Ozonides and Epoxides from Ozonization of Pyrethroids

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Ozonization of pyrethroids as solutions or thin films yields products proposed to be epoxides from the 2,2-dihalovinyl substituents of deltamethrin and permethrin and transitory ozonides from these compounds and more stable ozonides from the 2-methyl-1-propenyl and 2-chloro-3,3,3-trifluoropropenyl substituents of phenothrin and descyanocyhalothrin, respectively. The unstable epoxydeltamethrin from ozonization is identified by ^1H nuclear magnetic resonance spectroscopy and chemical ionization-mass spectroscopy and by reversion to deltamethrin on treatment of reaction mixtures with triphenylphosphine. Degradation of the ozonides yields the corresponding caronaldehyde in each case and trifluoroacetyl chloride from the chlorotrifluoropropenyl analogues. The ozonolysis mixtures are direct acting but weak bacterial mutagens presumably due to their epoxide and ozonide components.

Chrysanthemates with the 2-methyl-1-propenyl substituent are cleaved on ozonization (Ueda and Matsui, 1970) and photooxidation (Ruzo et al., 1982) to yield the corresponding caronaldehyde ester. The putative ozonide intermediate is reported to form on plants treated with phenothrin (Nambu et al., 1980). Chrysanthemate analogues with 2,2-dibromovinyl or 2,2-dichlorovinyl substituents are much more resistant to photooxidation, yielding the corresponding caronaldehyde (Ruzo and Casida, 1982), a keto lactone (Ruzo, 1983), and a substituted bromo epoxide (Ruzo and Casida, 1980).

Mutagens are generated on photooxidation and ozonization of several alkenes. Haloalkene epoxides are known or suspected mutagens and carcinogens (Kline et al., 1982; Van Duuren et al., 1983), and they are also possible intermediates on ozonization of haloalkenes (Griesbaum and Brüggemann, 1972). Formaldehyde and possibly other mutagens are formed on ozonization of propylene (Shepson et al., 1985). Neither pyrethroids (Miyamoto, 1976; Ruzo and Casida, 1977) nor their photoproducts are detected as mutagens except for an α-keto epoxide formed in the alcohol moiety of allethrin (Kimmel et al., 1982). The mutagenicity has not been examined for pyrethroid derivatives formed on ozonization.

This report considers the reactions of selected pyrethroids with ozone and the mutagenicity of the reaction mixtures.

MATERIALS AND METHODS

Chemicals. Designations for the pyrethroids and their derivatives and reaction products are given in Figure 1 and Table I. Pyrethrins (highly refined pyrethrum extract; 40% pyrethrins I and 46% pyrethrins II) were supplied by McLaughlin Gormley King Co. (Minneapolis, MN), the synthetic pyrethoids or their acid moieties were supplied by the basic manufacturers, and [$^{13}CH_3$]-*trans*-phenothrin was supplied by M. A. Brown (Brown et al., 1985). Methyl esters were prepared by treating the carboxylic acids with diazomethane. Spectral data for descyanocyhalothrin are as follows: ^{19}F nuclear magnetic resonance (NMR) (dioxane) δ 13.91; ^1H NMR (chloroform-*d*) δ 1.3 (2 × 3 H, s), 2.02 (1 H, d), 2.19 (1 H, t), 5.12 (2 H, s), 6.82 (1 H, d), 7.0–7.4 (9 H, m); CI-MS *m/z* 425 [$(MH)^+$, 14], 405 [$(M - F)^+$, 18], 183 [100].

