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S-Chloroallyl Thiocarbamate Herbicides: Chemical and Biological Formation and Rearrangement of Diallate and Triallate Sulfoxides

Ingolf Schuphan[1] and John E. Casida*

S-Chloroallyl diisopropylthiocarbamate sulfoxides are obtained on treating the corresponding thiocarbamates such as diallate and triallate herbicides with equimolar *m*-chloroperbenzoic acid in chloroform at −15 °C and workup at low temperature. Within 10–120 min at 40 °C, sulfoxides with 3-chloro-, *cis*- and *trans*-2,3-dichloro-, and 2,3,3-trichloroallyl substituents undergo a [2,3] sigmatropic rearrangement, followed by a 1,2-elimination reaction to yield acrolein, 2-chloroacrolein, and 2-chloroacrylyl chloride, respectively, plus diisopropylcarbamoylsulfenyl chloride. The carbamoylsulfenyl chloride decomposes to diisopropylcarbamoyl chloride. These sulfoxides are also very reactive with glutathione, yielding *S*-diisopropylcarbamoylglutathione. Metabolism of the diallate isomers proceeds via their sulfoxides to form 2-chloroacrolein in the mouse hepatic microsomal oxidase system and 2,3-dichloro-2-propene-1-sulfonic acid in mice and rats in vivo and their liver oxidase preparations. The herbicidal activities of diallate and triallate probably result from the metabolically formed sulfoxides acting as carbamoylating agents for critical enzyme thiol groups or liberating chloroallyl-containing toxicants such as 2-chloroacrolein.

Herbicidal thiocarbamates are conveniently divided into two classes, the *S*-alkyl or *S*-benzyl compounds (e.g., EPTC, butylate, benthiocarb, etc.) and the *S*-chloroallyl derivatives [e.g. $(i\text{-}C_3H_7)_2NC(O)SCH_2CCl{=}CR_1R_2$; R_1 = H, R_2 = Cl, *cis*- and *trans*-diallate; R_1 = R_2 = Cl, triallate). The first class but not the second gives easily detectable amounts of sulfoxide derivatives in microsomal monooxygenase reactions (Casida et al., 1974, 1975a,b; Chen et al., 1979), yet both classes are probably metabolized via their sulfoxides since they form *S*-dialkylcarbamoyl derivatives of glutathione (GSH) and the corresponding mercapturic acids as major metabolites in rats (Chen et al., 1979; Hubbell and Casida, 1977). The sulfoxide derivatives of the *S*-alkyl and *S*-benzyl compounds are considered to be important intermediates in their her-

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Table I. TLC R_f Values for Diallate Isomers and Triallate and Their Sulfoxide and Sulfone Derivatives on Silica Gel F-254 Chromatoplates at 4 °C

compound	TLC R_f in indicated solvent system: cyclohexane-diisopropyl ether (3:1)	acetone-hexane (1:1)
cis-diallate	0.44	0.70
trans-diallate	0.48	0.68
triallate	0.48	0.74
cis-diallate sulfoxide[a]	0.00	0.46
trans-diallate sulfoxide[a]	0.00	0.47
triallate sulfoxide[a]	0.00	0.48
cis-diallate sulfone	0.21	0.65
trans-diallate sulfone	0.20	0.66

[a] Decompose on chromatography at 20 °C.

bicidal action (Casida et al., 1974, 1975a,b). The S-chloroallyl thiocarbamates but not the other types are mutagenic in bacterial systems, requiring microsomal oxidation for this activity (De Lorenzo et al., 1978; Sikka and Florczyk, 1978).

The present report and a preliminary communication (Schuphan and Casida, 1979) on this study examine the chemistry and action of S-(3-chloroallyl) thiocarbamate sulfoxides with emphasis on the diallate isomers and triallate and the unique reactions that differentiate the two classes of thiocarbamate herbicides.

MATERIALS AND METHODS

Chemicals. *Criteria of Purity and Identification.* Chromatography and spectroscopy were used to determine the purity and structure of the chemicals of interest. *cis*- and *trans*-Diallate were individually analyzed by gas chromatography (GC) with an electron capture (EC) detector using 2% XE 60 on Chromosorb W (AW, DMCS, 80–100 mesh) in a column (180 cm × 2 mm i.d.) operated at 130 °C with N_2 at 60 mL/min. Thin-layer chromatography (TLC) used silica gel 60 F-254 precoated chromatoplates of 0.25-mm layer thickness (E. Merck, Darmstadt, Germany) with detection of unlabeled compounds by ultraviolet light, iodine vapor or $KMnO_4$ (0.5% in 1% aqueous Na_2CO_3) and of labeled compounds by autoradiography. Relevant R_f values are given in Tables I and II and in the Supplementary Material (see Supplementary Material Available paragraph). ^{14}C metabolites were identified by two-dimensional TLC cochromatography with unlabeled standards from synthesis using various combinations of five different solvent systems.

Nuclear magnetic resonance (NMR) spectra were obtained with a Perkin-Elmer R32B 90 MHz spectrometer coupled to a Nicolet TT-7 Fourier transform unit using $CDCl_3$ or acetone-d_6 solutions and tetramethylsilane (Me_4Si) as the internal standard. Infrared (IR) spectra and chemical ionization–mass spectra (CI–MS) using isobutane as the reagent gas were obtained as previously reported (Hubbell and Casida, 1977).

Spectral data supporting the structures of all chemicals described below are tabulated in the Supplementary Material. Each compound gave an NMR spectrum consistent with its proposed structure. It also gave the anticipated $[M + 1]^+$ peak or isotope cluster pattern on CI–MS with the following exceptions: the sulfoxides are not of sufficient stability for analysis; diisopropylamine hydrochloride gives $[M - Cl]^+$ as its base peak. Compounds were additionally identified by C=O and S→O bands in their IR spectra.

