Photolysis of AMDRO Fire Ant Insecticide Active Ingredient Hydramethylnon (AC 217,300) in Distilled Water[1]

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AMDRO fire ant insecticide, the active ingredient of which is AC 217,300 [hydramethylnon; tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone [3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)-phenyl]ethenyl]-2-propenylidene]hydrazone], is an amidinohydrazone type of insecticide. Photodegradation of AC 217,300 ^{14}C labeled at the benzylic and pyrimidine-2 positions was carried out separately under borosilicate-filtered xenon arc lamps at 27 °C as a suspension in distilled water. AC 217,300 degraded rapidly in water under the light. The half-life of AC 217,300 was calculated as 42 min. The photoproducts of AC 217,300 were characterized with both ^{14}C- and ^{13}C-labeled starting materials. Identifications of the photolytic products were accomplished by CIMS in both the PI and NI modes along with the HPLC and TLC of the photoproduct with the standard reference compound. Compounds 1,5-bis(α,α,α-trifluoro-*p*-tolyl)-1,4-pentadien-3-one (photoproduct **1**), α,α,α-trifluoro-*p*-toluic acid (photoproduct **2**), and *p*-(trifluoromethyl)cinnamic acid (photoproduct **3**) were characterized as photoproducts of benzylic ^{14}C-labeled AC 217,300. In addition, 6,7,8,9-tetrahydro-7,7-dimethyl-3-[*p*-(trifluoromethyl)styryl]-4*H*-pyrimido[2,1-*c*]-*as*-triazin-4-one (photoproduct **4**) was characterized as a photoproduct from both benzylic and pyrimidine-2 ^{14}C-labeled AC 217,300.

INTRODUCTION

A series of amidinohydrazones was described by Lovell (1979) as a new class of insecticides that is selectively toxic to insects with chewing or sponging mouthparts. One member of this series, AC 217,300 [hydramethylnon; tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone [3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]-ethenyl]-2-propenylidene]hydrazone] (Figure 1), is now registered in the United States under the trade name AMDRO fire ant insecticide for the control of the red imported fire ant (*Solenopsis invicta*) (Banks et al., 1981; Williams et al., 1980) and COMBAT insecticide and MAXFORCE insecticide for the control of cockroaches. Field trials with AC 217,300 baits for control of the big-headed ant (*Pheidole megacephala*) and subterranean termites (*Coptotermes formosanus*) have also been promising (Su et al., 1980, 1982). AC 217,300 is selectively toxic to insects whose mode of feeding results in ingestion of the toxicant and shows little or no toxicity by foliar application to insects with piercing sucking mouth parts. AC 217,300 is also relatively nontoxic to insects where exposure was limited to cuticular contact. AC 217,300 is a slow-acting stomach poison and is very active against imported fire ants when used in a 0.88% bait utilizing soybean oil as an attractant on corn grit carrier (Lovell, 1979; Technical Information, 1980). The oral LD_{50} of AC 217,300 for the male rat is 1131 mg/kg of body weight; thus, the compound is considered to be a safe material for mammals.

The fate and behavior of a pesticide, once it has entered the environment, can be influenced by photodegradation. The action of sunlight may destroy or chemically alter the pesticide. Vander Meer et al. (1982) reported a rapid decomposition of AC 217,300 during daylight hours under ambient summer climatic conditions, but no decomposition during evening hours. Vander Meer et al. also reported that no thermal decomposition of AC 217,300 was detected in the absence of light; therefore, the decomposition was attributed to photolysis. Their paper was not aimed at residue analysis nor determination of breakdown products. In the present study, experiments were conducted with AC 217,300, the active ingredient of AMDRO fire ant insecticide, to determine its photostability and to identify photodegradation products.

MATERIALS AND METHODS

Isotopes and Chemicals. For identification of photoproducts of AC 217,300, both carbon-14- and carbon-13-labeled AC 217,300 were employed. Experments were performed on a mixture of one part carbon-13-labeled AC 217,300 and one part unlabeled AC 217,300 containing sufficient carbon-14-radiolabeled AC 217,300 to allow ready detection and measurement of AC 217,300 and its photodegradation products by conventional radiotracer techniques. By virtue of the carbon-12/carbon-13 ratio, the mixture provided doublet ion peaks in the mass spectra of isolated AC 217,300 and its photodegradation products. These doublets assist in distinguishing ions due to the photodegradation products from those derived from nonlabeled contaminants, even after an extensive and elaborate sequence of purification (Ku et al., 1979; Pohl et al., 1975). Benzylic (Bullock, 1978a) and pyrimidine-2 (Bullock, 1978b) carbon-14 radiolabeled and carbon-13 labeled (Bullock, 1979) AC 217,300 were obtained from the Agricultural Research Division, American Cyanamid Co., Princeton, NJ. The locations of the isotope labels are shown in Figure 1. The specific activity of the two-radiolabeled compounds was 22.98 and 23.71 μCi/mg, respectively, with a radiochemical purity of >99% as determined by two-dimensional thin-layer chromatography (TLC) and autoradiography. In the photolysis study of the benzylic-labeled material, it should be noted that carbon-14 was incorporated at both benzylic atoms but that carbon-13 was incorporated at only one of the benzylic carbon atoms. The presence of carbon-13 at only one benzylic carbon atom takes on significance in explaining mass spectra obtained on several photoproducts derived from the benzylic-labeled starting material. Samples of the following potential photodegradation products were obtained from the Amercian Cyanamid Co. chemical library file: 1,5-bis(α,α,α-trifluoro-*p*-tolyl)-1,4-pentadien-3-one (photoproduct **1**); α,α,α-trifluoro-*p*-toluic acid (photoproduct 2); *p*-(trifluoromethyl)cinnamic acid (pho-

Agricultural Research Division, American Cyanamid Company, Princeton, New Jersey 08540.

[1]AMDRO is a registered trademark of American Cyanamid Co.

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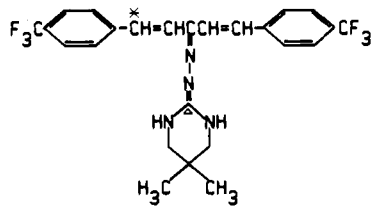


AC 217,300 (MW 494)

Figure 1. Chemical structure of AMDRO fire ant insecticide active ingredient hydramethylnon (AC 217,300). Key: *, position of carbon-14 label at benzylic position; Δ, position of carbon-14 label at pyrimidine-2 position.

toproduct 3); 6,7,8,9-tetrahydro-7,7-dimethyl-3-[*p*-(trifluoromethyl)styryl]-4*H*-pyrimido[2,1-*c*]-*as*-triazin-4-one (photoproduct 4).

