Identification of Mutagenic Photoproducts of the Pyrethroids Allethrin and Terallethrin

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Allethrin and terallethrin exposed as thin films to light and air are converted in low yields to potent mutagenic derivatives (85–127 revertants/nmol in the Ames *Salmonella typhimurium* TA100 assay) identified by spectroscopic methods as esters of 1-cyclopropyl-4-hydroxy-5-methyl-6-oxabicyclo-[3.1.0]hexan-2-one formed by di-π-methane rearrangement of the allyl group followed by epoxidation of the hydroxycyclopentenone moiety. The mutagenic photoproduct of terallethrin was synthesized by peroxy acid oxidation of the corresponding hydroxycyclopropylcyclopentenone. Other mutagenic photoproducts of allethrin may contain the indicated alcohol moiety esterified with various oxidized derivatives of chrysanthemic acid. Metabolic activation assays with the TA100/S9 system revealed mutagen formation from the cyclopropyl derivatives of allethrin and terallethrin but not from the parent pyrethroids. Other commercial pyrethroids examined yield photoproduct mixtures in sunlight with no more than 10% of the mutagenic activity of photodecomposed allethrin and terallethrin.

Pyrethroids in general show no direct mutagenic activity in a variety of assays (Miyamoto, 1976; Ruzo and Casida, 1977). All pyrethroids studied thus far yield a large number and considerable diversity of metabolites and photoproducts which also need to be evaluated for mutagenic potential. Allethrin is reported to have mutagenic activity in two in vitro screens, i.e., the *Salmonella* assay and the chromosomal aberration test in Chinese hamster cells when treated with liver S9 mix for activation (Matsuoka et al., 1979). It is therefore important to clarify the possible mutagenesis of pyrethroids and their metabolites and photoproducts with emphasis on allethrin.

This investigation uses the Ames *Salmonella typhimurium* assay (Ames et al., 1975) to evaluate conditions under which mutagenic photoproducts and metabolites arise from pyrethroid insecticides and to monitor the isolation of mutagenic photoproducts of allethrin and related compounds.

MATERIALS AND METHODS

Chromatography. Thin-layer chromatography (TLC) utilized silica gel F_{254} chromatoplates prewashed with

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acetone and of 0.25-mm gel thickness (for analysis) or 0.5-mm gel thickness (for preparative isolations) with development as follows: (A) carbon tetrachloride–ether (3:1); (B) hexane–ether (4:1, two developments). Compounds were detected by viewing under UV (254 nm) or spraying with 4-(p-nitrobenzyl)pyridine reagent to reveal epoxides as blue-violet spots (Hammock et al., 1974). In preparative isolations, the purified products were recovered from the gel by extraction with ether and then stored in hexane at –20 °C.

High-pressure liquid chromatography (HPLC) utilized the Waters Associates 6000A instrument equipped with a 254-nm absorbance detector and a μPorasil column (7.8 mm i.d. × 30 cm) eluted with chloroform–acetonitrile mixtures at a flow rate of 2 mL/min.

Spectroscopy. Chemical ionization mass spectra (CI-MS) were obtained with a Finnigan 3200 instrument coupled to a computerized data system by using methane (~0.8 torr) as the reagent gas, an ionization voltage of 40–70 eV, and the inlet probe at 40–120 °C as appropriate. Masses and relative intensities are given for quasi-molecular ions $(M + 1)^+$ and several major fragments. ^{1}H nuclear magnetic resonance (NMR) spectra were obtained at 250 MHz in deuteriochloroform. Chemicals shifts (δ) are reported as downfield from tetramethylsilane and coupling constants (J) are given in hertz.
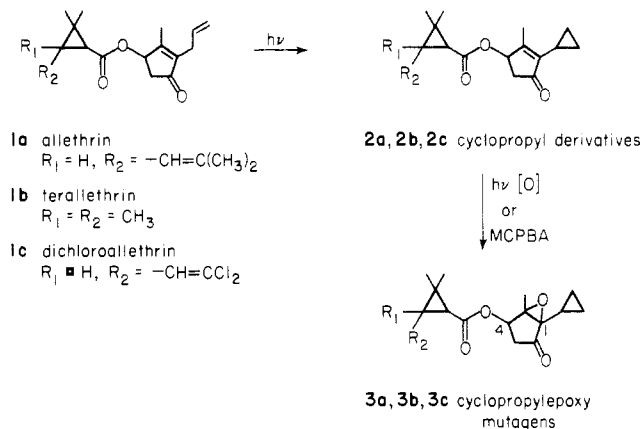
Figure 1. Photochemical pathways of S-bioallethrin, terallethrin, and dichloroallethrin involved in their conversion to mutagenic esters of 1-cyclopropyl-4-hydroxy-5-methyl-6-oxabicyclo[3.1.0]-hexan-2-one.

