Toxicity of Benzoyl chloride (2,4,6-trichlorophenyl) hydrazone (Banamite) and Potential Metabolites to Twospotted Spider Mites and Potency as Inhibitors of Rat Liver Monoamine Oxidase[1]

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Benzoyl chloride (2,4,6-trichlorophenyl) hydrazone or Banamite is a new acaricide being developed by The Upjohn Company to control mites attacking tree fruits and nuts, small fruits, and ornamentals. We have been studying the metabolism of this phenylhydrazone compound in twospotted spider mites, Tetranychus urticae Koch, a primary target species. During the course of these studies 2,4,6-trichlorophenylhydrazine was isolated from mites treated with Banamite-^{14}C (1). Hydrazines are known inhibitors of monoamine oxidase (2), thus it seemed possible that 2,4,6-trichlorophenylhydrazine was involved in the toxic action of Banamite acaricide. This paper reports the toxicity of Banamite and potential metabolites to twospotted spider mites and their potency as inhibitors of rat liver monoamine oxidase.

MATERIALS AND METHODS

An analytical grade sample of Banamite was provided by The Upjohn Company, Kalamazoo, Michigan. Benzoic acid 2-(2,4,6-trichlorophenyl) hydrazide was synthesized by refluxing benzoyl chloride with 2,4,6-trichlorophenylhydrazine, and benzaldehyde 2-(2,4,6-trichlorophenyl) hydrazone was obtained by condensing benzaldehyde with 2,4,6-trichlorophenylhydrazine (3). Each compound was recrystalized from ethanol and each migrated as a single zone on TLC (silica gel GF_{254}, n-hexane:ethyl acetate, 80:20). Benzohydroxamic acid was prepared as described by Hauser (4). Benzoic acid, benzamide, benzoylhydrazine, benzaldoxime, 2,4,6-trichlorophenylhydrazine, and 2,4,6-trichloroaniline were obtained from commercial sources.

Twospotted spider mites of an organophosphate susceptible strain were reared in the laboratory on bean plants. Mites were harvested from plants by brushing infested leaves with a soft brush. The toxicity of Banamite and potential metabolites to mites was evaluated by the slide-dip technique (5) as used by Ahmad and Knowles (6). Mortality was recorded after 24 hr.

Monoamine oxidase was obtained from freshly dissected rat liver as described by Aziz and Knowles (7). For monoamine oxidase assay the colorimetric technique of Weissbach et al. (8) was used. The procedure for studying the inhibitory potency of Banamite and related compounds was identical to that reported previously (7).

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TABLE 1

Toxicity of Banamite and potential metabolites to twospotted spider mites and potency as inhibitors of rat liver monoamine oxidase

Compound	Structure	Toxicity to spider mites LC_{50}, ppm[a]	Inhibition of rat liver MAO I_{50}, M[b]
Banamite	Ph-CCl=N-NH-$PhCl_3$-2,4,6	12	1.4×10^{-3}
Benzaldehyde 2-(2,4,6-trichlorophenyl) hydrazone	Ph-CH=N-NH-$PhCl_3$-2,4,6	110	1.2×10^{-4}
Benzoic acid 2-(2,4,6-trichlorophenyl) hydrazide	Ph-C(O)-NH-NH-$PhCl_3$-2,4,6	320	$> 1.0 \times 10^{-4}$
2,4,6-Trichlorophenyl hydrazine	H_2N-NH-$PhCl_3$-2,4,6	21	4.7×10^{-5}
2,4,6-Trichloroaniline	H_2N-$PhCl_3$-2,4,6	460	$> 1.0 \times 10^{-3}$
Benzoylhydrazine	Ph-C(O)-NH-NH_2	79	$> 1.0 \times 10^{-3}$
Benzoic acid	Ph-C(O)OH	> 1000	$> 1.0 \times 10^{-3}$
Benzamide	Ph-C(O)-NH_2	485	$> 1.0 \times 10^{-3}$
Benzaldoxime	Ph-CH=NOH	417	$> 1.0 \times 10^{-3}$
Benzohydroxamic acid	Ph-C(O)NHOH	> 1000	-

[a] Slide-dip technique; mortality recorded at 24 hr.

[b] Concentration in moles/liter giving 50% inhibition of rat liver MAO preparation.

RESULTS AND DISCUSSION

Table 1 gives the toxicity of Banamite and potential metabolites to twospotted spider mites and their potency as inhibitors of rat liver monoamine oxidase. Banamite was the most toxic compound with an LC_{50} of 12 ppm. Other toxic compounds included benzaldehyde 2-(2,4,6-trichlorophenyl) hydrazone (LC_{50} 110), 2,4,6-trichlorophenylhydrazine (LC_{50} 21), and benzoylhydrazine (LC_{50} 79). Benzoic acid 2-(2,4,6-trichlorophenyl) hydrazide, 2,4,6-trichloroaniline, benzoic acid, benzamide, benzaldoxime, and benzohydroxamic acid were relatively nontoxic with LC_{50} values greater than 300 ppm.

Benzaldehyde 2-(2,4,6-trichlorophenyl) hydrazone and 2,4,6-trichlorophenylhydrazine were inhibitors of rat liver monoamine oxidase yielding I_{50} values of 1.2×10^{-4} M and 4.7×10^{-5} M, respectively (Table 1). For comparison the I_{50} value for iproniazid, a classical monoamine oxidase inhibitor, was 6.3×10^{-6} M under identical conditions (7).

2,4,6-Trichlorophenylhydrazine has been isolated from spider mites treated with Banamite-^{14}C (1). It is toxic to twospotted spider mites, and it is a relatively potent inhibitor of rat liver monoamine oxidase (Table 1). Thus, it seems likely that this hydrazine metabolite is involved in the toxic action of Banamite acaricide in twospotted spider mites. However, confirmation must wait until difficulties encountered in the assay of spider mite monoamine oxidase have been overcome.

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