Thiocarbamates and Carbamoyl Derivatives. Diallate was fractionated according to Schuphan and Ebing (1977) to obtain the cis isomer (mp 40–42 °C; 99.5% purity by GC–ECD) which was further purified by recrystallization an additional three times from hexane (mp 42–43 °C; >99.9%). *trans*-Diallate was obtained from a cis,trans isomer mixture by repeated fractional crystallization from hexane at −10 to −25 °C to ultimately obtain colorless crystals, mp −8 to −10 °C, consisting of 92% *trans*- and 8% *cis*-diallate. These diallate samples were free of 2-chloroacrolein based on two criteria: none was detected on CI–MS analysis of a low-temperature distillate at 15 mm of a 1-g sample in a closed trapping system described later; no mutagenic activity was evident with *Salmonella typhimurium* strain TA 100 without microsomal activation (Ames et al., 1975) under conditions where 0.05% 2-chloroacrolein impurity would give significant numbers of revertants (Schuphan and Casida, 1979; Schuphan et al., 1979). [2-*allyl*-^{14}C]Diallate (9.5 mCi/mmol) was provided by Monsanto Co. (St. Louis, MO).

Triallate from Chem Service (Westchester, PA) was recrystallized two times from hexane yielding slightly yellow crystals of mp 30–32 °C. S-(3-Chloroallyl) diisopropylthiocarbamate [bp 120–125 °C (2.5 mm); $n^{20}{}_D$ = 1.5138] was prepared in 42% yield by reacting diisopropylamine, carbonyl sulfide, and *cis,trans*-1,3-dichloropropene (Fluka AG, Buchs, Switzerland), in a molar equivalent ratio of 2:1:1, in *tert*-butyl alcohol and product recovery according to the general procedure of Tilles (1959).

Diisopropylcarbamoylsulfenyl chloride was prepared on an 0.5-mol scale by a method based on Bögemann et al. (1955). The thiocarbamate salt from reaction of equimolar diisopropylamine, NaOH, and carbonyl sulfide in water (85 mL) at 0 °C was oxidized by slow addition of equimolar $K_3Fe(CN)_6$ in water (600 mL) at 0 °C. The precipitated

Table II. TLC R_f Values for 2-Chloroacrolein 2,4-Dinitrophenylhydrazone, 2,4-Dinitrophenylhydrazine, 2-Chloroacrylic Acid, and Dichloroallylsulfonic Acid on Silica Gel F-254 Chromatoplates at 25 °C

solvent system	TLC R_f of indicated compound	
	2-Chloroacrolein-DNPH	DNPH
cyclohexane-diisopropyl ether (3:1)	0.07	0.12
toluene	0.29	0.06
methylene chloride	0.45	0.19
dioxane	0.56	0.48
ethyl acetate	0.69	0.25
	2-Chloroacrylic acid	Dichloroallylsulfonic acid
ethyl acetate-chloroform-acetic acid (50:50:1)	0.27	0.00
1-propanol-chloroform-water (17:6:3)	0.29	0.28
ethyl acetate-methanol-acetic acid (50:25:1)	0.50	0.71
1-butanol-acetic acid-water (6:1:1)	0.61	0.42
methanol	0.76	0.66

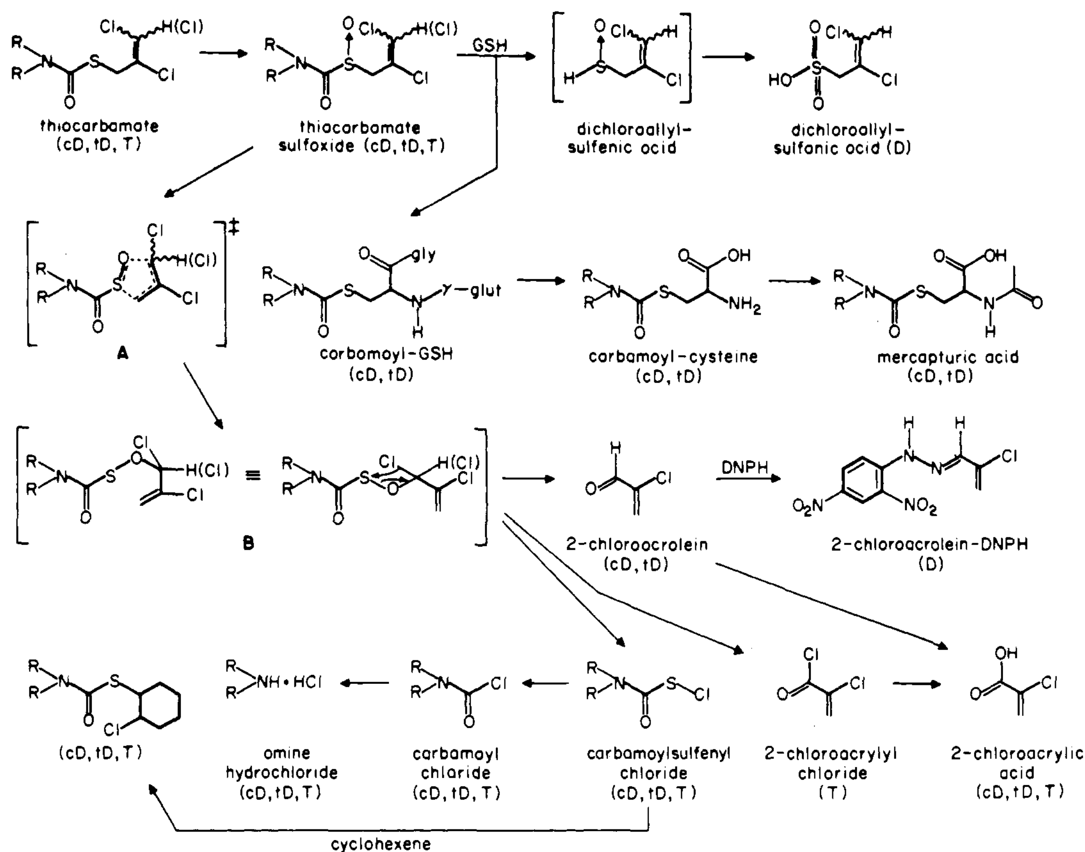

Figure 1. Sulfoxidation of *cis*-diallate (cD), *trans*-diallate (tD), and triallate (T) in peracid and biological systems and subsequent reactions of the sulfoxides. Derivatives of certain reaction products are also shown, i.e., 2-chloroacrolein-DNPH and the cyclohexene adduct of the carbamoylsulfenyl chloride. Designations in parentheses under compound names are the thiocarbamates for which the product has been demonstrated. Abbreviations: R = isopropyl; gly = glycine; glut = glutamate; DNPH = 2,4-dinitrophenylhydrazine.