Spectroscopy and Chromatography. NMR (Bruker WM 300) spectra for solutions in chloroform-*d*, dioxane-d_8, or cyclohexane-d_{12} were obtained at 300 MHz (^1H), 75.5 MHz (^{13}C), or 282 MHz (^{19}F), and chemical shifts are reported (ppm) downfield from tetramethylsilane (^1H, ^{13}C) or trifluoroethanol (CF_3CH_2OH) (^{19}F). Emphasis in ^1H NMR monitoring of ozonization reactions is given to the region below 4 ppm due to the complexity of signals at higher field attributable to the diastereomeric character of the multicomponent mixtures involved. MS (Hewlett-Packard 5985B) with CI (methane at 0.8 torr, 230 eV) or electron impact (EI) (70 eV) involved samples introduced via direct inlet with heating (60–150 °C) or by gas chromatography (GC). GC–MS used a 10-m methyl silicone high-performance capillary column with helium as the carrier gas (1 mL/min) and temperature programming (80–240 °C/min). Electron capture (EC)-GC (Hewlett-Packard 5830A) involved the SP 2100 (Supelco) glass column (0.6 m, 3 mm) operated at 240 °C with argon/methane (19:1) as the carrier gas (40 mL/min), giving a

Table I. Bacterial Mutagenicity of Ozonized Pyrethroids and Related Compounds

compd (stereo)	mutagenicity,[a] revertants/μg
Chrysanthemates	
allethrin (1*R*,*trans*,α*S*)	1.0–1.1
cyphenothrin (1*RS*,*cis*,α*RS*)	0.5–1.2
ethyl chrysanthemate (1*RS*,*cis*,*trans*)	0.6–0.8
phenothrin (1*RS*,*trans*)	2.4 ± 0.2
pyrethrins	0.9–1.5
resmethrin (1*RS*,*trans*)	<0.5–1.2
tetramethrin (1*RS*,*trans*)	<0.5–0.5
Dihalovinyl Analogues	
cypermethrin (1*R*,*cis*,α*S*)	0.5–0.6
deltamethrin (1*R*,*cis*,α*S*)	3.2 ± 0.6
methyl 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate	0.7–3.2
permethrin (1*R*,*cis*)	0.8–1.4
permethrin (1*R*,*trans*)	1.3–3.0
Chlorotrifluoropropenyl Analogues	
descyanocyhalothrin (1*R*,*cis*,*E*)	0.9–3.8
methyl 3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate (1*R*,*cis*,*E*)	[b]
Acid Moieties Lacking Alkene Substituent	
methyl 2-(4-chlorophenyl)-3-methylbutyrate	<0.2
methyl *N*-[2-chloro-4-(trifluoromethyl)phenyl]valinate	<0.2
methyl 2,2,3,3-tetramethylcyclopropanecarboxylate	<0.2

[a] *S. typhimurium* strain TA100 preplating assay for direct mutagenic activity. Range of values for two or three independent experiments or means ± standard errors for 6–15 experiments. The values for each compound before ozonization are <0.05 revertant/μg [see also Kimmel et al. (1982)]. [b] Mutagenicity ≤0.1 revertant/μg before ozonolysis. Bactericidal activity of the ozonized product precluded sensitive mutagenesis assay.

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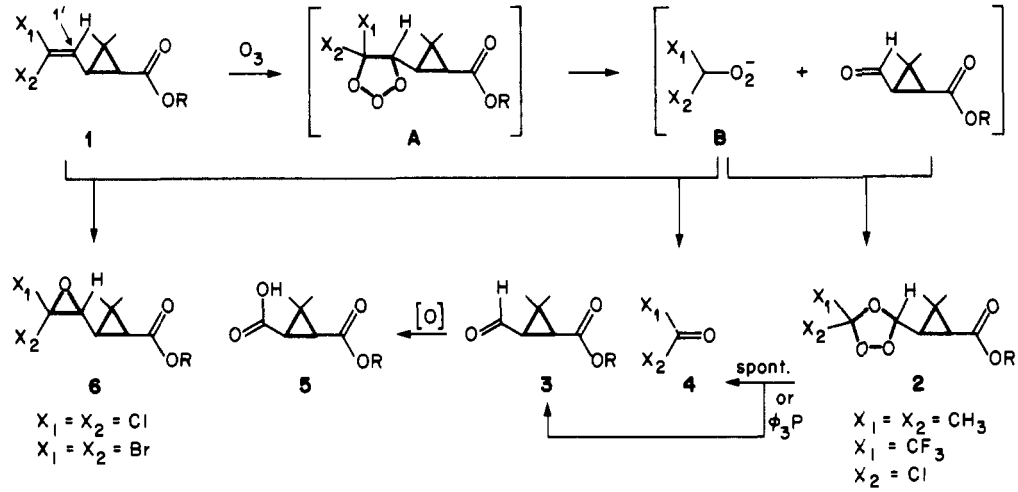