Irradiation. The experiments were conducted in a Mallory environmental chamber (Mallory Engineering Inc., Salt Lake City, UT) equipped with Atlas xenon arc light systems (Atlas Electric Devices Co., Chicago, IL) using borosilicate inner and outer filters to simulate natural sunlight, at 6000 W, at 27 °C. On the basis of manufacturer's specification (Atlas Bulletin, No. 1183, 1975), constant light output of 6500 W with the above-mentioned filter combination at 48 cm is 114 340 mW/cm^2 with the following spectral distribution: 1500 (<340 nM); 5750 (340–400 nM); 58 700 (400–750 nM); 48 400 (>750 nM) mW/cm^2. The output is comparable to noon summer sunlight in Chicago, IL, of 141 800 mW/cm^2 with the following spectral distribution: 1040 (<340 nM); 5260 (340–400 nM); 59 800 (400–750 nM); 75 700 (>750 nM).

Solubility of AC 217,300 in water is approximately 5–7 ppb. Due to the extremely low solubility of AC 217,300 in water, the test compound was suspended in distilled water. One milliliter of a methanol solution containing 1 mg of tracer-labeled AC 217,300 was pipetted into a 125-mL Vycor flask (Corning Glass Works, Corning, NY) containing 50 mL of distilled water while stirring on a magnetic stirrer. The mixture with a nominal concentration of 20 ppm in the stoppered Vycor flask was stirred continuously while exposed to the xenon arc light for various time intervals. A parallel control experiment was run at various periods in the dark in the environmental chamber. Separate photolysis studies were performed for benzylic and pyrimidine-2 carbon-14-labeled AC 217,300.

Extraction of the Photolysates. At the end of each exposure period, a 50-mL solvent mixture of ethyl acetate–anhydrous diethyl ether (1/1, v/v) was poured into the Vycor flask that contained photolysis mixture. The mixture was then transferred to a separatory funnel. The Vycor flask was thoroughly washed with 50 mL of the solvent mixture. The flask wash was added to the separatory funnel. After separation, the aqueous fraction was reextracted with 50 mL of the solvent mixture. The aqueous phase was then acidified with hydrochloric acid to pH 2 and followed by two extractions with 50 mL of the solvent mixture. The aqueous phase was adjusted to pH 8 by adding sodium bicarbonate and extracted twice with 50 mL of the solvent mixture.

The combined organo extracts were filtered through anhydrous sodium sulfate. After the removal of solvent by rotary evaporator at approximately 25 °C, the residue was redissolved in a small amount of a solvent mixture of hexane–2-propanol–methanol (85/5/10, v/v/v). The amount of carbon-14 radioactivity in the aqueous, organophilic, and the concentrated organophilic fractions was determined by scintillation counting of aliquots in an AQUASOL-2 scintillator (New England Nuclear, Boston, MA). The carbon-14 radioactivity in the concentrated organophilic fraction was adjusted to 900 000–1 000 000 cpm/mL with the same solvent mixture for high-pressure liquid chromatographic analysis (HPLC). The extraction process was conducted in a laboratory of subdued room light to prevent further photolytic degradation.

HPLC Analysis. The analysis of the organophilic fraction was carried out with a Micromeritics Model 7000 liquid chromatograph (Micromeritics Instrument Corp., Norcross, GA) using a series of five columns (lengths 250 mm, o.d. 6.35 mm, i.d. 4.6 mm) of PARTISIL-10 PAC, a polar bonded phase in which a cyanoamino moiety is bonded to PARTISIL-10 via a siloxane bond (Whatman Inc., Clifton, NJ). The sample (107 μL) was injected and eluted with a solvent mixture of hexane–2-propanol–methanol (85/5/10, v/v/v) for 80 min at a flow rate of 1 mL/min at which time the AC 217,300 had emerged; then, a linear acidic acid gradient was initiated over a period of 60 min with a solvent mixture of hexane–acetic acid–2-propanol–methanol (83/2/5/10, v/v/v/v). At the end of the linear gradient time, the elution with the acidified solvent was continued for an additional 2 h. The effluent from the column was monitored by UV at 254 nm and for radioactivity by on-line mixing of the column effluent stream with an AQUASOL-2 scintillator via a Milton Roy pump (Milton Roy Co., Riviera, FL) at a rate of 6 mL/min. The resulting solution was then passed through a modified Packard flow monitor (Packard Instrument Co., Downers Grove, IL) to measure carbon-14 radioactivity via a Packard Model 3002 scintillation spectrometer and a Tennelec TC 596 rate meter (Tennelec, Oak Ridge, TN) with a time constant of 10 s. The UV response at 254 nm and carbon-14 radioactivity were recorded. The effluent from the flow monitor was collected into fractions (2 min/fraction) for more precise quantitation of carbon-14 radioactivity by conventional liquid scintillation counting method.

Isolation and Identification of Photolysis Products. Identification of photolytic products was determined by demonstration of identical cochromatography of the unlabeled reference compounds with the radioactive spots and where appropriate by HPLC or TLC isolation and purification of the radiocomponents followed by mass spectral analysis.

Thin-Layer Chromatographic Analysis (TLC). Thin-layer chromatography was performed on commercial precoated analytical silica gel 60F254 (0.25-mm thickness) plates, preparative silica gel (1.00-mm thickness) plates (E. Merck Co., Darmstadt, Germany), and $KC_{18}F$ reversed phase plates (Whatman Inc., Clifton, NJ). Silica gel TLC plates were developed in either of the following solvent systems: (a) chloroform; (b) acetonitrile–2-propanol (166/34, v/v); (c) acetonitrile–2-propanol–acetic acid (166/34/5, v/v/v); (d) chloroform–acetone–diethylamine (180/90/3, v/v/v); (e) toluene–*p*-dioxane–acetic acid (270/90/3, v/v/v). Reversed-phase plates were developed in a (f) methanol–water–acetic acid (140/60/3, v/v/v) solvent system.

Reference compounds were spotted on plates prior to application of the radioactive solutions. Radiolabeled compounds were visualized by radioautography on Kodak SB-5 single-coated blue-sensitive X-ray film (Eastman Kodak Co., Rochester, NY). The nonradioactive standards were located under ultraviolet light. Quantitation of radioactivity was determined by scraping the radioactive spots from the TLC chromatograms and counting in the AQUASOL-2 scintillation cocktail as gel.

