Resolution and Reactions of the Chiral Isomers of *O*-Ethyl *S*-Phenyl Ethylphosphonodithioate (Fonofos) and Its Analogues

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The chiral isomers of *O*-ethyl ethylphosphonothioic acid, *O*-ethyl *S*-phenyl ethylphosphonodithioate (fonofos), and *O*-ethyl *S*-*p*-bromophenyl ethylphosphonodithioate (*p*-bromo analogue of fonofos) were prepared and their absolute configurations were established by x-ray diffraction analysis and chemical correlation. Also prepared were the chiral isomers of *O*-ethyl *S*-phenyl ethylphosphonothioate (fonofos oxon), *O*-ethyl *S*-*p*-bromophenyl ethylphosphonothioate (*p*-bromo fonofos oxon), phenyl and *p*-bromophenyl ethyl(ethoxy)phosphinyl disulfides. The absolute configurations of the enantiomers of these compounds were assigned by relating them to the configurations of the corresponding *O*-ethyl ethylphosphonothioic acids. Oxidation of $(S)_P$-fonofos by *m*-chloroperoxybenzoic acid took place stereoselectively, resulting predominantly in $(R)_P$-fonofos oxon and $(R)_P$-phenyl ethyl(ethoxy)phosphinyl disulfide. Conversely, $(R)_P$-fonofos gave $(S)_P$-oxon and $(S)_P$-disulfide. Oxidation to the oxon proceeded with retention, but oxidation to the disulfide occurred with inversion.

Biological oxidations are of considerable significance in the in vivo modification of pesticides in plants and animals. The various metabolic reactions which take place, e.g., olefin epoxidation, thioether oxidation, N- or O-dealkylation, aliphatic and aromatic hydroxylation, and phosphorothionate desulfuration (P=S to P=O conversion), result in both activated and detoxified products and are mediated by the mixed function oxidases (MFO) (Ullrich, 1972; Wilkinson and Brattsten, 1972; Fukuto and Metcalf, 1969). Because of the importance of MFO, a variety of chemical model oxidation systems have been examined in seeking correspondence between products of biological oxidation and those obtained from exclusively chemical systems. These include the systems of Fenton (1894), Udenfriend et al. (1954), peroxytrifluoroacetic acid (Ptashne et al., 1971), and *m*-chloroperoxybenzoic acid (McBain et al., 1971a; Fahmy and Fukuto, 1972; Wustner et al., 1972; Bellet and Casida, 1974; Wustner et al., 1974).

Owing to the poor anticholinesterase activity of phosphorothionate (P=S) triesters, activation to the corresponding P=O ester is essential for intoxication by phosphorothionate insecticides. Mechanistic studies on the transformation of P=S to P=O esters by MFO and model chemical oxidation systems have recently received considerable attention (Ptashne et al., 1971; Ptashne and Neal, 1972; McBain et al., 1971b; Wustner et al., 1972; Herriott, 1971). Previous investigations in this and other laboratories (Wustner et al., 1972; McBain et al., 1971b) on the *m*-chloroperoxybenzoic acid oxidation of *O*-ethyl *S*-phenyl ethylphosphonodithioate (fonofos) revealed that fonofos was converted into a variety of compounds, the principal products being fonofos oxon (*O*-ethyl *S*-phenyl ethylphosphonothioate) and phenyl ethyl(ethoxy)phosphinyl disulfide, an unusual oxidative rearrangement product. Because of the chiral nature of the phosphorus atom in fonofos, the oxidation reaction was reexamined with the resolved $(R)_P$ and $(S)_P$ isomers of fonofos. This report is concerned with the resolution, determination of absolute configuration, and the stereochemical aspects of the *m*-chloroperoxybenzoic acid oxidation and alkaline hydrolysis of the chiral isomers of fonofos.

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MATERIALS AND METHODS

General. Optical rotations were determined with a Rudolph Model 80 high precision polarimeter (±0.02°) at the sodium D line (589 nm) or with a Karl Zeiss photoelectric polarimeter (±0.005°) at 578 and 546 nm. Proton magnetic resonance (^{1}H NMR) spectra were determined in Varian Model A-60, Model A-60D, or Model T-60 spectrometers. Spectra were determined as 5–10% solutions in carbon tetrachloride, deuteriochloroform, or deuterioacetone using 1% tetramethylsilane as the internal standard. Infrared spectra were obtained with a Perkin-Elmer Model 137 or Model 621 grating spectrophotometer. Mass spectra were recorded on a Finnigan Model 1015 GC–mass spectrometer interfaced with a digital computer controlled data acquisition and reduction system (System-150).