material was washed with water at 5 °C, crystallized from ethanol–water at 20 °C and the crystals of bis(diisopropylcarbamoyl) disulfide (75% yield) were quickly dried between filter paper and stored at -18 °C. This disulfide in CCl_4 at 10 °C gave the carbamoylsulfenyl chloride on uptake of nearly 1 molar equivalent of Cl_2. The product was stored at -18 °C. For proof of structure, excess cyclohexene (50 μL) was added to the carbamoylsulfenyl chloride (0.5 mmol) in CCl_4 (0.5 mL) and the mixture was held 2 h at 50 °C prior to solvent evaporation, yielding the adduct of diisopropylcarbamoylsulfenyl chloride to the double bond of cyclohexene (Figure 1) (for a related reaction, see Zumach and Kühle, 1970).

Diisopropylcarbamoyl chloride was prepared by reacting 2 molar equivalents of diisopropylamine with phosgene in benzene at -5 °C. Removal of the precipitate by filtration and the benzene by distillation at 14 mm and then fractionation gave the desired product at 67 °C (2 mm) (61% yield; mp 57 °C).

2-Chloroacrolein and Related Compounds. Chlorination of acrolein at 5 °C according to Shostakovskii et al. (1967) followed by distillation gave 2,3-dichloropropionaldehyde [52% yield, bp 36 °C (7 mm), n^{20}_D = 1.4808). 2-Chloroacrolein [bp 33 °C (30 mm), n^{20}_D = 1.4615) was obtained in 51% yield on dehydrochlorination of the dichloropropionaldehyde at 5 °C by addition of 1.3 molar equivalents of diethylaniline and then distillation and redistillation. NMR ($CDCl_3$) δ 9.46 (s, 1, -HC=O), 6.59 (d, 1, J = 2.0 Hz, =CH_2), and 6.43 (d, 1, J = 2.0 Hz, =CH_2). Reaction with 2,4-dinitrophenylhydrazine (DNPH) reagent [5% w/v DNPH in 85% H_3PO_4–ethanol (3:2) mixture] and recrystallization from ethyl acetate gave the hydrazone (2-chloroacrolein-DNPH; mp 167 °C). The trimer was obtained as a solid on holding 2-chloroacrolein in D_2O for several days at 20–40 °C.

2-Chloroacrylyl chloride [bp 63 °C (118 mm)] was prepared by reacting equimolar 2-chloroacrylic acid (Polysciences, Inc., Warrington, PA) and $SOCl_2$ in the presence of a catalytic amount of dimethylformamide for 2 h at 90 °C, then distillation and redistillation. 2-Chloroacrylic acid: NMR ($CDCl_3$) δ 11.4 (s, 1, COOH), 6.68 (d, 1, J = 1.4 Hz, =CH_2), and 6.15 (d, 1, J = 1.4 Hz, =CH_2). 2-Chloroacrylyl chloride: NMR ($CDCl_3$) δ 6.93 (d, 1, J = 2.5 Hz, =CH_2) and 6.45 (d, 1, J = 2.5 Hz, =CH_2).

Sodium *cis,trans*-dichloroallylsulfonate was obtained by the general procedure of Schuphan et al. (1977) on reacting equimolar *cis,trans*-1,2,3-trichloropropene (Pfalz and Bauer, Flushing, NY) and Na_2SO_3 in distilled water at 100 °C. The sodium sulfonate was recrystallized from distilled water giving a 30:70 cis/trans isomer ratio in the first crystalline fraction and a 45:55 ratio in the second fraction. The sulfonic acid (30:70 cis/trans mixture; mp 8–30 °C) was obtained by passing the first fraction through a column of acid-washed Dowex W-X 200–400 mesh cation exchanger. NMR (free acid, acetone-d_6) cis isomer, δ 8.9 (s, 1, -SO_3H), 6.84 (s, 1, =CHCl), and 4.07 (s, 2, CH_2); trans isomer, δ 8.9 (s, 1, -SO_3H), 6.69 (s, 1, =CHCl), and 4.14 (s, 2, CH_2).

Peracid Oxidation of Thiocarbamates and Their Sulfoxides. The following procedures were carried out in a cold room (4 °C) using acetone–dry ice for further cooling of samples as appropriate. The thiocarbamate (1 mmol) in CH_2Cl_2 or $CDCl_3$ (1.5 mL) at -20 °C was treated with equimolar *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2 or $CDCl_3$ (4 mL) at -10 °C. After 10 min at -10

to −5 °C, the reaction mixture was extracted at 0 °C with 5% aqueous Na_2CO_3 solution (2 mL × 2) and water (2 mL × 3) and finally dried (Na_2SO_4, 10–20 min, −5 to 0 °C). A crystalline product was obtained on evaporation of the solvent below 0 °C using a vigorous stream of N_2 and touching the residual colorless syrup with a glass rod at −10 °C. For establishing purity, NMR spectra were taken immediately after transferring ~10 mg of the crystals to a precooled (−20 °C) NMR tube and addition of $CDCl_3$ (350 μL) at −20 °C.

A similar procedure was used to treat the thiocarbamate or sulfoxide with 2–2.5 or 1 molar equivalent of MCPBA, respectively.