Mass Spectrometric Analysis (MS). The purified photodegradation products were analyzed by chemical

Table I. Distribution of Radioactivity in Different Extract Fractions after Exposure of Distilled Water with Benzylic Carbon-14-Labeled AC 217,300 to Simulated Sunlight

	% appl radioact rec at various time pts, min						
fraction	0	30	45	60	75	90	90
organophilic	98.1	98.0	93.0	96.6	96.4	88.1	102
aqueous	<0.1	0.7	0.7	0.7	1.3	1.2	<0.1
total	98.2	98.7	93.7	97.3	97.7	89.3	102.1

Table II. Distribution of Radioactivity in Different Extract Fractions after Exposure of Distilled Water with Pyrimidine-2 Carbon-14-Labeled AC 217,300 for 60 min to Simulated Sunlight

fraction	% appl radioact rec
organophilic	89.0
aqueous	6.2
total	95.2

ionization mass spectrometry (CIMS) in both the positive-ion (PI) and negative-ion (NI) modes on a Finnigan Model 4023 GC–MS–DS equipped with a pulsed positive-ion negative-ion chemical ionization (PPINICI) accessory (Finnigan Corp., Sunnyvale, CA). The mass spectrometric parameters were as follows: source pressure, 0.4 torr; source temperature, 250 °C; electron energy, 100–150 eV; conversion dynode voltages, ±3000 V; electron multiplier voltage, 800–900 V; preamplifier range, 10^{-7} amp/V; mass spectrometer manifold temperature, 70 °C. Spectra were alternatively acquired at 2 s/scan in the positive-ion mode (m/z 60^{+}–600^{+}) and in the negative-ion mode (m/z 40^{-}–600^{-}).

Gas chromatography–CIMS (GC–CIMS) analyses were carried out on a 6 ft × 2 mm (i.d.) glass column packed with 3% SP 2100 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, PA). Methane was used as the GC carrier gas and as the CI reagent gas. No separator or enrichment device was used. In addition to the preceding mass spectrometry and data acquisition parameters, the following gas chromatographic operating conditions were used: CH_4 flow rate, 15 mL/min; source pressure, 0.4 torr; injector temperature, 250 °C; GC–MS interface temperature, 200 °C; column oven temperature, 200 °C.

Radioanalysis. Carbon-14 radioactivity in various samples was determined by a liquid scintillation counting technique using a Searle Mark III liquid scintillation system (Tracor Analytical Inc., Austin, TX) and an Intertechnique MULTI-MAT system consisting of a Model SL 30 liquid scintillation spectrometer and MICRODATA 1600 computer (Intertechnique, Fairfield, NJ).

RESULTS AND DISCUSSION

Radioactivity Balance. Data for the distribution of carbon-14 radioactivity in the organophilic and aqueous phases after exposure of distilled water separately with benzylic and pyrimidine-2 carbon-14-labeled AC 217,300 to simulated sunlight are presented in Tables I and II, respectively. The data show nearly quantitative recovery of the applied radioactivity from the water up to 75 min. Even after 90-min exposure, the overall recovery was about 90% of the applied radioactivity from the water with benzylic carbon-14-labeled AC 217,300 (Table I).

Radioactivity Profile of the HPLC Effluent. On-line monitoring of the carbon-14 radioactivity of the HPLC effluent showed that benzylic and pyrimidine-2-carbon-14-labeled AC 217,300 degraded very rapidly as a suspension in distilled water into at least 15 and 8 measurable components in addition to the parent compound, respectively. Radioactivity in each HPLC effluent component was quantitatively determined and reconstructed the radioactive profiles from the liquid scintillation data. The HPLC profiles obtained from the 90-min light exposure and control (dark) with carbon-14-labeled benzylic and 60-min light exposure with carbon-14-labeled pyrimidine-2 samples are shown in Figure 2. The percentage distributions of various HPLC degradation peaks containing radioactive photolytic degradation products derived from benzylic and pyrimidine-2 carbon-14-labeled AC 217,300 in distilled water under simulated light are presented in Tables III and IV, respectively. As shown in the tables, at least 16 radioactive HPLC peaks were derived from the benzylic carbon-14 label, and 9 radioactive peaks were derived from the pyrimidine-2 carbon-14 label. The HPLC chromatographic peak, identified as IX, was the major

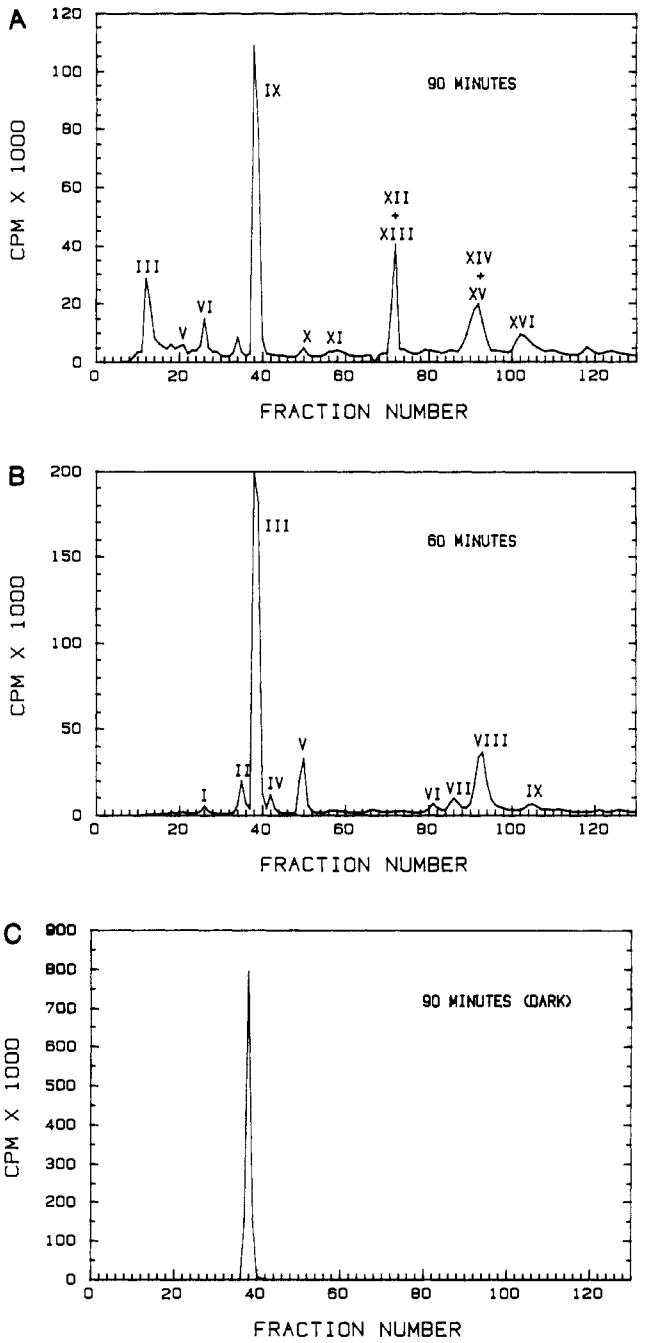


Figure 2. High-pressure liquid chromatogram of the photolytic decomposition products derived from carbon-14-labeled AC 217,300 in distilled water under xenon arc light. The figures represent the distribution of radioactivity determined by LSC: (2A) benzylic carbon-14-labeled AC 217,300; (2B) pyrimidine-2 carbon-14-labeled AC 217,300; (2C) benzylic carbon-14-labeled AC 217,300 kept in the dark.