Figure 1. Ozonization reactions of 3-(2,2-disubstituted vinyl)-2,2-dimethylcyclopropanecarboxylates 1 involving intermediate molozonides A, Criegee intermediates B, and ozonides 2. Epoxide formation may also occur by direct addition of ozone. R = 3-phenoxybenzyl: $X_1 = X_2 = CH_3$, (1*RS*)-*trans*-phenothrin; $X_1 = X_2 = Cl$, (1*R*)-*cis*-permethrin; $X_1 = CF_3$, $X_2 = Cl$, (1*RS*)-*cis*-descyanocyhalothrin. R = α-cyano-3-phenoxybenzyl: $X_1 = X_2 = Br$, deltamethrin.

retention time of 9.7 min for deltamethrin.

Ozonization. Reactions were carried out as solutions or thin films to 10–99% conversion. The pyrethroid or derivative (4 mg) dissolved in dioxane-d_8, cyclohexane-d_{12}, or other solvent (2 mL) as specified was treated for 40 s with a stream of dry ozone (1.75 mmol/min) generated from a Welsbach T-816 laboratory ozonator (Welsbach Ozone Systems Co., Philadelphia, PA). Alternatively, the test compound (4 mg) was added in acetone to a 50-mL round-bottom flask, and the solvent was evaporated to yield a thin film of 30–60 μg/cm^2. Care was taken to minimize volatilization loss of the methyl and ethyl esters. The sample flask was placed in an ice bath and treated with the ozone gas stream for 1 min. The temperature was maintained at 10 °C or below until analysis. The reaction mixtures were examined directly by NMR comparison with standards and by MS, or they were evaporated to dryness and redissolved in an appropriate solvent for column, thin-layer, or high-pressure liquid chromatography or for Ames assay. Some reaction mixtures in dioxane-d_8 were treated with triphenylphosphine (1.0 equiv relative to the starting pyrethroid) for 72 h at 25 °C to cleave the ozonides (Fieser and Fieser, 1967) or to convert the epoxides to the starting alkenes (March, 1985) or with a catalytic amount of zinc chloride in 1 N hydrochloric acid to destroy the ozonides.

Mutagenicity Testing. The assays used *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 (Maron and Ames, 1983) provided by B. N. Ames (Department of Biochemistry, University of California, Berkeley) and the preplating technique (Barber et al., 1983) in which the bacteria are plated in 2 mL of top agar and (plus histidine) incubated for 6 h at 37 °C and the compounds then applied in a second 2-mL portion of agar (minus histidine). Following ozonization of the pyrethroid or derivative as a thin film (see above), residual ozone, a potent bactericide, was removed by flushing the reaction flask with nitrogen. The reaction products were dissolved in dioxane (0.5 mL) and appropriate aliquots subjected to assay. Dioxane does not interfere with the Ames assay in amounts up to 50 μL.

RESULTS

General Observations. Table I includes some pyrethroids and related compounds with an alkene substituent in the acid moiety (chrysanthemates and their dihalovinyl and chlorotrifluoropropenyl analogues) and others lacking an alkene substituent. When examined as thin films, compounds of the first type react with ozone and those of the second type do not on the basis of GC–MS analysis [methyl[chloro(trifluoromethyl)phenyl]valinate] or NMR examination (the chrysanthemates phenothrin, cyphenothrin, and ethyl chrysanthemate and each of the dihalovinyl and chlorotrifluoropropenyl analogues). As detailed below, the alkene-containing pyrethroids and derivatives yield mutagenic products and the nonalkene compounds do not. Emphasis is therefore given to the more reactive chrysanthemates and their dihalovinyl and chlorotrifluoropropenyl analogues.