These reactions were conveniently monitored by NMR using a several step procedure. First, the exact chemical shifts were recorded for the thiocarbamate (0.05 or 0.1 mmol) in $CDCl_3$ (350 μL with 1% Me_4Si). Second, the solution was concentrated to 100 μL with a stream of N_2. Third, a cold solution of MCPBA in $CDCl_3$ (250 μL with 1% Me_4Si) was added at −15 to −20 °C. Finally, the cold tube was taken immediately after MCPBA addition or 5 or 10 min later and quickly transferred into the NMR probe (40 °C) and a spectrum taken within 2–5 s.

Reactions of Thiocarbamate Sulfoxides. The NMR monitoring procedure given above was modified only by taking additional spectra on the thiocarbamate–MCPBA mixture or the isolated thiocarbamate sulfoxide at 40 °C over a time span of a few seconds to several hours. For convenience, the NMR tube was returned to an acetone–dry ice bath as necessary to assist in monitoring the reaction or recording the spectra and the precise chemical shifts. When these spectra revealed essentially complete decomposition of the sulfoxides at 40 °C, the samples were subjected to distillation at low temperature in a closed system at ~20 mm. The NMR tube containing the reaction mixture was connected to a small glass Y-tube allowing distillation at ~20 °C within a few minutes of $CDCl_3$ and volatile compounds with collection in a new NMR tube cooled to −70 °C. The distillate and the residue were examined by NMR, CI–MS, and IR comparisons with standard compounds from synthesis as described above. Samples of *cis*- and *trans*-diallate sulfoxides and triallate sulfoxide were also examined by NMR and CI–MS after holding 24 h in $CDCl_3$ at 40 °C, with or without distillation. Crystalline material in the distillation residue was subjected to NMR and CI–MS analyses with or without recrystallization (water and $CHCl_3$).

For IR monitoring of thiocarbamate sulfoxide degradation, spectra were recorded for CCl_4 solutions held various times at 22 °C, and comparisons were made on changes in the region of the C═O bands (1675–1770 cm^{-1}) and loss of the S→O band (1070–1075 cm^{-1}).

cis- or *trans*-Diallate sulfoxide (0.05 mmol) was treated with equimolar GSH in 350 μL of D_2O–acetone (d_6) (1:1) at 40 °C with NMR and TLC monitoring of the reaction products (for methods, see Chen et al., 1979).

Metabolism of Diallate. In order to analyze for the possible presence of 2-[^{14}C]chloroacrolein as a microsomal metabolite, a method was developed to trap this compound as its DNPH derivative for TLC analysis. DNPH derivatives are very useful in TLC separation of closely related aldehydes (Stahl and Jork, 1969). *cis,trans*-[^{14}C]Diallate (0.4 μmol) was added in ethanol (8 μL) to a mixture of hepatic microsomes from male albino mice (20% liver fresh weight equivalent, prepared according to Chen and Casida, 1978) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) (0 or 3.6 μmol) in 1.5 mL of 0.1 M sodium phosphate buffer (pH 7.4). The reaction mixture in a two-necked vial of 10-mL volume was incubated with shaking for 10 min at 37 °C while continuously sucking a stream of air through the incubation medium and three trapping vials, each of 2-mL volume. The first vial was an empty security trap to prevent droplet escape and the other two each contained 0.5 mL of DNPH reagent (see above). To facilitate transfer of 2-[^{14}C]-chloroacrolein formed as a diallate metabolite, unlabeled 2-chloroacrolein (1 μmol in 10 μL of ether) was injected into the reaction mixture after the first 10 min of incubation and then 5 min later an additional 10 μL was added and the reaction mixture was held 15 min at 42 °C. Precipitate in the middle trap was transferred quantitatively to a small sintered glass (1 cm) funnel, washed with cold ethanol (20 μL), and dissolved from the funnel by slow addition of ethyl acetate (1 mL) at 25 °C; elevated temperatures were avoided because of possible product polymerization. The adduct was analyzed for total radiocarbon content [combustion and liquid scintillation counting (LSC) of an aliquot] and for composition (TLC cochromatography). The third trap contained insignificant amounts of radiocarbon and hydrazone relative to the middle trap.

In separate enzymatic studies, analyses were made for 2-[^{14}C]chloroacrylic acid and [^{14}C]dichloroallylsulfonic acid as possible metabolites using rat liver microsomal preparations replacing mouse liver preparations, but otherwise as above, and substrate levels of 0.04, 0.4, and 4 μmol with incubation for 1 h in 25 mL Erlenmeyer flasks. The incubated reaction mixtures were adjusted to pH 3 by addition of HCl and extracted with ether (1 vol × 3). $(NH_4)_2SO_4$ was added to saturate the aqueous phase which was then extracted with ether–ethanol mixture (2:1, 1 vol × 3). The ether and ether–ethanol extracts were individually analyzed by two-dimensional TLC and the aqueous phase and precipitate (unextracted residue) were subjected to combustion and LSC.

cis,trans-[^{14}C]Diallate was administered orally by stomach tube at 0.1 mg/kg to a male albino rat and intraperitoneally (ip) at 0.2 mg/kg to a male albino mouse. The urine was collected for 72 and 12 h, respectively, in appropriate metabolism cages. The whole urine, its ether and ether–ethanol extracts, and the remaining aqueous phase, prepared as above, were subjected to TLC.

Herbicidal Assays. Oat seedlings (cultivar Curt, *Avena sativa*) with roots of 1–3-mm length were placed in petri dishes (9-cm diameter), each containing a filter paper (Whatman no. 1) and 1 mL of water. Water (1 mL) containing an acetone solution (10 μL) of the test compound was then added, and the petri dishes were covered and held under continuous fluorescent lighting for 48 h. Measurements were made on the lengths of the shoot and each root for comparison with controls containing acetone only. The experiments involved five seedlings per dish, duplicates within each series, and two experiments are averaged in the tabulated results.