Chemicals.

Allethrin (1a) (Schechter et al., 1949), terallethrin (1b) (Matsui and Kitahara, 1967), dichloro-allethrin (1c) (Farkaš et al., 1959), and some of their de-rivatives are shown in Figure 1. Commercial pyrethroids were obtained from their basic manufacturers (Gammon et al., 1981). (1R,trans)-Chrysanthemic acid and (1R,-trans)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid were gifts from Roussel-Uclaf (Paris, France), 2,2,3,3-tetramethylcyclopropanecarboxylic acid was from Shell Development Co. (Modesto, CA), and al-lethrolone was from Sumitomo Chemical Co. (Osaka, Ja-pan). The identity and purity of each sample were con-firmed by CI-MS and by TLC or gas chromatography.

Allethronyl acetate (bp 92 °C/0.2 mmHg) was irradiated (Pyrex, 300 nm) at 3 mM in degassed hexane for 16 h to give the cyclopropyl derivative (80% yield) (Bullivant and Pattenden, 1976), which was purified by column chroma-tography (silicic acid, 5% ether in hexane): CI-MS 195 (M + 1, 28), 135 (M − 59, 100). The acetate was hydrolyzed on treatment with K_2CO_3 in methanol (10% w/v, 25 °C, 1 h; >90% yield): CI-MS 153 (M + 1, 100). The resulting cyclopropyl derivative of allethrolone or allethrolone itself was used to prepare 1b, 1c, and 2a–c (Figure 1) on reaction with the corresponding acid chloride in benzene with equivalent pyridine at reflux (3 h). The esters (>90% yield; appropriate CI-MS and NMR) were purified by addition of ether, washing with saturated NaCl, and col-umn chromatography (Florisil, hexane).

Photolysis Procedures. Thin films (~0.3 mg/cm²) of pyrethroid in Petri dishes with Pyrex covers were exposed to sunlight or UV light (RPR 3500 lamps, Rayonet reactor, Southern N. E. Ultraviolet Co., Middletown, CT), both procedures yielding similar photoproduct mixtures. The irradiated films in each 14-cm diameter dish were rinsed with hexane (4 mL × 4) to obtain the apolar products and then with acetone (4 mL × 4) to obtain the polar products.

Mutagenesis Assays. Standard procedures were used for Ames assays and preparation of the S9 mix (Ames et al., 1975). The TA100 strain was used unless specifically indicated otherwise. Test compounds and fractions were dissolved in dimethyl sulfoxide for assay.

RESULTS

Mutagenic Activities of Pyrethroids and Their Photoproduct Mixtures. Eleven pyrethroids showed trace or no mutagenic activity in the TA100 assay (Table I). Five photoproduct mixtures formed on exposure of thin films of these pyrethroids to sunlight for 1–24 h showed definite mutagenic activity with varying times for

Table I. Mutagenic Activity in the Ames S. typhimurium TA100 Assay of Pyrethroids and Their Photoproduct Mixtures Formed on Exposure of Thin Films to Sunlight for Up to 24 Hours

pyrethroid	revertants/μg after indicated time[a]			
	0 h	1 h	6 h	24 h
S-bioallethrin	<0.05	0.8	2.0	0.3
allethrin[b]	0.1	0.7	1.9	1.5
pyrethrins[c]	<0.05	<0.05	<0.05	0.2
tetramethrin[b]	<0.05	0.2	<0.05	<0.05
permethrin[b]	<0.05	<0.05	0.2	0.1

[a] Compounds with <0.05 revertants/μg at 0, 1, 6, and 24 h are as follows: 90–95% technical-grade resmethrin, cypermethrin, and fenvalerate; 95+% kadethrin, delta-methrin, and fenpropathrin. [b] 90–95% technical grade. [c] Pyrethrum extract consisting of 40% pyrethrins I and 46% pyrethrins II.
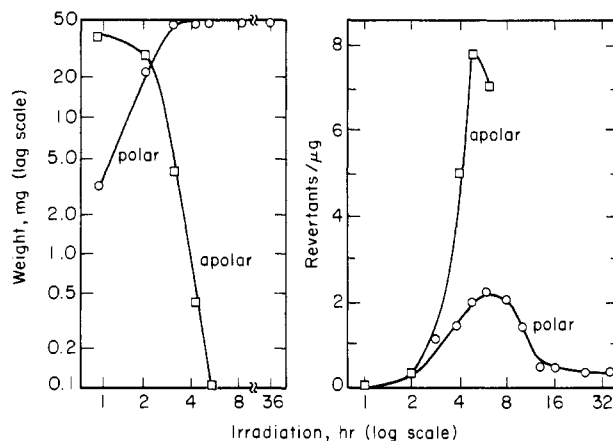