Carefully selected reaction conditions are required to obtain products of interest, based on results with deltamethrin and descyanocyhalothrin. Acetonitrile, acetone, dichloromethane, methanol, and tetrahydrofuran are not suitable solvents for ozonization due to reaction of ozone with the solvent, instability of the products (even at <0 °C), or incompatibility in the Ames test. Ozonization in hydrocarbon solvents gives similar results at −60 to +10 °C. The products are relatively stable when generated in hexane, cyclohexane, or dioxane or as thin films but decompose on attempted chromatography. Precipitates are sometimes obtained from reaction mixtures in hexane, but their composition is similar to that of the supernatant. *Caution*: Explosions occurred on two occasions when precipitates from ozonized descyanocyhalothrin were dried under vacuum.

Chrysanthemates. Ozonization of (1*RS*)-*trans*-phenothrin in cyclohexane-d_{12} or as a thin film to >95% conversion yields a mixture containing carboxaldehyde **3** [^{1}H NMR δ 9.57, CHO; CI-MS *m/z* 325 [(MH)$^+$, 18], 183 [100]]; carboxylic acid **5**, identified as the methyl ester (diazomethane) GC–CI-MS [*m/z* 355 [(MH)$^+$, 11], 183 [100]]; acetone (**4**) (^{1}H NMR δ 2.01); and small and variable amounts of formic acid (δ 7.91). No epoxyphenothrin is detected. Formation of ozonide **2** from *trans*-phenothrin is suggested by the presence of an additional product giving a fragment at *m/z* 365 by EI- or CI-MS (direct introduction), possibly by loss of hydrogen peroxide from **2**, and two ^{1}H NMR doublets at δ 6.80 and 6.82 (*J* = 9.6 Hz), appropriate for an ozonide, which disappear on treatment with triphenylphosphine, increasing the yield of **3** and of acetone. Reaction of [$^{13}CH_3$]-*trans*-phenothrin with ozone and inspection by ^{13}C NMR (cyclohexane-d_{12}) reveal small amounts of acetic acid (δ 20.8) plus signals at δ 21.0 and

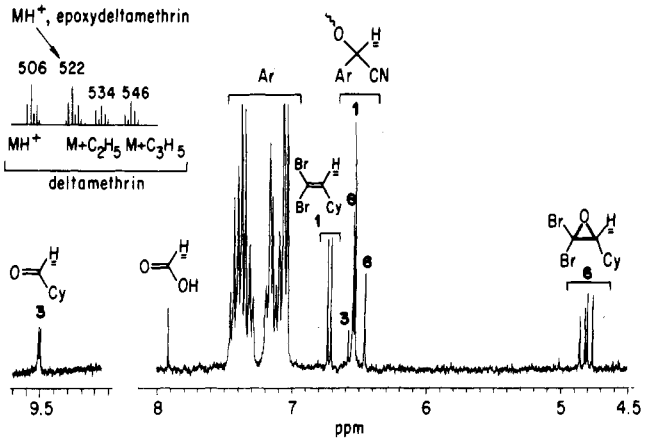

Figure 2. Partial ^1H NMR (dioxane-d_8) and CI-MS spectra of deltamethrin ozonized as a thin film. Ar = 3-phenoxyphenyl. Cy = 3-substituted 2,2-dimethylcyclopropanecarboxylate. ^1H NMR assignments are indicated by partial structures with reference to compounds designated by numbers in Figure 1. The δ 9.0–9.7 region is shown with a ~4-fold sensitivity enhancement.

21.2 tentatively assigned to the ozonides. Ozonization of cyphenothrin used for comparison gives an m/z 390 fragment, consistent with loss of hydrogen peroxide from the corresponding ozonide.