RESULTS

Peracid Oxidation of Mono-, Di-, and Trichloroallyl Diisopropylthiocarbamates. Sulfoxides of the *S*-(3-chloroallyl) thiocarbamates were prepared by a method used previously for *S*-alkyl and *S*-benzyl compounds (Casida et al., 1974, 1975a; Tilles and Casida, 1975) with care to work at low temperature to minimize decomposition. *cis*- and *trans*-Diallate and triallate are almost quantitatively converted to sulfoxide derivatives on treatment with equimolar MCPBA in CH_2Cl_2 or $CDCl_3$ at −15 °C. The reaction with MCPBA is significantly faster with *cis*-diallate than with *trans*-diallate or triallate, al-

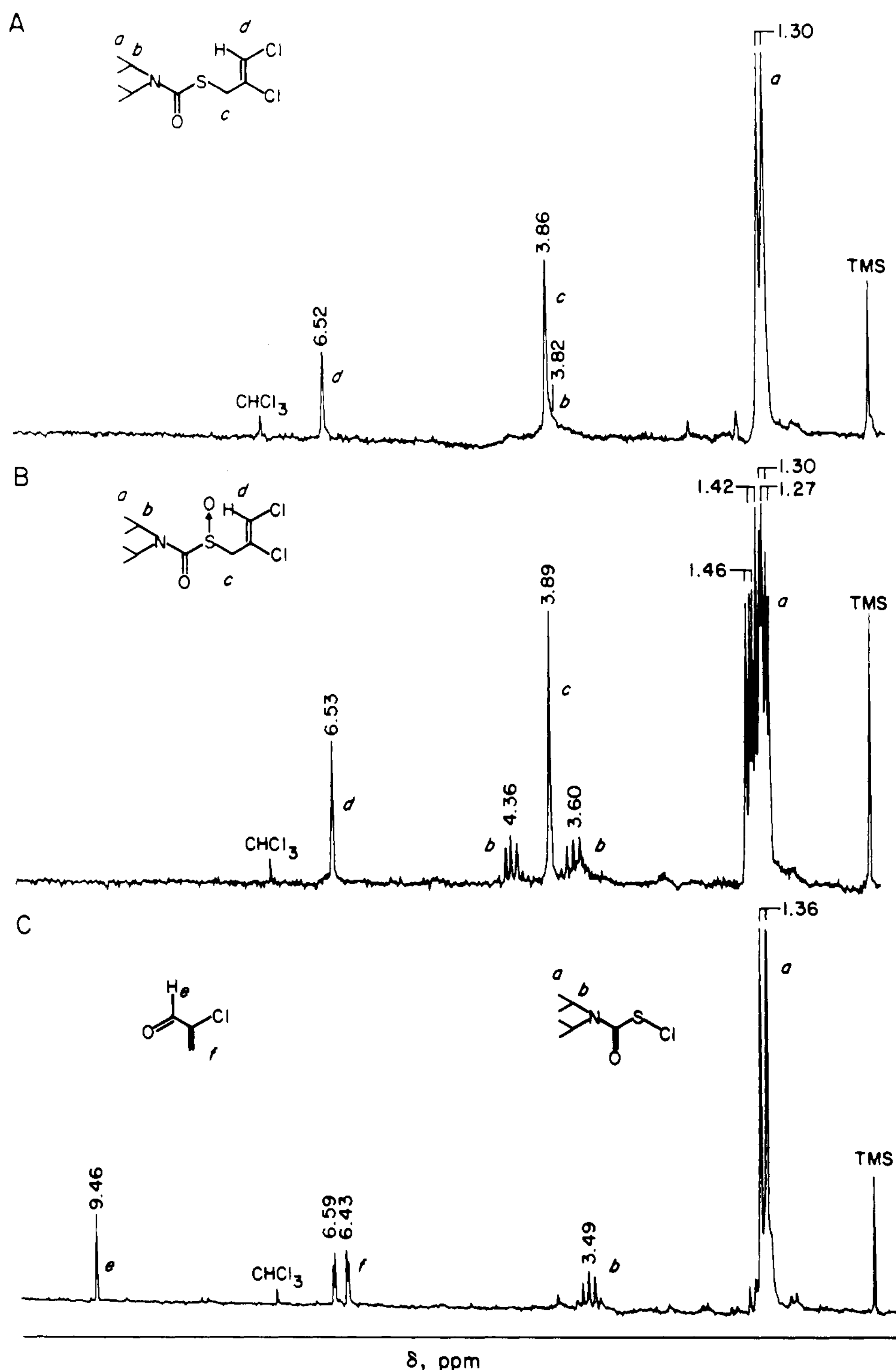

Figure 2. NMR spectra and assignments of $CDCl_3$ solutions of (A) *cis*-diallate, (B) *cis*-diallate sulfoxide, and (C) 2-chloroacrolein plus diisopropylcarbamoylsulfenyl chloride from decomposition of the sulfoxide on holding 10 min at 40 °C.

though in each case it is essentially complete within 5 min under the monitoring conditions used. With 2.5 molar equivalents of MCPBA the reaction at −15 °C stops at the sulfoxide, whereas at 20–30 °C it proceeds to give >50% sulfone in 1 h. The sulfones are also obtained, but in lower yield, on treating the sulfoxides with a second molar equivalent of MCPBA at >20 °C. The higher sulfone yields on rapid oxidation of the thiocarbamates with excess MCPBA may be associated with a shorter exposure time of the sulfoxide intermediate to the elevated temperatures required for sulfone formation which also inevitably lead to sulfoxide decomposition (see below).

