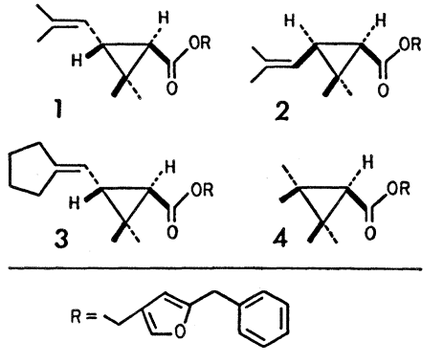
Pyrethroid Insecticides: Esterase Cleavage in Relation to Selective Toxicity

Abstract. *The ester group of primary alcohol chrysanthemates is cleaved by mouse hepatic microsomal esterases, more rapidly for the* (+)-trans *than for the* (+)-cis *isomers. Substrate-specificity and inhibition studies in vivo establish that these pyrethroid-hydrolyzing esterases probably contribute to the low mammalian toxicity of bioresmethrin and other* (+)-trans *chrysanthemate insecticide chemicals derived from primary alcohols.*

Pyrethroids are becoming increasingly important as insect control agents because they possess a unique combination of desirable properties including exceptional insecticidal activity, low mammalian toxicity, rapid biodegradation, and little or no persisting residues. One of these compounds, bioresmethrin **(1)** [the (+)-*trans* isomer], has the highest selectivity ratio known for any insecticide chemical; it is 32,000 times more toxic (on a milligram per kilogram basis) to houseflies when applied topically than to rats when given orally (*1*). Modification of bioresmethrin by making the (+)-*cis*-chrysanthemate (**2**), the (+)-*trans*-ethanochrysanthemate (3), or the tetramethylcyclopropanecarboxylate (**4**) results in very effective insecticides, but the desirable selectivity ratio is greatly reduced, to a value of 130- to 470-fold (*1*).



It is known that (±)-*trans*-resmethrin is metabolized in living rats by cleavage of the ester group and oxidation of the alcohol moiety (*2*). We find that an esterase (or esterases) cleaves the ester group of bioresmethrin and related insecticide chemicals and that this esterase is important in conferring low mammalian toxicity.

Mouse liver microsomes, either fresh preparations (*3*) or acetone powders (*4*), containing 15 to 20 mg of protein were incubated with the pyrethroid (100 nmole) in 2.5 ml of tris-HCl buffer (50 m*M*, *p*H 7.5) at 37°C for periods of time varying from 2.5 to 30 minutes. The rate of substrate loss was quantitatively analyzed by subjecting organic extracts prepared from the incubated mixtures to gas-liquid chromatography [3 percent SE-30 on Gaschrom Q (80 to 100 mesh); glass tubing 1.8 m by 3.2 mm (inside diameter); column temperature program, 180° to 280°C at 10°C/min; N_2, 20 cm^3/min; flame ionization detector; retention times vary from 4.3 to 11 minutes for the various pyrethroids studied]. Alternatively, the products of [^{14}C]bioresmethrin metabolism were analyzed by thin-layer chromatography and cochromatography with authentic unlabeled compounds obtained by synthesis (*5*).

Both types of microsomal preparations, fresh microsomes and acetone powders, hydrolyze bioresmethrin, producing the corresponding alcohol and acid as the only detected products. When reduced nicotinamide adenine dinucleotide phosphate (NADPH, 4.0 μmole) is added to fresh microsomal preparations, bioresmethrin is transformed to several metabolites including chrysanthemum dicarboxylic acid and others formed by the oxidation of the isobutenyl moiety, indicating that oxidases are also involved in the metabolism of this substrate. To study only the oxidase reactions, fresh microsomes are first incubated with 4.0×10^{-5} *M* paraoxon, which completely inhibits the esterase activity, before NADPH is added. The contribution of oxidative metabolism over and above the esterase attack can be evaluated by determining the effect of added NADPH on the rate of pyrethroid metabolism by fresh microsomes. The esterase reactions are most conveniently studied by using acetone powders of microsomes since these preparations lack oxidase activity even when NADPH is added.