Figure 2. Conversion of S-bioallethrin to polar derivatives and photoproducts mutagenic in the Ames S. typhimurium TA100 assay on exposure as thin films to sunlight.

maximum potency, i.e., 1 h for tetramethrin, 6 h for al-lethrin and permethrin, and 24 h for pyrethrins. The photolyzed mixtures derived from S-bioallethrin (single isomer) and technical allethrin (eight isomer mixture with 10% impurities) were 10-fold more potent (~2 rever-tants/μg) than those from other pyrethroids (up to 0.2 revertant/μg). An allethrin photoproduct mixture giving 2.0 revertants/μg with the TA100 strain gave only 0.03 revertant/μg with the TA98 strain. These findings served to focus attention on allethrin and the TA100 strain in further studies. They also suggested that the critical re-action(s) takes (take) place in the allethronyl moiety, speculation supported by the finding that allethronyl acetate yields a mutagenic photoproduct mixture.

Optimizing Conditions for Mutagen Formation on Photolysis of Allethrin and Related Compounds. S-Bioallethrin photoproduct mixtures reached maximum mutagenic activity after 6–8 h of sunlight exposure with the same approximate peak for total mutagenic activity and specific activities for the apolar and polar fractions (Figure 2). Polar photoproducts predominate at longer irradiation times, and the mutagenic activity is greatly decreased with exposures of 14 h or longer. The finding of both apolar and polar mutagen fractions indicated that two or more components contribute to the mutagenesis. Further studies emphasized the apolar fraction because it contained fewer components and they were more easily separable.

The cyclopropyl derivative of allethrin (2a) is a major photoproduct and contains the only identified modification of the alcohol moiety (Bullivant and Pattenden, 1976; Ruzo et al., 1980). It was therefore of interest to compare al-

Allethrin and Terallethrin Mutagenic Photoproducts

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Table II. Mutagenic Activity in the Ames *S. typhimurium* TA100 Assay of *S*-Bioallethrin, Terallethrin, Dichloroallethrin, and Their Cyclopropyl Derivatives following Exposure of Thin Films to Sunlight for Four Hours

	revertants/μg			% apo-lar
compound	total	apo-lar[a]	po-lar[a]	by wt[a]
S-bioallethrin (1a)	1.7	4.5	1.7	0.8
cyclopropylallethrin (2a)	2.8	4.5	2.1	29
terallethrin (1b)	3.2	7.6	0.8	35
cyclopropylterallethrin (2b)	18	18.4	6.1	95
dichloroallethrin (1c)	1.5	5.7	1.4	2.2
cyclopropyldichloroallethrin (2c)	29	38.8	22.7	40

[a] Hexane-soluble portion designated as apolar fraction and remainder as polar fraction.

lethrin (1a) with its cyclopropyl derivative (2a) and with related compounds containing more photostable acid moieties, i.e., terallethrin (1b), dichloroallethrin (1c), and their cyclopropyl derivatives (2b and 2c) (Table II). The overall mutagenic activities (total revertants per microgram) were similar for *S*-bioallethrin, terallethrin, and dichloroallethrin, further indicating the importance of the alcohol moiety. In contrast, photolyzed mixtures from the cyclopropyl derivatives gave higher (2a) to much higher (2b and 2c) mutagenic potency. The cyclopropyl derivatives also gave a greatly increased yield by weight of apolar products (3–36-fold). These findings reaffirmed the importance of alcohol moiety modifications and suggested that the cyclopropyl derivatives may be intermediates in mutagen formation. They also verified the importance of acid moiety stability in enhancing the yields of apolar mutagens.

Isolation of Mutagens from Photolysis of Allethrin, Terallethrin, Dichloroallethrin, and the Cyclopropyl Derivatives of Terallethrin and Dichloroallethrin. *S*-Bioallethrin (1a) (3 g) irradiated as thin films for 4 h yielded 600 mg of apolar fraction and 2400 mg of polar fraction with specific activities of 4 and 1–2 revertants/μg, respectively. HPLC of the apolar fraction with chloroform–acetonitrile (99:1) revealed many products eluting in the R_t range of 6–14 min, including a very minor component (R_t = 11.5 min, UV absorbance) with all of the

mutagenic activity (Ames assay). The mutagen was eluted completely before 1a and 2a, which required chloroform-acetonitrile (49:1) or more polar mixtures for elution. One HPLC purification of the mutagen increased its specific activity to 120 revertants/μg, and repurification on HPLC yielded 0.14 mg of product of 400 revertants/μg for CI-MS and NMR examinations.