The identities of the sulfoxides and sulfones are readily evident from their NMR spectra. The chemical shifts of the methyl group protons are diagnostic for the presence of the chiral center at sulfur and the degree to which there is restricted rotation around the amide N–CO bond. Thus, the coalescence temperatures for thiocarbamates, thiocarbamate sulfoxides, and thiocarbamate sulfones are −1–7, 52–61, and 93–102 °C, respectively (Tseng and Below, 1977). The methyl groups of the parent thiocarbamates show a doublet in their NMR spectra at 40 °C (Figure 2), whereas at temperatures below 10 °C two doublets are evident due to restricted rotation around the N–CO bond. Spectra of the sulfoxides reveal four doublets even at 40 °C due to the four methyl groups that are nonequivalent because of the chiral center at the sulfur and the restricted rotation around the amide bond. The sulfones at 40 °C

have only two doublets for the four methyl groups (nonequivalence for methyl groups between but not within the two diisopropyl moieties) as a result of the loss of the chiral center on sulfone formation while retaining restricted rotation around the amide bond.

Reactions of Mono-, Di-, and Trichloroallyl Diisopropylthiocarbamate Sulfoxides. The sulfoxides of *cis*- and *trans*-diallate and triallate are unstable at room temperature, but at −15 °C they can be isolated and held a few hours or days in relatively high purity. NMR monitoring of $CDCl_3$ solutions at 40 °C reveals essentially complete breakdown of the three sulfoxides in 10, 60, and 120 min, respectively. Sulfoxides of *cis*- and *trans*-diallate degrade to 2-chloroacrolein at a similar rate in D_2O (as an emulsion) or D_2O–acetone-d_6 (1:1) as in $CDCl_3$.

The relative stability of *cis*- and *trans*-diallate sulfoxides and triallate sulfoxide is fully supported for CCl_4 solutions by IR monitoring of their decomposition (S→O loss and changes in C=O bands). Due to their instability, these sulfoxides can be analyzed by TLC at 4 °C but not at 20 °C (Table I).

The NMR spectrum of thermally degraded *cis*-diallate sulfoxide is a combination of those of diisopropylcarbamoylsulfenyl chloride and 2-chloroacrolein (Figure 2). On holding the sulfoxide for 24 h the reaction mixture often assumes the characteristic turbidity of elemental sulfur, in agreement with an observation of Bögemann et al. (1955) on a related compound, and the liberation of sulfur is confirmed by elemental analysis. Analyses of reaction mixtures held 24 h at 40 °C establish the presence of diisopropylcarbamoyl chloride (CI-MS with support by NMR) and diisopropylamine hydrochloride (isolated as a crystalline material, NMR, CI-MS).

The decomposition products of *trans*-diallate sulfoxide are identical with those from the cis isomer, i.e., almost quantitative liberation of 2-chloroacrolein and diisopropylcarbamoylsulfenyl chloride. The sulfoxides of triallate and *S*-(3-chloroallyl) diisopropylthiocarbamate decompose to give the carbamoylsulfenyl chloride plus 2-chloroacrylyl chloride (NMR, CI-MS) and acrolein (NMR), respectively.

cis-Diallate sulfoxide reacts quickly with GSH to give the *S*-diisopropylcarbamoyl-GSH derivative (NMR and TLC cochromatography for identification) without the liberation of 2-chloroacrolein (NMR).

Reactions of 2-Chloroacrolein. No reaction is evident within 20 min at 60 °C between 2-chloroacrolein and equimolar MCPBA in $CDCl_3$ but there is ~50% yield of 2-chloroacrylic acid after 10 h at 50 °C. 2-Chloroacrolein is slowly trimerized in D_2O, i.e., ~20% trimer after 3 days at 40 °C and 75% trimer after 7 days at 20 °C.

Metabolites of [*allyl*-^{14}C]Diallate. The mouse hepatic microsomal system converts diallate to 2-chloroacrolein based on the volatilization, trapping, and derivatization method involving two-dimensional TLC cochromatography of the 2-chloroacrolein-DNPH derivative in various combinations of five different solvent systems (Table II). The 2-chloroacrolein yield was 0.04% in the absence of NADPH and 0.9, 1.6, and 2.3% in three separate experiments with NADPH fortification. Another metabolite, dichloroallylsulfonic acid, was detected in the incubated mouse microsome mixture.

The rat liver microsomal system gave dichloroallylsulfonic acid as a major metabolite and no detectable 2-chloroacrylic acid based on two-dimensional TLC cochromatography involving five solvent systems as above (Table II). At the normal substrate level (0.40 μmol), the products detected, each dependent on NADPH fortification, were as follows: unmetabolized substrate (52%) appearing mostly in the ether extract; dichloroallylsulfonic acid (9% recovery in the ether–ethanol extract); two unidentified metabolites (7%) chromatographing between the aforementioned compounds and extracted with both ether and ether–ethanol; water-soluble metabolites (9%) and unextracted products (23%). When the substrate levels were 0.04 and 4 μmol, the dichloroallylsulfonic acid recoveries in the ether–ethanol extract were 22 and 6%, respectively.

Rat urine contained 43, 51, and 57% of the orally administered radiocarbon at 12, 24, and 72 h, respectively. TLC examination of the 0–24 h urine revealed one major metabolite in the ether extract and another appearing in both the ether–ethanol extract and the remaining water-soluble products. The latter metabolite accounting for 43% of the administered radiocarbon cochromatographed with dichloroallylsulfonic acid in five different TLC solvent systems used in two dimensions (Table II). The less polar metabolite (2% of the administered radiocarbon) chromatographed in the region of but failed to cochromatograph with 2-chloroacrylic acid. No diallate appeared in the urine.

The urine of the ip-treated mouse with 33% of the administered radiocarbon at 12 h contained dichloroallylsulfonic acid (75% of the urinary radiocarbon identified by TLC as above), a material chromatographing similar to but not identical with 2-chloroacrylic acid (0.9%) and compounds of higher polarity [remaining at the origin on TLC in the 1-butanol–acetic acid–water (6:1:1) solvent system; see Table II].