Dihalovinyl Analogues. Deltamethrin gives two major products at 70% conversion as a thin film (Figure 2) or in dioxane-d_8, i.e. aldehyde **3** [^1H NMR δ 9.61 (d, J = 6 Hz); CI-MS m/z 350 [$(MH)^+$, 18]] and a second product in greater yield proposed to be epoxydeltamethrin (**6**). The latter compound analyzed in the mixture by CI-MS shows $[MH]^+$ m/z 522 (based on ^{81}Br), and ^1H NMR reveals two doublets at δ 4.78 and 4.85 (J = 11.1 Hz) as appropriate for epoxydeltamethrin (Figure 2). Epoxidation rather than ozonide formation is further supported by three observations: the upfield shift of the C-1′ proton; a positive 4-(*p*-nitrobenzyl)pyridine test (Hammock et al., 1974); regeneration of deltamethrin on triphenylphosphine treatment (March, 1985) as established by ^1H NMR (increase in δ 6.70–6.75 signals due to the deltamethrin vinylic proton and an associated decrease in δ 4.78 and 4.85 signals of the corresponding proton of epoxydeltamethrin; see Figure 2) and by EC-GC analysis of ozonized mixtures before and after triphenylphosphine addition. Formic acid is a minor product.

(1*R*)-*cis*-Permethrin behaves similarly to deltamethrin, giving two products on treatment with ozone to 30% conversion in the solid phase, the major one being aldehyde **3** (characterized as above) and the minor one exhibiting a pair of ^1H NMR doublets at δ 5.18 and 5.20 indicative of epoxypermethrin. Decomposition with triphenylphosphine does not increase the yield of **3** but instead appears to regenerate **1**. The products from permethrin are more reactive than those from detamethrin.

Chlorotrifluoropropenyl Analogues. Reaction of descyanocyhalothrin with ozone to >75% conversion in dioxane-d_{12} or as a thin film proceeds ~30-fold slower than reaction of *trans*-phenothrin with ozone based on ^1H NMR. At 70% conversion the ozonolysis products in dioxane-d_8 or cyclohexane-d_{12} or as a thin film consist of **2**–**5** (Figure 1). The ozonides **2** are characterized by ^{19}F and ^1H NMR (Figure 3) and by their breakdown to the caronaldehyde ester **3** [^1H NMR δ 9.57 (CHO, d, J = 6 Hz); CI-MS as above] and trifluoroacetyl chloride (**4**) (^{19}F NMR identical with standard compound) on standing or on treatment with triphenylphosphine (Fieser and Fieser, 1967). **4** adds water to give trifluoroacetic acid (Figure 3).

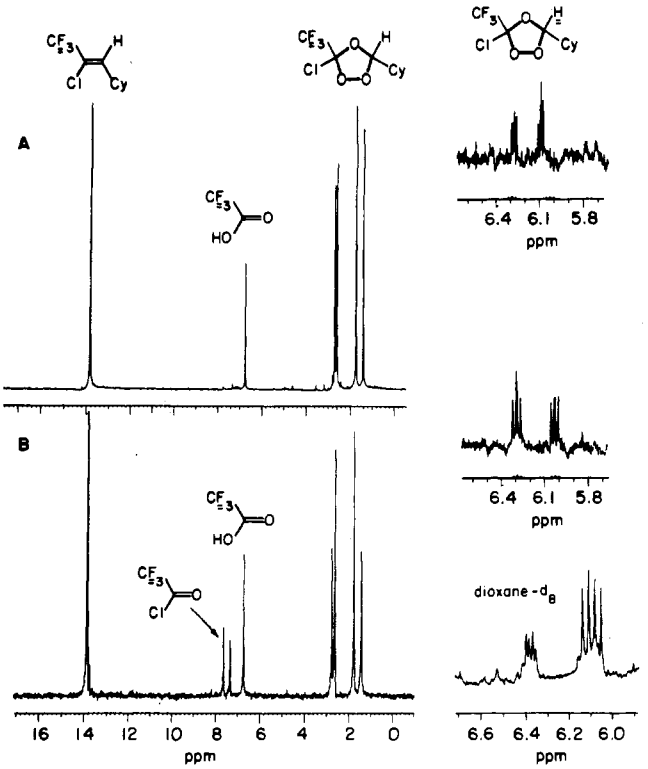

Figure 3. ^{19}F NMR (cyclohexane-d_{12}) spectra of methyl (1*RS*)-*cis*-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate (**A**) and descyanocyhalothrin (**B**) ozonized as thin films. Trifluoroacetic acid is formed by hydrolysis of trifluoroacetyl chloride. Partial ^1H NMR (cyclohexane-d_{12}) spectra are also shown for ozonized methyl ester and descyanocyhalothrin. Cy = 3-substituted 2,2-dimethylcyclopropanecarboxylate. The lower right spectrum illustrates improved resolution in dioxane-d_8.