Cyclopropylterallethrin (2b) (600 mg) on photolysis of thin films for 6 h gave 150 mg of apolar fraction, which was subjected to TLC (solvent system A) with recovery of the upper band evident under UV light (Table III). Repurification in the same manner gave 1.2 mg of product of 290 revertants/μg for CI-MS and NMR spectroscopy. The same product (CI-MS) was recovered with terallethrin (1b) as starting material.

Dichloroallethrin and its cyclopropyl derivative (1c and 2c) were irradiated on a few milligrams scale, and the appropriate products isolated by TLC (solvent system A) were subjected to CI-MS, giving identical compounds from 1c and 2c.

Identification and Synthesis of Mutagens from Allethrin, Terallethrin, and Dichloroallethrin. The purified mutagenic products derived from each of 1a–c, 2b, and 2c gave a blue-violet spot (R_f values as indicated for 3a–c, Table III) on spraying TLC plates with the 4-(nitrobenzyl)pyridine reagent, suggesting that they contained epoxide substituents. CI-MS of 3a–c revealed incorporation of oxygen, i.e., (M + 1)$^+$ with 16 mass units greater than that of the parent pyrethroid. Mutagens 3a–c have unmodified acid moieties since their acylium ions were the same as those of the parents (Table III). Each mutagen therefore contains an epoxide of allethrolone or an isomeric material esterified with the original acid moiety. NMR of the mutagenic products from 1a and 2b confirmed the unmodified acid moieties and established that changes in the alcohol moiety included the absence of vinylic protons, the presence of cyclopropyl protons, and a shift in the allylic methyl group (Table III).

The spectroscopic data (Table III) are consistent with 3a and 3b as the structures of the allethrin and terallethrin mutagens. Attempts to prepare 3a proved unsuccessful because of the reactivity of the isobutenyl substituent toward oxidation. Fortunately, it was possible to synthesize 3b and 3c. Thus, 2b and 2c were treated with 10 equiv of *m*-chloroperoxybenzoic acid (MCPBA) in deuteriochloroform at 25 °C over a period of 24 h. The ep-

Table III. Chromatographic and Spectroscopic Properties of Allethrin (1a), Terallethrin (1b), Dichloroallethrin (1c), and Their Photoproducts

com-pound	TLC R_f A	TLC R_f B	CI-MS (rel intensity) (M + 1)$^+$	acylium ion	other fragments	NMR (CDCl$_3$), δ[a] H$_a$	H$_b$	H$_c$	H$_d$	H$_e$[b]
1a	0.38	0.42	303 (14)	151 (34)	163 (12), 135 (100)	5.68	2.88	2.25	2.04	
2a	0.43	0.46	303 (33)	151 (54)	163 (96), 135 (100)	5.60	2.77	2.12[b]	2.07	1.1, 0.7–0.9
3a	0.48	0.53	319 (7)	151 (100)	179 (13)	5.24	2.75	2.01	1.54	0.5–0.9
1b	0.37	0.40	277 (32)	125 (30)	163 (22), 143 (69), 135 (100)	5.68	2.86	2.27	2.03	
2b	0.41	0.44	277 (19)	125 (33)	163 (14), 143 (15), 135 (100)	5.59	2.78	2.19	2.06	1.1, 0.7–0.9
3b	0.47	0.52	293 (6)	125 (100)	151 (48)	5.25	2.73	2.02	1.54	0.5–0.9
1c	0.34	0.37	343 (4)	191 (12)	163 (45), 135 (100)					
2c	0.40	0.40	343 (8)	191 (62)	209 (40), 135 (100)					
3c	0.46	0.48	359 (6)	191 (41)	209 (26), 151 (100)					