Table III. Distribution of HPLC Peaks Containing Radioactive Photolytic Degradation Products Derived from Benzylic Carbon-14-Labeled AC 217,300 in Distilled Water under Xenon Arc Light

HPLC degrdn peak	approx HPLC ret time, min	% inj radioact rec as photoproducts at various time pts, min					
		0	30	45	60	75	90
I + II	18–20	0.08	0.61	0.49	0.38	1.07	0.63
III	24	0.03	3.98	5.51	5.33	6.83	7.63
IV + V	33–38	0.07	1.51	2.13	2.03	2.79	3.14
VI	50	0.11	1.58	2.67	2.36	1.08	3.06
VII	56	0.04	1.12	0.64	0.82	4.17	0.62
VIII	66	0.18	1.65	2.11	2.12	1.91	2.05
IX	75	102.40	62.20	43.90	46.30	23.90	21.60
X	98	0.19	2.17	2.79	2.43	1.96	1.75
XI	112	0.32	2.00	2.80	2.42	3.05	3.10
XII + XIII	143–147	0.34	5.88	5.41	4.70	7.56	8.00
XIV + XV	160–178	0.24	5.19	9.35	9.51	9.97	10.43
XVI	190	0.37	4.25	4.95	3.90	5.70	5.85
others[a]		1.43	7.32	11.00	11.13	14.34	15.61
total		105.80	99.46	93.75	93.43	84.33	83.47

[a] Measurable radioactivity not detected as peak(s).

Table IV. Distribution of HPLC Peaks Containing Radioactive Photolytic Degradation Products Derived from Pyrimidine-2 Carbon-14-Labeled AC 217,300 in Distilled Water under Xenon Arc Light for 60 min

HPLC degradn peak	approx HPLC ret time, min	% inj radioact rec	HPLC degradn peak	approx HPLC ret time, min	% inj radioact rec
I	50	1.6	VI + VII	161–172	5.5
II	69	3.6	VIII	186	14.9
III	76	39.2	IX	210	4.3
IV	87	2.1	others[a]		15.3
V	98	6.7	total		93.2

[a] Measurable radioactivity not detected as peak(s).

component identified at any given time interval from the benzylic label, and none of the other peaks exceeded 10% of the total injected radioactivity (Table III). With the exception of HPLC peaks III and VIII from the pyrimidine-2 label, none of the peaks exceeded 10% of the total injected radioactivity (Table IV). The data from the tables showed that for both labels 93% or more of the injected radioactivity into HPLC was accounted for in the column effluent through 60-min exposure. The radioactivity recovered from the HPLC column was about 84% of the injected radioactivity for the 75- and 90-min samples of the benzylic label.

Rate of Photodegradation of AC 217,300. The rate of photodegradation of AC 217,300 was determined from the data obtained with benzylic carbon-14-labeled AC 217,300. Radioactivity in each HPLC effluent component was quantitatively determined by liquid scintillation counting (Table III). The amount of AC 217,300 remaining at various time intervals was calculated as carbon-14 radioactivity under HPLC peak IX divided by the total carbon-14 radioactivity injected into HPLC system. Regression analysis of log (percent AC 217,300 remaining) vs. exposure time (min) (Figure 3) gave the following equation:

$$\log (\%\ \text{AC 217,300 remaining}) = 2.0184 - 0.0076237(\text{exp time, min})$$

$$n = 6 \qquad r = 0.97$$

where n = number of data points used in the analysis and r = correlation coefficient. Thus, the exposure time required ($T_{1/2}$) to degrade AC 217,300 to half the amount was calculated as 41.9 min. The rate constant was calculated as 1.65×10^{-2} min^{-1} with $\lambda = \ln[(100/50)/T_{1/2}]$. Figure 3 indicated that the photodegradation of AC 217,300 in distilled water as a suspension followed by first-order kinetics over a period of 90 min.

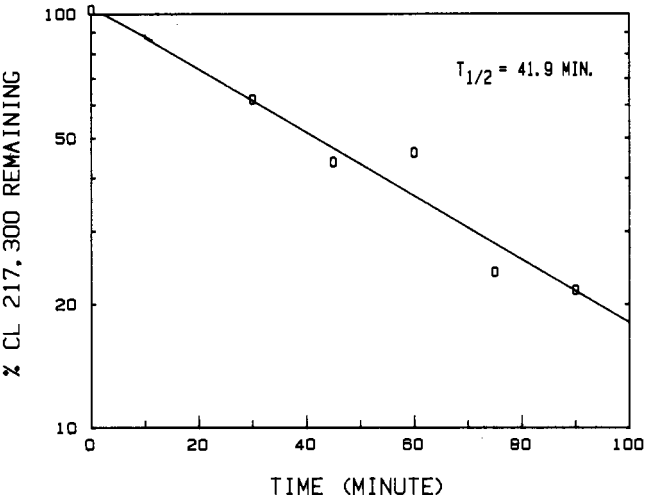


Figure 3. Rate of photodegradation of benzylic carbon-14-labeled AC 217,300 in distilled water as a suspension under xenon arc light.

Table V. TLC Analysis of the Benzylic Carbon-14-Labeled AC 217,300 HPLC Peaks for Radioactive Components

HPLC degradn peak[a]		TLC findings	
ID	% total inj radioact	compn	% total inj radioact
III	7.6	photoproduct 1	6.9
		others	0.8
VI	3.1	photoproduct 1[b]	1.7
		photoproduct 4	0.3
		others	1.0
IX	21.6	AC 217,300	19.9
		others	1.8
XII + XIII	8.0	photoproduct 2	4.1
		photoproduct 3	1.5
		others	2.4
XIV + XV	10.4	unknown 1	2.8
		unknown 2	2.2
		unknown 3	2.6
		unknown 4	1.1
		others	1.7
XVI	5.9	unknown 1	2.0
		unknown 5	2.7
		others	1.2

[a] Ninety-minute exposure time (from Table III). [b] Has same TLC R_f value as photoproduct 1.