The rate of bioresmethrin hydrolysis by microsomal acetone powders was compared with the rate of hydrolysis of 14 other pyrethroids; in each case, gas-liquid chromatography was used to determine the rate of loss of the pyrethroid substrate. The enzymatic hydrolysis of each substrate is blocked by paraoxon; therefore, the study concerns the substrate-specificity of one or more esterases in the preparations. Bioresmethrin (**1**) is hydrolyzed at a rate of 351 ± 79 pmole per milligram of protein per minute while the hydrolysis rates of the other pyrethroids designated above are as follows (relative to bioresmethrin taken as 100): (+)-*cis*-resmethrin (**2**), 12; the (+)-*trans*-ethano analog of resmethrin (**3**), 45; the tetramethylcyclopropanecarboxylate analog of resmethrin (**4**), 25. Comparison of the rates of hydrolysis of the (+)-*trans* and (+)-*cis* isomers of resmethrin is shown in Fig. 1. The (−) isomers of *trans*- and *cis*-resmethrin are hydrolyzed at rates similar to the corresponding (+) isomers but not at identical rates. The (+)-*trans* isomers of other highly insecticidal pyrethroids are also hydrolyzed more rapidly than the corresponding (+)-*cis* isomers by the indicated factors: the ethanochrysanthemate analog of resmethrin by 4-fold; the chrysanthemates of 3,4,5,6-tetrahydrophthalimidomethanol, 3-phenoxybenzyl alcohol, and 5-propargyl-2-furylmethanol by 3-, 5- and more than 50-fold, respectively. The only substrate cleaved more rapidly than bioresmethrin is 5-propargyl-2-furylmethyl (+)-*trans*-chrysanthemate. Neither the (+)-*trans* nor the (+)-*cis* isomer of allethrin, a chrysanthemate of a secondary alcohol, is hydrolyzed by the esterase preparation (that is, less than 1/1000 the rate of bioresmethrin).

The finding that the (+)-*trans* isomers of compounds **1** and **3** are hydrolyzed by the microsomal acetone powder preparations from four to eight times more rapidly than the corresponding (+)-*cis* isomers may be of toxicological significance because these (+)-*trans* compounds are at least tenfold less toxic to mice than the corresponding (+)-*cis* compounds. Thus, detoxification by esterase action appears to be a factor contributing to the low toxicity of the (+)-*trans* isomers.

The significance of esterases in the in vivo detoxification of pyrethroids was examined as follows: the intraperitoneal LD_{50}'s (lethal dose, 50 percent effective) of the pyrethroids and the esterase activity of hepatic microsomes on pyrethroid substrates in normal mice were compared with the LD_{50}'s and esterase activities in mice treated 6 hours earlier with *S,S,S*-

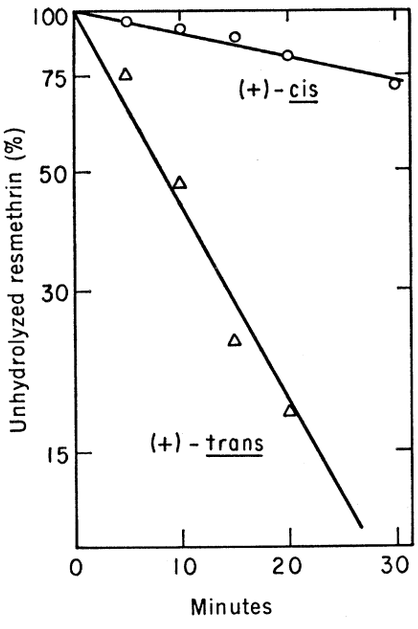


Fig. 1. Specificity of esterases for hydrolysis of (+)-*trans*-resmethrin (bioresmethrin) and (+)-*cis*-resmethrin. Acetone powder preparations of mouse hepatic microsomes were used at a concentration of 17.8 mg of protein per reaction.