Herbicidal Activity. Sulfoxides of *cis*- and *trans*-diallate and triallate are less potent than their nonsulfoxidized precursors in inhibiting root and shoot growth of oat seedlings (Table III). Of the products derivable by oxidation of the diallate isomers or triallate which were tested, only 2-chloroacrolein falls in an activity range of interest; it equals or exceeds the potency of the diallate isomers in inhibiting root growth. The product mixtures after the rearrangement–elimination reactions (aged diallate and triallate sulfoxides) are similar in herbicidal activity to the freshly prepared compounds, indicating that externally applied sulfoxides probably degrade before entering the plant or arriving at the site of action. Thus, it is not possible from assays of this type to make a meaningful judgment on the potential herbicidal activity of the sulfoxides because of their instability.

Table III. Potency of *cis*- and *trans*-Diallate and Triallate and Their Sulfoxides and Other Derivatives or Degradation Products in Inhibiting Root and Shoot Elongation of Oat Seedlings

compound	ppm for 50% inhibition	
	root	shoot
cis-diallate	9	7
trans-diallate	20	8
triallate	22	12
cis-diallate sulfoxide[a]	52	30
trans-diallate sulfoxide[a]	40	25
triallate sulfoxide[a]	60	25
2-chloroacrolein[b]	7	>100
2-chloroacrylyl chloride	90	>100
2-chloroacrylic acid	70	>100
diisopropylcarbamoyl chloride	>100	>100
diisopropylamine hydrochloride	>100	>100

[a] Aqueous solution aged by holding 2 h at 50 °C prior to addition to germinated seedlings is similar in activity to freshly prepared sulfoxide solution. [b] Acrolein is of similar potency.

DISCUSSION

Figure 1 shows a series of products derived from *cis*- and *trans*-diallate and triallate that originate from reaction sequences requiring sulfoxide formation as the initial step. The sulfoxides are easily obtained by oxidation of these thiocarbamates with MCPBA, providing the reactions are carried out at low temperature to avoid or minimize product decomposition. They are not of sufficient stability for direct detection in microsomal monooxygenase systems or in vivo, so their possible presence as transitory intermediates in biological systems must be judged by the presence of other metabolites that form preferentially or only via the sulfoxides. [*allyl*-^{14}C]Diallate-treated rats and mice excrete [*allyl*-^{14}C]dichloroallylsulfonic acid which may originate via the sulfoxide and the sulfenic and sulfinic acids among other pathways. The [^{14}C=O]carbamoylcysteine derivative and [^{14}C=O]mercapturic acid are rat urinary metabolites of *cis*- and *trans*-[^{14}C=O]diallate and the carbamoyl-GSH compound is formed in the mouse hepatic microsomal oxygenase system fortified with GSH (Chen et al., 1979). Of even greater relevance and importance is the finding of 2-chloroacrolein as a diallate metabolite in the microsomal oxygenase system. Formation of 2-chloroacrolein depends on the sulfoxide as an intermediate as discussed below.

Sulfoxides of 3-chloroallyl thiocarbamates are quite unstable compounds due to a facile [2,3] sigmatropic rearrangement reaction via A to give the unstable *S,O*-(1-chloroallyl) thiocarbamate sulfenate ester B (Figure 1). This is followed by a 1,2-elimination reaction, giving acrolein from the 3-chloroallyl thiocarbamate sulfoxide, 2-chloroacrolein from the diallate sulfoxide isomers, and 2-chloroacrylyl chloride from triallate sulfoxide, in addition to the carbamoylsulfenyl chloride from each sulfoxide. In contrast, rearrangement of a related *S*-(2-chloroallyl) thiocarbamate sulfoxide yields the corresponding *S,O*-(2-chloroallyl) thiocarbamate sulfenate ester (Schuphan and Casida, 1979), which in this case is a stable compound since it lacks the 1-chloro substituent necessary for the elimination reaction.

Diallate and triallate sulfoxides undergo two distinct alternative types of reactions important to their biological activity: (1) rearrangement–elimination as above to form 2-chloroacrolein and 2-chloroacrylyl chloride, respectively; (2) carbamoylation of tissue thiols with liberation of 2,3-dichloroallylsulfenic acid. There is a close interrelationship between the reactions of the sulfoxides and the GSH level. The rearrangement–elimination reaction sequence occurs only with that portion of the sulfoxide that does not react with GSH or undergo direct hydrolysis. In forming carbamoyl-GSH, the sulfoxide lowers tissue levels of free GSH. Because of their instability, the sulfoxides most likely react within the cell where they are formed and even compartmentalized reactions within the cell may be important. In rats, the detoxification pathway via GSH carbamoylation accounts for a high proportion of an oral dose of *cis*- and *trans*-diallate based on the amounts of excreted cysteine and mercapturic acid conjugates (Figure 1) and mercaptoacetic acid conjugate (Chen et al., 1979). The alternative pathway via 2-chloroacrolein is of relatively minor quantitative significance in rats, but it may be of major toxicological importance because of the potency of 2-chloroacrolein as a mutagenic agent (Schuphan and Casida, 1979; Schuphan et al., 1979).

Diallate and triallate are probably proherbicides that undergo bioactivation to the sulfoxides or via the sulfoxides to other metabolic inhibitors, e.g., 2-chloroacrolein and 2-chloroacrylyl chloride. It is proposed that the herbicidal activities of *S*-alkyl and *S*-benzyl thiocarbamates result from their sulfoxide derivatives (Casida et al., 1974) carbamoylating thiol sites important in fatty acid biosynthesis (Lay and Casida, 1976). An analogous argument can be made for diallate and triallate since they are similar to the other thiocarbamate herbicides in their effects on sensitive plants (Fang, 1975; Wilkinson, 1978). However, it is also plausible that diallate serves only as a liposoluble precursor for 2-chloroacrolein which may contribute to or be the cause of the herbicidal action. The herbicide could conceivably have both types of action. The relation of chemical reactivity to herbicidal activity is further complicated by the observation of antagonistic effects between the diallate isomers (Rummens et al., 1975). It is interesting in this context to note that *cis*-diallate sulfoxide undergoes the rearrangement–elimination reactions perhaps ten-fold faster than the trans isomer and triallate sulfoxide and that a portion of the sulfoxide dose is probably used up in GSH conjugation. It therefore appears that *cis*- and *trans*-diallate and triallate have several types of biological activities that may depend on different features of the reactivity of their sulfoxide metabolites.