Analysis of HPLC Effluent Peaks for Radioactive Components by TLC. The benzylic carbon-14-labeled AC 217,300 HPLC chromatographic peaks from a 90-min light exposure and identified as peaks III, VI, IX, XII +

Table VI. TLC Analysis of the Pyrimidine-2 Carbon-14-Labeled AC 217,300 HPLC Peaks for Radioactive Components

HPLC degradn peak[a]		TLC findings	
ID	% total inj radioact	compn	% total inj radioact
II	3.6	photoproduct 4	1.4
		AC 217,300	1.6
		others	0.6
III	39.2	AC 217,300	36.0
		others	3.2
V	6.7	unknown 1	3.3
		others	3.4
VI + VII	5.5	unknown 2	2.2
		unknown 3	1.5
		others	1.9
VIII	14.9	unknown 2	0.7
		unknown 3	8.8
		others	5.5

[a] Sixty-minute exposure time (from Table IV).

XIII, XIV + XV, and XVI (Figure 2A; Table III) were analyzed quantitatively for radioactive components by thin-layer chromatography, and the results are summarized in Table V. The HPLC peak III showed one major component and was designated as photoproduct 1. HPLC peak VI showed photoproducts 1 and 4 as radioactive components. The major component of HPLC peak IX was identified by cochromatography as AC 217,300. HPLC peak XII + XIII showed photoproducts 2 and 3 as radioactive components. HPLC peaks XIV + XV and XVI showed at least four and two radioactive components, respectively, and were designated as unknowns. No attempt was made to identify these minor radioactive unknowns.

The pyrimidine-2 carbon-14-labeled AC 217,300 HPLC chromatographic peaks from a 60-min light exposure and identified as peaks II, III, V, VI + VII, and VIII (Figure 2B; Table IV) were analyzed for radioactive components by TLC, and the results are presented in Table VI. The HPLC peak II showed photoproduct 4 and AC 217,300 as radioactive components. The major component of HPLC peak III was identified as AC 217,300. Peaks V–VIII showed at least three radioactive unknowns in various proportions. Control experiment run at a 90-min time period without simulated lights in the environment chamber showed no breakdown of AC 217,300 during the above procedure (Figure 2C).

Identification of Photoproducts by MS and TLC. Two-dimensional development of HPLC peak IX (benzylic label) and peak III (pyrimidine-2 label) on a silica gel TLC plate showed the major component of the peak cochromatographed with AC 217,300. From the photodegradation extract, the product corresponding to AC 217,300 was isolated on a preparative silica gel TLC plate. The isolate was characterized by NICI (CH_4) and cochromatography. Figure 4A shows the ion map obtained from the PICI (CH_4) analysis of 1 μg of AC 217,300. The full-scale response for the ion map is 1000, and no ions of any significant intensity are observed for AC 217,300. In sharp contrast, the ion map generated from NICI (CH_4) of the same sample is shown in Figure 4B. The full-scale response for this ion map is 20 000, and the sample signal was present for about 6 min. The ion doublet at m/z 494$^-$, 495$^-$ is the M$^{\cdot-}$ of AC 217,300 and dominates the NICI (CH_4) mass spectrum (Figure 5). Analysis of the photoproducts of AC 217,300 and other compounds of similar structure indicated that compounds retaining the conjugated system gave excellent NICI (CH_4) response (Hunt et al., 1976; Stout and Steller, 1984) and poor PICI (CH_4) response (Field, 1980; Stout et al., 1983), while compounds

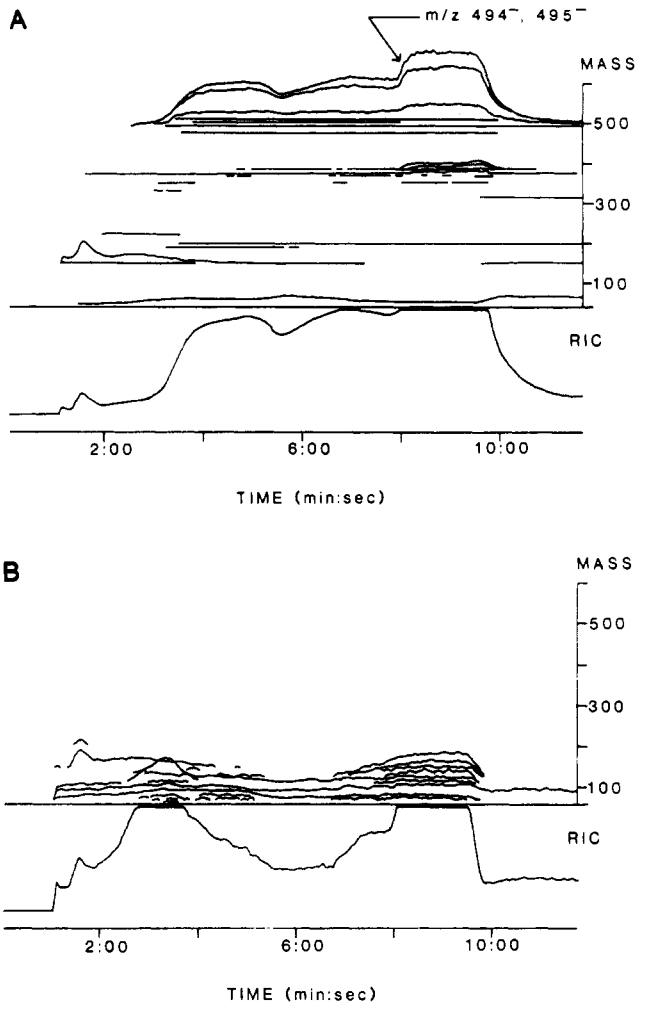


Figure 4. Ion maps from (4A) PICI (CH_4) analysis and (4B) NICI (CH_4) analysis of 1 μg of AC 217,300 isolated from the photolysis mixture.

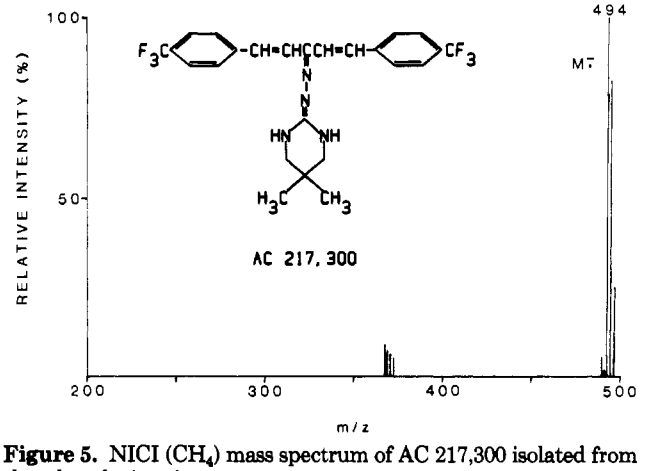


Figure 5. NICI (CH_4) mass spectrum of AC 217,300 isolated from the photolysis mixture.

containing only the pyrimidine ring behaved conversely. Coupling this response behavior with the isotope labels located at the benzylic carbon and on the pyrimidine ring proved invaluable in elucidating the structures of unknown photoproducts.