Descyanocyhalothrin and the corresponding methyl ester undergo the same ozonization reactions as thin films on the basis of ^1H and ^{19}F NMR (Figure 3) except for the isomeric distribution on formation of **2**; i.e., while the four ^{19}F NMR singlets corresponding to each isomer of the E/Z,1*RS* mixture are of approximately equal area in the methyl ester (Figure 3A), the phenoxybenzyl ester shows some stereospecificity to ozone addition (Figure 3B). In some experiments the isomers exhibiting ^1H NMR signals at δ 6.0–6.2 comprise >70% of the diastereomeric ozonides obtained as thin films (Figure 3B), but less isomeric differences are observed on ozonization in cyclohexane-d_{12}.

Mutagenesis. The mutagenicity (revertants/μg) of a pyrethroid/ozone reaction mixture is dependent on the assay conditions, the ozonization procedure, and the compound involved. The mutagenicity of ozonized deltamethrin is as follows in direct comparisons of the same sample with four *S. typhimurium* strains: 0.8 with TA97, <0.1 with TA98, 5.3 with TA100, and 2.3 with TA102. The preplating assay enhances the mutagenicity, e.g. 1.1 for ozonized deltamethrin without preplating. All other results reported are therefore with strain TA100 and the preplating assay. Dioxane proved to be more suitable than acetonitrile, acetone, hexane, or tetrahydrofuran as a nonreactive solvent suitable as a carrier for the Ames assay.

Mutagenic products are formed on ozonization of several pyrethroids and derivatives of their acid moieties (Table I). As indicated above, compounds with alkene substituents in the acid moeity give higher mutagenicity following ozonization than those lacking an alkene substituent. The mutagenicity of the chrysanthemates and their dihalovinyl and chlorotrifluoropropenyl analogues is generally increased on ozonization from <0.2 to 0.5–4 revertants/μg.

The mutagenic products are unstable in the presence of triphenylphosphine, i.e. 6-fold loss of mutagenicity for the descyanocyhalothrin/ozone reaction mixture.

DISCUSSION

Plausible mechanisms for the ozonization reactions of pyrethroids are shown in Figure 1. The relative yields of ozonides, epoxides, and aldehydes depend in part on the substituent undergoing reaction but also on variations in the thickness of the exposed films, the ozone concentration, and inherent product instability. Both epoxides and ozonides are formed with the dihalovinyl analogues while only ozonides are detected with the chrysanthemates and the chlorotrifluoropropenyl analogues. Epoxide formation may involve direct addition of ozone (Murray and Suzui, 1973; Ruzo et al., 1982). Alternatively it may result from peroxygenated species **B** (the "Criegee intermediate") reacting with starting material **1** to give **4** and **6** to a greater extent than **B** reacts with the caronaldehyde to give **2** (Agopovich and Gillies, 1983). Stereoselectivity on ozonolysis of the chlorotrifluoropropenyl analogues may be due to steric hindrance in molozonide and ozonide formation. Detection of the ozonide on phenothrin-treated leaves (Nambu et al., 1980) may be favored by the apolar environment of the cuticular hydrocarbons since in our studies the products are most stable in alkane solvents.

Ozonization processes are of potential environmental relevance, e.g. atmospheric oxidations (Hatakeyama et al., 1985), wastewater treatment procedures (Peyton et al., 1982; Richard and Brener, 1984), and mutagen formation in surface waters (Van Hoof et al., 1985). The present study establishes that ozone degrades many pyrethroids via transitory epoxides and ozonides and that these oxidation products may contribute to the bacterial mutagenicity of the reaction mixtures. Ozonolysis as an environmental process or waste disposal procedure may generate hazardous intermediates. Although illustrated here with pyrethroids, the toxicological relevance of ozonization processes should also be considered with other natural products and environmental pollutants.

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