were 0.1 μ g (cabbage looper guts), 18–88 μ g (fish microsomes) or 1 μ g (other cases). The cofactor NADPH was added at 0 or 2.2 μ moles except with fish microsomes where 1.2 μ mole was used along with glucose-6-phosphate and glucose-6-phosphate dehydrogenase as a NADPH-regenerating system. Incubations at 30°C (fish and insect preparations) and 37°C (mammalian preparations) were carried out with shaking in air for 30 to 120 min. The ether-soluble metabolites of each substrate labeled with radiocarbon in both the acid and alcohol moieties were identified by thin-layer cochromatography with authentic unlabeled standards from synthesis^{14–17)} or by cleavage to acid and alcohol fragments for cochromatography with appropriate standards.¹⁷⁾

RESULTS

The substrates used with each enzyme preparation and NADPH are shown in Table 1. These enzymes without NADPH fortification hydrolyze the *trans*-pyrethroids more extensively than the corresponding *cis*-pyrethroids. With the preparations examined (all except

the fish microsomes), these esterases are completely inhibited on incubation with 1×10^{-4} M tetraethyl pyrophosphate for 10 min at 25°C.

Both the *trans*- and *cis*-pyrethroids undergo oxidation on addition of NADPH to the enzyme preparations, except for *trans*-permethrin with looper enzyme. The site specificity in these oxidations is given in Table 1. Several points are worthy of note. The *trans*-methyl group is preferentially hydroxylated over the *cis*-methyl group with *trans*-permethrin and housefly enzyme and with *cis*-permethrin and carp and looper enzymes. The 4'-position is usually the major site of hydroxylation in the alcohol moiety. Other important hydroxylation sites are the 6-position in the permethrin isomers with mouse and housefly enzymes and the 5-position in cypermethrin and decamethrin with mouse enzyme. Hydroxylation at the 2'-position is detected only with *cis*-permethrin using mouse microsomes treated with tetraethyl pyrophosphate. The highest degree of site specificity is with the trout en-

Fig. 2 Oxidation of *trans*-permethrin at the geminal-dimethyl substituent to yield hydroxymethyl, carboxaldehyde and carboxylic acid derivatives with or without hydrolysis. R = H or 3-phenoxybenzyl.

Table 1 Site specificity in oxidation of permethrin and cypermethrin isomers and decamethrin by microsomal preparations from various organisms.

Organism*	Products from hydroxylation at indicated site, amount**					
	Methyl group in acid moiety		Site in alcohol moiety			
	<i>trans</i>	<i>cis</i>	2'	4'	5	6
<u>[1RS, <i>trans</i>]-Permethrin</u>						
Mouse	4	4	0	3	0	3
Rat	2	3	0	3	0	0
Carp	2	3	0	4	0	0
Trout	1	1	0	4	0	0
Housefly	3	0	0	2	0	2
Looper	0	0	0	0	0	0
<u>[1RS, <i>cis</i>]-Permethrin</u>						
Mouse	4	4	1***	3	0	2
Rat	3	3	0	4	0	0
Carp	3	1	0	4	0	0
Trout	1	0	0	4	0	0
Housefly	2	3	0	2	0	1
Looper	3	0	0	3	0	0
<u>[1RS, <i>trans</i>, αRS]-Cypermethrin</u>						
Mouse	2	3	0	3	1***	0
<u>[1RS, <i>cis</i>, αRS]-Cypermethrin</u>						
Mouse	4	3	0	3	3	0
<u>Decamethrin [1R, <i>cis</i>, αS]</u>						
Mouse	3	3	0	3	2	0

* Microsome-plus-soluble fraction of looper guts, microsome fraction of housefly abdomens-plus-thoraces, and microsome fraction of liver in all other cases.

** Amounts are indicated as: 0 = <0.1%; 1 = 0.1–0.5%; 2 = 0.6–1.9%; 3 = 2.0–9.9%; 4 = 10–36%. They are based on the identified ester metabolites and their hydrolysis products.

*** Detected only with microsomes treated with tetraethyl pyrophosphate to inhibit esterase activity.

zyme in hydroxylation of the permethrin isomers at the 4'-position.

Some unanticipated metabolites were encountered in those studies. *Trans*-permethrin is converted by mouse liver microsomes to a large number of products resulting from oxidation of the intermediate hydroxymethyl derivatives to the corresponding aldehydes and carboxylic acids, with or without hydrolysis (Fig. 2). Similar oxidations occur with *cis*-permethrin in mouse and rat microsomes and with the cypermethrin isomers and decamethrin in mouse microsomes. Looper preparations metabolize *trans*- and *cis*-permethrin to the glycine conjugates of the acid moieties and of 3-phenoxybenzoic acid, indicating that this microsome-plus-soluble fraction contains glycine and appropriate cofactors to carry out

the relevant series of reactions. Rat enzyme may cleave the diphenyl ether group of *trans*-permethrin, possibly via 2'-hydroxy-*trans*-permethrin,¹⁶⁾ since the corresponding 3-hydroxybenzyl ester is detected. Small amounts of the cyanohydrin cleavage product are found on mouse enzyme metabolism of the cypermethrin isomers and decamethrin. Ester cleavage of the *cis*-cyano-pyrethroids by the mouse enzyme appears to involve both esteratic and oxidative mechanisms.

Quantitative data will be presented in complete reports on these investigations.^{17,18)}

DISCUSSION

Esterases and oxidases effective in metabolizing dihalovinyl pyrethroids appear to be widely distributed in animal systems since they

are present in all of the enzyme preparations examined. With both insects and mammals the inhibition of pyrethroid-hydrolyzing esterases with suitable organophosphorus esters and of oxidases with appropriate methylenedioxy-phenyl compounds often results in large increases in pyrethroid potency, *i.e.* high degrees of synergism.^{11,19)} This indicates that most if not all of these metabolic reactions are detoxification processes. Several sites of attack are involved in metabolism and probably in detoxification of each pyrethroid. Therefore, the goal of enhancing insecticidal potency by introducing substituents into the pyrethroid to block all major biodegradation sites is not easily achieved. These structural changes may also increase the vertebrate toxicity of a class of insecticides normally considered to be of minimal hazard for mammals.

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要 約

trans- および *cis*-permethrin の酵素的酸化を、マウス、ラット、コイ、ニジマスの肝臓、イエバエ胸部部のマイクロソームならびに cabbage looper 中腸のマイクロソーム+上清を用いて、NADPH 存在下で調べたところ、*trans*-methyl, *cis*-methyl ならびに phenoxybenzyl 基の 2', 4', 6 の位置が水酸化され、種間に酸化位置の選択性があることが確認された。イエバエのマイクロソームは *trans*-permethrin の *trans*-methyl を水酸化し、コイと looper の酵素も *cis*-permethrin の *trans*-methyl を選択的に水酸化する。permethrin はほぼすべての酵素標品で、アルコール部分に関しては

主として 4' の位置で水酸化され、マウスとイエバエでは 6 の位置も水酸化される。マウスは *cis*-permethrin の 2' の位置も水酸化する。cyano-pyrethroid である *trans*- ならびに *cis*-cypermethrin と decamethrin はマウス・マイクロソームにより *trans*-methyl, *cis*-methyl およびアルコール部分の 4' と 5 の位置が水酸化され、permethrin で水酸化される 6 の位置の代りに 5 の位置が水酸化されることが特徴である。*trans*-permethrin の *trans*-methyl と *cis*-methyl はさらにアルデヒド、カルボン酸に酸化され、それ等のエステルならびに加水分解物が代謝産物として得られた。

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