tributyl phosphorotrithioate (DEF) at a dose of 50 mg/kg. In mice DEF increases the toxicity of malathion by inhibiting the malathion-detoxifying esterases (*6*); DEF also increases the acute toxicity to mice of compounds **2** and **4** by 3- to 5-fold and of compound **3** by more than 188-fold, but it does not alter the toxicity of bioresmethrin. When hepatic microsomes from mice given preliminary treatment with DEF are assayed without NADPH fortification, their activity relative to microsomes from untreated controls is inhibited by 95 percent for bioresmethrin hydrolysis and by 86 percent for hydrolysis of the (+)-*trans*-ethano derivative. The increased toxicity of the ethano derivative to the treated mice may result from inhibition of detoxifying esterases, but the toxicity of bioresmethrin is not increased even with more complete esterase inhibition; therefore, some other factor or factors must also contribute to the low mammalian toxicity of bioresmethrin.

Possibly, a portion of the detoxifying action involves oxidase attack in addition to esterase cleavage occurring in different degrees on bioresmethrin and the ethano derivative. Neither piperonyl butoxide nor sesamex, known inhibitors of the microsomal oxidases (*7*), alters the susceptibility of mice to poisoning by bioresmethrin or the ethano derivative when the synergists are administered intraperitoneally at 150 to 170 mg/kg 30 minutes prior to the pyrethroid. This finding indicates that oxidation of these pyrethroids is either not important in their detoxification or that it is not the only detoxifying mechanism. These alternative hypotheses were examined by enzyme studies.

When fresh microsomes from untreated mice are used, the rate of metabolism of bioresmethrin is increased by 1.3-fold on addition of NADPH but that of the ethano analog is not affected. It appears that the cyclopentylidenemethyl moiety of the ethano derivative is not as readily oxidized as the *trans*-methyl group of the isobutenyl moiety; therefore, the hepatic microsomes of normal mice metabolize the ethanochrysanthemate almost entirely by hydrolysis rather than by the combined esterase and oxidase action occurring with bioresmethrin. When fresh microsomes from DEF-treated mice are used and NADPH is added, the rate of bioresmethrin metabolism is 4.7-times greater than that of the ethano derivative. Thus, it is probable that in DEF-treated mice the enzymes in the fresh microsomes remaining active in bioresmethrin metabolism are largely oxidases while with the ethano derivative they are largely esterases. Therefore, the low mammalian toxicity of bioresmethrin appears to result from the combined attack of esterases and oxidases that lead to rapid detoxification even when the activity of one or the other of the two enzyme systems is inhibited.

The esterase hydrolyzing bioresmethrin are not restricted to liver microsomes. They are also found in other subcellular fractions of mouse liver, and other mouse tissues including the brain, and in certain insect homogenates (*8*).

An understanding of the enzymatic mechanisms for pyrethroid detoxification is important for the continuing use of these extremely effective insecticide chemicals and for the development of even more potent and selective materials. The naturally occurring form of chrysanthemic acid, the (+)-*trans* isomer, combined with suitable primary alcohols yields highly insecticidal esters that are of low mammalian toxicity (*1*) in part because of the ease of hydrolysis of these pyrethroids by mammalian liver esterases.

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Oxygen Dissociation in Human Erythrocytes: Its Response to Hyperbaric Environments

Abstract. *Hyperbaric nitrogen at a pressure of 100 atmospheres increases significantly the affinity of erythrocytic hemoglobin for oxygen. This results in oxygen dissociation curves which are shifted to lower than normal oxygen partial pressures. The pressure effect is completely reversible and can therefore be observed only during exposure of the blood cells to the hyperbaric gas.*

The ability of red blood cells (RBC's) to bind and to release oxygen is probably best described by their oxygen dissociation curves. If such curves are determined in freshly collected human RBC's while they are exposed to an elevated nitrogen pressure, an increase in the affinity of erythrocytic hemo-