[a] Coupling constants are as follows: for hydroxycyclopentenones 1a, 2a, 1b, and 2b, $J_{H_a\text{-}H_b}$ = 2.0, $J_{H_b\text{-}H_c}$ = 18.7, and $J_{H_a\text{-}H_c}$ = 6.1; for epoxide derivatives 3a and 3b, $J_{H_a\text{-}H_c}$ = 6.6 and $J_{H_b\text{-}H_c}$ = 18.7. [b] Multiplet, coupling obscured.
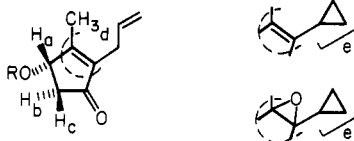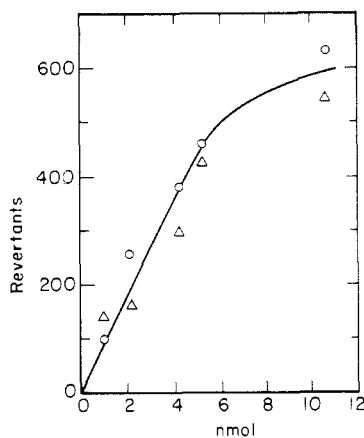
Figure 3. Mutagenic activity in the Ames S. typhimurium TA100 assay of a cyclopropylepoxy derivative of terallethrin (3b) as isolated from cyclopropylterallethrin photoproduct mixtures (O) and obtained on synthesis (△).

oxides were isolated by TLC (solvent system A) and characterized by CI-MS and in the case of **3b** by NMR as well. The yields were low (2–10%). Synthetic **3b** and **3c** were identical with the isolated mutagenic photoproducts in their spectroscopic properties (Table III) and in the case of **3b** also in mutagenic activities (Figure 3). The mutagenic activities were 400 revertants/μg or 127 revertants/nmol for **3a** and 290 revertants/μg or 85 revertants/nmol for **3b**. In comparison, synthetic **3b** yielding 290 revertants/μg with the TA100 strain gave less than 5 revertants/μg with the TA98, TA1535, TA1537, and TA1538 strains.

Mutagenic Derivatives of S-Bioallethrin, Terallethrin, and Their Cyclopropyl Derivatives from Exposure to Sunlight and Metabolism by the Liver S9 Mix. In comparison with the allyl derivatives **1a** and **1b**, the cyclopropyl derivatives **2a** and **2b** yield photoproduct mixtures of greater mutagenic activity. The same relationship applies to metabolic activation experiments where the highest mutagenic activity was obtained from cyclopropylallethrin (**2a**) (Table IV).

DISCUSSION

The photochemical pathway for mutagen formation from allethrin, terallethrin and related compounds is shown in Figure 1. This process clearly requires the re-thronyl moiety with an allyl side chain.

Maximum mutagen yields can be calculated by assuming that the only mutagens are cyclopropylepoxy derivatives and that their specific activity is unaltered on modifying the acid moiety. This gives a yield of 0.4% from allethrin and 1.1% from terallethrin. A much lower yield is obtained for apolar mutagens **3b** and particularly **3a** with unmodified acid moieties, since in the latter case the isobutenyl substituent is much more susceptible than the alcohol moiety to photooxidation, i.e., epoxides, alcohols, aldehydes, acids, and hydroperoxides are formed (Ruzo et al., 1980, 1982). Several factors probably contribute to the low yields. The di-π-methane rearrangement is relatively inefficient at sunlight wavelengths (Ruzo et al., 1980). The α,β-unsaturated ketone deactivates the hydroxycyclo-

Table IV. Mutagenic Activity in the Ames S. typhimurium TA100 Assay of S-Bioallethrin, Terallethrin, and Their Cyclopropyl Derivatives following Exposure as Thin Films to Sunlight and Metabolism by the Liver S9 Mix

| | revertants/μg | | | |
| | sunlight | | metabolism | |
compound	0 h	4 h	−S9	+S9
S-bioallethrin (1a)	<0.05	1.7	<0.1	<0.1
cyclopropylallethrin (2a)	<0.05	2.8	<0.1	1.9
terallethrin (1b)	<0.05	3.2	<0.1	<0.1
cyclopropylterallethrin (2b)	<0.05	18	<0.1	0.3

pentenone double bond toward electrophilic reaction and the cyclopropyl group may provide steric hindrance to oxidation. The recovered yields are also low due to the instability of the epoxides; e.g., **3a** readily undergoes acid-catalyzed alcoholysis and decomposes under some TLC and column chromatographic conditions.

The identified mutagenic photoproducts of allethrin and terallethrin may also be minor microsomal oxidase metabolites of their cyclopropyl derivatives (i.e., **2a** and **2b**) based on the S9-activated Ames test. In assays of this type, care must be taken to use allethrin free of its cyclopropyl derivative. Identification of allethrin and terallethrin mutagenic photoproducts should facilitate studies on their possible occurrence under actual use conditions and relevance, if any, in nonbacterial systems.

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