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Supplementary Material Available: Analytical data for *cis*- and *trans*-diallate and for triallate and their sulfoxides, sulfones, and other derivatives or degradation products (4 pages). Ordering information is given on any current masthead page.

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Dissipation of the Experimental Aquatic Herbicide Fluridone from Lakes and Ponds

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The dissipation of the aquatic herbicide fluridone, 1-methyl-3-phenyl-5-[3-trifluoromethyl)phenyl]-4(1*H*)-pyridinone, has been determined in experiments conducted in small ponds at three different geographic regions in the United States and in Gatun Lake of the Panama Canal. Fluridone dissipated rapidly from the water, with a half-life averaging 5 days. The dissipation was due in part to deposition on hydrosoil and uptake by aquatic plants, although evidence is presented to suggest photolysis as a contributing mechanism. The accumulation and dissipation patterns of fluridone on hydrosoil were highly variable. The herbicide demonstrated a very low potential for bioconcentration in fish, zooplankton, and aquatic plants.

Fluridone, 1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1*H*)-pyridinone, is an experimental herbicide currently being developed by Elanco Products Co., a Division of Eli Lilly and Co. Fluridone has demonstrated potential as both a terrestrial herbicide for use on cotton (Waldrep and Taylor, 1976, 1977; Webster et al., 1977) and as an aquatic herbicide with unique activity against aquatic vascular plants at low application rates (McCowen at al., 1979; Sanders et al., 1979; Arnold, 1979; Parka et al., 1978).

The absorption, translocation, and metabolism of fluridone in several agronomic crops has been reported (Berard et al., 1978), and mode of action studies have been published (Bartels and Watson, 1978; Devlin et al., 1978; Berard et al., 1978). The dissipation of fluridone when applied as an aquatic herbicide to a Canadian pond has also been reported (Muir and Grift, 1978). In the Canadian study, fluridone exhibited a half-life of 4–7 days in the water and greater than 3 months in the hydrosoil. In the same study duckweed (*Lemna minor*) was reported to concentrate the herbicide by a factor of 85 compared to the concentration in the water, although this amount was estimated to represent less than 1% of the total amount of herbicide applied to the pond (Muir and Grift, 1978).

In this paper the dissipation of fluridone from water and hydrosoil is reported for studies conducted in small ponds located in Michigan, New York, and Florida and in Gatun Lake of the Panama Canal. The residue level of fluridone in fish, zooplankton, and aquatic plants is also reported for some of the small pond experiments.

EXPERIMENTAL SECTION

Methods of Application and Plot Description. The initial study with fluridone was conducted at Lake City, Michigan, in June, 1976 (McCowen et al., 1979). The pond was 0.04 ha in size with an average depth of 1.1 m. Fluridone formulated as a 4 lb/per gallon aqueous suspension (4AS) was subsurface applied (SSA) with a conventional CO_2 sprayer at a rate of 0.1 ppm relative to the total water column, which was equivalent to 1.12 kg/ha.

A second trial was begun at Ithaca, New York, in May, 1977 (McCowen et al., 1979). The ponds in this experiment were 0.07 ha in size with an average depth of 0.9 m. Fluridone 4AS was applied either by SSA or by layering the herbicide on the bottom of the pond with a hand-held CO_2 sprayer. With the SSA technique, fluridone was applied at 0.3 ppm relative to the total water column, which was equivalent to 2.7 kg/ha. With the layered technique, fluridone was applied at 0.3 ppm relative to the bottom one-third of the water column, which was equivalent to 0.9 kg/ha.

A third trial was begun in April, 1977, at Orlando, Florida, with a 0.16-ha pond averaging 0.7 m deep (Arnold, 1977). Fluridone 4AS was applied at 0.3 ppm relative to the total water column, which was equivalent to 2.0 kg/ha.

A fourth trial was begun at Hialeah, Florida, in September, 1977, with 0.4-ha and 0.8-ha ponds averaging 5.2 m deep (Arnold, 1979). The herbicide was applied to the bottom of the ponds via an airboat equipped with a two-stage piston pump and weighted, trailing hoses. The application rates were 1.1 and 1.7 kg/ha, which were equivalent to 0.02 and 0.03 ppm relative to the total water column.

Several trials were begun in January, 1978, in selected areas of Gatun Lake, a man-made reservoir in the Panama Canal (Sanders et al., 1979). Fluridone 4AS was applied below the water surface from an airboat equipped with a conventional spray pump and weighted, trailing hoses 6.1 m long. Fluridone formulated as a 5% pellet (5P) was surface applied (SA) using a rotary spreader mounted on the front of an airboat. The pellets sank to the hydrosoil. Both formulations were applied at 1.7 and 6.7 kg/ha to plots which varied in size from 0.7 to 1.0 ha and in depth from 2.1 to 8.2 m.

Residue Sampling Procedures. Residue samples were collected for analysis at regular intervals following the application of fluridone. In the Michigan trial, water samples were collected just below the water surface. In the other experiments, samples were collected at various depths on each sampling date. Water samples were preserved with 0.5 mL of concentrated sulfuric acid per liter and stored at 4°C until analyzed. (Previous studies had established the stability of fluridone under these conditions.)

Three to ten hydrosoil subsamples were collected with a soil sampler containing removable 2.54 cm i.d. plastic tubes. These cores were taken at depths of 0–7.6 to 0–25.4 cm, depending on the texture and hardness of the hydrosoil. Upon receipt, hydrosoil subsamples from individual tubes were combined and excess water was removed

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