Photoproduct 1 was isolated from the photodegradation extract on a silica gel TLC plate. The isolate was analyzed by GC–NICI and generated the ion map shown in Figure 6. Both major peaks and a third smaller one all generated the same ion doublet at m/z 370$^-$, 371$^-$ corresponding to

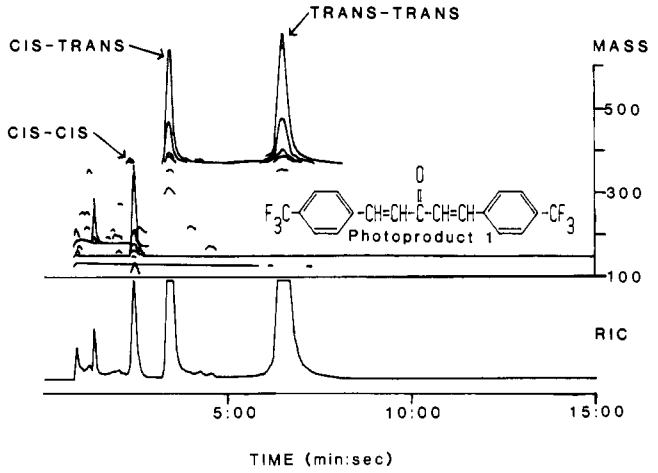


Figure 6. Ion map from GC–NICI analysis of photoproduct 1.

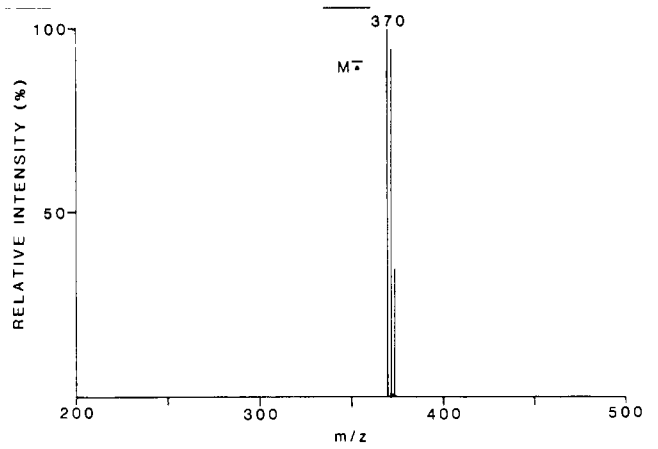


Figure 7. NICI (CH_4) mass spectrum of photoproduct 1.

the $M^{\bullet-}$ (Figure 7) of the photoproduct 1. The isolated photoproduct 1 was cochromatographed on TLC with the synthesized material, 1,5-bis(α,α,α-trifluoro-*p*-tolyl)-1,4-pentadien-3-one. The three GC peaks arise from the cis and trans isomers that can exist at each double bond of the dienone. Subsequently, it was determined that the trans,trans orientation about the double bonds was present in the parent AC 217,300 and that the last eluting labeled peak cochromatographed with the *trans,trans*-dienone. Photolysis of the *trans,trans*-dienone in 9% DME–91% *n*-heptane under incandescent light was also found to generate this same isomeric mixture. From this later study, it was further determined that the other major peak in the chromatogram was the *cis,trans*-dienone and that the early eluting minor peak was the cis,cis isomers.

Photoproduct 2 was isolated from the photodegradation extract using a reversed-phase TLC plate. The PICI (CH_4) and NICI (CH_4) mass spectra of photoproduct 2 are shown in Figure 8. The isolate showed ion doublets at m/z 191$^+$, 192$^+$; 171$^+$, 172$^+$; and 190$^-$, 191$^-$ corresponding to the (M + H)$^+$, (M + H − HF)$^+$, and $M^{\bullet-}$ ions. The isolated photoproduct 2 cochromatographed on TLC with the authentic compound α,α,α-trifluoro-*p*-toluic acid. Note that the carbon-12/carbon-13 ratios of the doublets are no longer 1/1 but are closer to 3/1. This change arises from carbon-13 being incorporated at 50 mol % at only one benzylic carbon. This point was emphasized in the Experimental Section. Although, for every two parent AC 217,300 molecules, one will have a carbon-13 at the benzylic carbon giving the 1/1 isotope ratio, there is only one carbon-13 for four benzylic carbon atoms. As illustrated in Figure 9, for a large population of parents cleaving

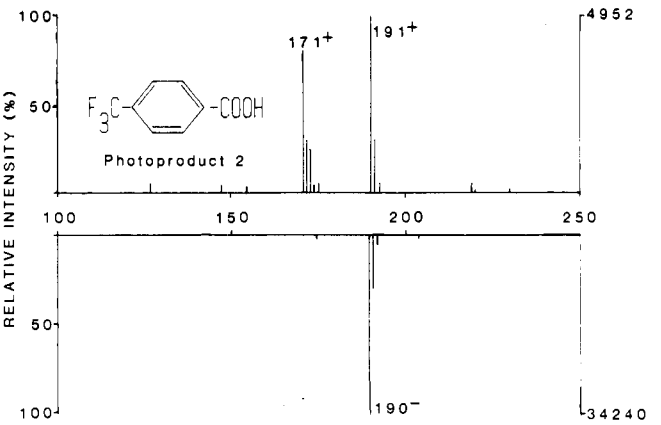


Figure 8. PICI (CH_4) and NICI (CH_4) mass spectra of photoproduct 2.

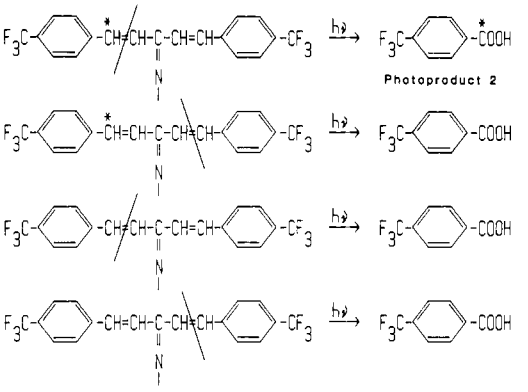


Figure 9. Description of the change in the carbon-12/carbon-13 ratio in forming photoproduct 2.

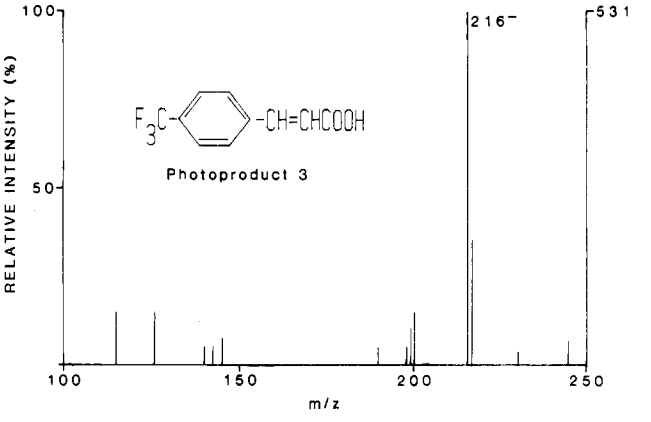


Figure 10. NICI (CH_4) mass spectrum of photoproduct 3.

randomly at the benzylic carbon to give the trifluoro-*p*-toluic acid, three molecules will be generated having carbon-12 at the benzylic carbon while only one will have carbon-13 at the benzylic carbon: thus, the 3/1 ratio of carbon-12/carbon-13 in the photoproduct. This change in the isotope ratio proved useful in determining the number of benzylic carbons retained in several photoproducts.

Photoproduct 3 was isolated from the photodegradation extract on a reversed-phase TLC plate. The mass spectral analysis of photoproduct 3 indicated that it was similar in structure to the trifluoro-*p*-toluic acid (photoproduct 2). The ion doublet at m/z 216$^-$, 217$^-$ with 3/1 isotopic ratio corresponding to the $M^{\bullet-}$ was observed (Figure 10). The isolated photoproduct 3 was cochromatographed on TLC with the authentic standard of *p*-(trifluoromethyl)-cinnamic acid.

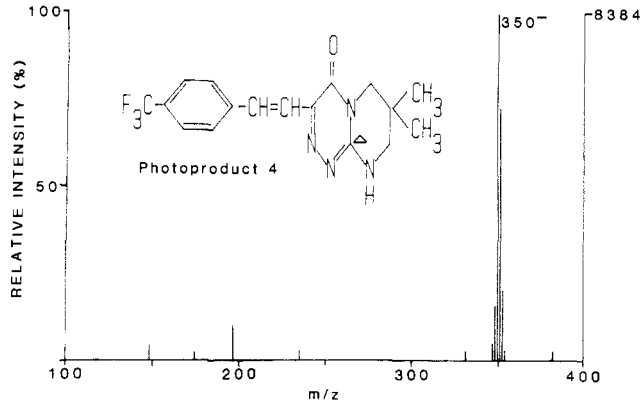


Figure 11. NICI (CH_4) mass spectrum of photoproduct 4.

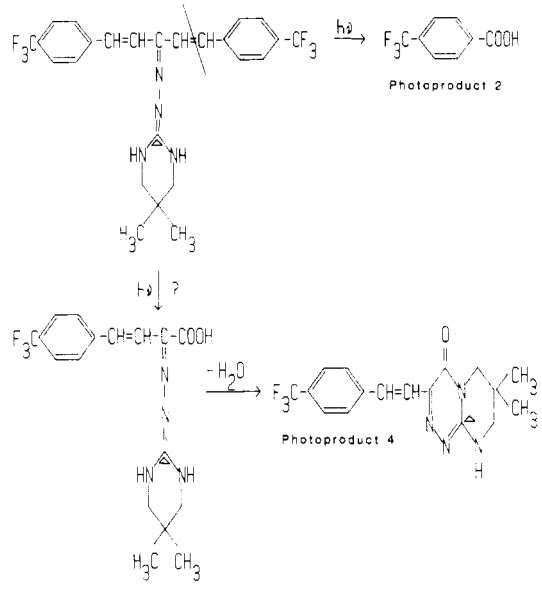


Figure 12. Proposed pathway for the formation of photoproduct 4.

Photoproduct 4 was isolated from the photodegradation extract on a silica gel TLC plate and was analyzed by MS. It was one of the more structurally interesting photoproducts of AC 217,300. The NICI (CH_4) mass spectrum of this photoproduct labeled in the pyrimidine-2 ring is shown in Figure 11. The ion doublet at m/z 350⁻, 351⁻ strongly suggested a compound having a molecular weight of 350. The NICI (CH_4) mass spectrum of benzylic-labeled photoproduct 4 showed a 3/1 ratio of m/z 350⁻/m/z 351⁻, indicating retention of only one benzylic carbon in the photoproduct. Considering that the trifluoro-*p*-toluic acid (photoproduct 2) could be formed by cleavage of the double bond attached to the benzylic carbon, the question was what could happen if an acid was formed in an analogous manner on the other side of the double bond. As shown in Figure 12, this acid could lose water by cyclizing with an amine group on the pyrimidine ring to give 6,7,8,9-tetrahydro-7,7-dimethyl-3-[*p*-(trifluoromethyl)-styryl]-4*H*-pyrimido[2,1-*c*]-*as*-triazin-4-one. This compound was synthesized and found to cochromatograph on TLC with photoproduct 4, confirming the structural proposal. Photoproduct 4 was characterized as a photodegradation product from both benzylic and pyrimidine-2 carbon-14-labeled AC 217,300.

Photoproduct unknown 3 was observed as an irradiation product of both benzylic (Table III) and pyrimidine-2 (Table IV) carbon-14-labeled AC 217,300. From the pyrimidine-2 carbon-14-labeled AC 217,300 photodegradation

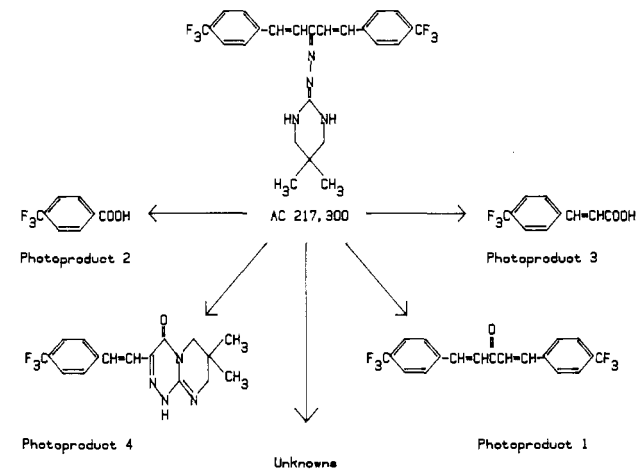
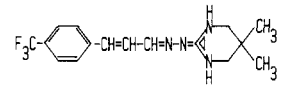


Figure 13. Suggested mechanistic scheme for the photolysis of benzylic and pyrimidine-2 carbon-14-labeled AC 217,300 in distilled water under borosilicate-filtered xenon arc light. Separate photolysis studies were conducted for benzylic carbon-14-labeled AC 217,300 and pyrimidine-2 carbon-14-labeled AC 217,300.

extract, unknown 3 was isolated on a silica gel TLC plate and was only partially characterized by MS. The pyrimidine-2 carbon-14-labeled material generated in ion doublet at m/z 326⁻, 327⁻, indicating a photoproduct with a molecular weight of 326. This photoproduct also showed retention of one benzylic carbon atom. A proposed structure consistent with this mass spectral data could result from reduction of a double bond in the structure shown below. This proposed structure has not yet been confirmed with a synthesized material.



The photoproducts of AC 217,300 have been characterized from both carbon-14- and carbon-13-labeled starting materials. Separate photolysis studies were conducted for benzylic and pyrimidine-2 carbon-14-labeled AC 217,300. Photoproducts retaining the conjugated aromatic system gave tremendous NICI (CH_4) response and poor PICI (CH_4) response.

The benzylic and pyrimidine-2 carbon-14-labeled AC 217,300 photodegraded very rapidly as a suspension in distilled water under simulated light under the described experimental conditions. The half-life of benzylic-labeled AC 217,300 was calculated as 42 min. Photodegradation products 1–4 were characterized as photodegradation products of AC 217,300 by TLC, HPLC, and MS. Photoproduct 4 and unknown 3 were observed as photodegradation products from both benzylic and pyrimidine-2 carbon-14-labeled AC 217,300. The photodegradation products of AC 217,300 represent compounds that would normally be expected from the structure of AC 217,300. An investigation of the insecticidal activity of each of the identified photodegradation products of AC 217,300 found none to be active against any insect species tested (Hollingshaus and Little, Jr., 1984). Compared with chlorinated hydrocarbon insecticides that persist in invertebrates and resist environmental degradation (Forsyth et al., 1983; Neithammer et al., 1984), AC 217,300 is fairly unstable outdoors (Apperson et al., 1984; Vander Meer et al., 1982) and particularly susceptible to photolysis. These characteristics of AC 217,300 serve to reduce its risk to nontarget organisms. A suggested mechanistic pathway for the photolysis of AC 217,300 in distilled water under simulated light is presented in Figure 13.

Registry No. *trans,trans*-1, 103836-71-1; *cis,trans*-1, 103836-73-3; *cis,cis*-1, 103836-74-4; **2**, 455-24-3; **3**, 2062-26-2; **4**, 103836-72-2; AMDRO, 67485-29-4; H_2O, 7732-18-5.

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Bioactive Aziridine Derivatives of Chrysanthemate Insecticides

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(1*R*,*trans*)-Phenothrin (**1**) and (1*R*,*trans*,α*RS*)-cyphenothrin (**1**-CN) are converted to their aziridine derivatives (**2**, **2**-CN) by oxidation of the 2-methyl-1-propenyl substituent to give a 1,2-diol, conversion to the unstable dimesylate, and reaction with aqueous ammonia. Lead tetraacetate catalyzed addition of *N*-aminophthalimide to the double bond of **1** and **1**-CN gives the *N*-phthalimidoaziridines (**4**, **4**-CN) which cleave with ethanolic hydrazine hydrate to generate the *N*-aminoaziridines (**3**, **3**-CN). The potency of these pyrethroids to houseflies, cockroaches, and mosquito larvae, both alone and with piperonyl butoxide, is generally **1** > **2** ≥ **3** > **4** for the phenothrin series and **1**-CN > **2**-CN ~ **3**-CN > **4**-CN for the more toxic cyphenothrin series. Only **1**-CN and **3**-CN are toxic when administered intracerebrally to mice. In contrast, the potency in inducing repetitive discharges in the abdominal nerve cord of the American cockroach is **2** and **3**-CN ≥ **3** > **1** ~ **4** > **2**-CN ≥ **1**-CN and **4**-CN. The high neuroactivity of the aziridines makes them candidate derivatizing agents to probe the target site.

The 2-methyl-1-propenyl substituent of chrysanthemates is important to their insecticidal activity and their metabolic and photochemical lability (Elliott and Janes, 1978). Derivatization of chrysanthemates to the corresponding epoxides, episulfides, and cyclopropanes generally reduces their insecticidal activity (Ueda et al., 1974; Ruzo et al., 1984) but enhances their in situ potency on the cockroach cercal sensory nerve (Gammon et al., 1983; Ruzo et al., 1984). The corresponding aziridinochrysanthemates are not reported, even though the aziridine functionality confers unique biological properties in several types of compounds, e.g. juvenile hormone mimics (Riddiford et al., 1971; Siddall et al., 1971), inhibitors of sterol biosynthesis (Corey et al., 1967), and antineoplastic agents such as the mitomycins (Lown, 1983; Danishefsky et al., 1985) and tetramin (Oettel, 1959). The present report considers the aziridine, *N*-aminoaziridine, and *N*-phthalimidoaziridine derivatives of phenothrin and cyphenothrin relative to toxicity and neurophysiological activity.

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MATERIALS AND METHODS

Spectroscopy. Chemical ionization mass spectrometry (CI-MS) utilized a Hewlett-Packard 5985B system with methane (0.8 torr) and ionization at 230 eV. Masses are given for the quasi-molecular ions $(M + 1)^+$. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker WM-300 instrument operated at 300 MHz using deuteriochloroform as solvent. Chemical shifts (δ) are reported downfield from tetramethylsilane.

Chromatography. Column chromatography on Kieselgel 60 F_{254} and preparative TLC on silica gel F_{254} chromatoplates utilized hexane–ethyl acetate (1:1) or chloroform–methanol (99:1) with detection by both viewing under ultraviolet light (254 nm) and coloration induced by standing in an iodine atmosphere. Product recovery (preparative TLC) involved gel extraction with ethyl acetate and filtration through fine sintered glass.

Chemicals. Structures, designations, and syntheses for the compounds are given in Figure 1. The required diols (**A**) were prepared by reacting (1*R*,*trans*)-phenothrin and (1*R*,*trans*,α*RS*)-cyphenothrin with a catalytic quantity of osmium tetroxide (5 mol %) with *N*-methylmorpholine

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