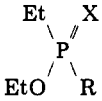
Thin-layer chromatography (TLC) was carried out on commerical Q1F silica gel plates (Quantum Industries, Fairfield, N.J.) and silica gel HLF_{254} plates (Analtech, Newark, Del.), 20 × 20 cm of 0.25 and 0.50 mm thickness, with inert binder and phosphor indicator. All melting and boiling points are uncorrected.

Synthesis. *O*-Ethyl ethylphosphonothioic acid (**1**), prepared according to Hoffman et al. (1959), was partially resolved via its diastereomeric quinine salts as described by Aaron et al. (1958). Further resolution of the (−)-(**1a**) and (+)-enantiomers (**1b**) of the acid was achieved by repeated recrystallization in ethyl acetate–hexane (2:1) of the corresponding salts prepared from (−)- and (+)-α-phenethylamine (Theilacker and Winkler, 1954). X-ray analysis was conducted on a purified sample of minus-rotating α-phenethylammonium salt of the acid (**2a**) as described later. Physical constants and values of optical rotation for the enantiomeric acids and α-phenethylammonium salts are presented in Table I, along with those of other relevant compounds.

Racemic **3** and optically active *O*-ethyl ethylphosphonochloridothioate (**3a** and **3b**) were prepared according to Michalski and Mikolajczyk (1964) from racemic or enantiomeric **1** and phosphorus pentachloride in carbon tetrachloride at −5 °C.

Enantiomeric *O*-ethyl *S*-phenyl ethylphosphonodithioate (fonofos, **4a** and **4b**) and its *p*-bromo analogue (**5a** and **5b**) were prepared by reacting the enantiomeric *O*-ethyl ethylphosphonochloridothioates (**3a** and **3b**) with the appropriate sodium arylthiolates in anhydrous 1,2-di-

Table I. Physical Constants and Values of Observed (Neat) and Specific Rotations in Degrees Arc for the Compounds of General Structure



Structure	No.	Abs config	% yield	Mp or bp (mm), °C	n_D^{24}	Obsd α_D^{24} (neat)	Specific[a] $[\alpha]^{24}_D$
X = S	1	Racemic	86	64-65 (0.10)	1.4885		
R = OH	1a	$(S)_P$	73	61-62 (0.08)	1.4887	−15.45	
	1b	$(R)_P$	75	64-65 (0.10)	1.4882	+14.82	
X = S	2a	$(S)_P$	74	104-105[b]			−12.78 (*c* 8.13)[c]
R = $O^-H_3N^+CH(CH_3)C_6H_5$	2b	$(R)_P$	68	104-105			+12.40 (*c* 7.43)[c]
X = S	3	Racemic	81	34-36 (0.7)	1.4921		
R = Cl	3a		86	26-27 (0.3)	1.4923	−82.50	
	3b		83	25-27 (0.3)	1.4920	+78.65	
X = S	4	Racemic	84	124-126 (0.1)	1.5894		
R = SC_6H_5	4a	$(S)_P$	93		1.5896	−181.50	+138.40 (*c* 0.301)[d]
	4b	$(R)_P$	92		1.5895	+155.98	−114.56 (*c* 0.309)
X = S	5	Racemic	82	31[b]			
R = SC_6H_4-*p*-Br	5a	$(S)_P$	96	31.5		−257.90[e]	+130.67 (*c* 17.02)[d]
	5b	$(R)_P$	92	31.5		+258.13	−129.53 (*c* 9.65)
X = O	6	Racemic	78	94-95 (0.005)	1.5453		
R = SC_6H_5	6a	$(S)_P$	52		1.5456		−121.70 (*c* 0.636)
	6b	$(R)_P$	61		1.5452		+112.6 (*c* 0.533)
X = O	7	Racemic	78	106-108 (0.10)	1.5836		
R = SC_6H_4-*p*-Br	7a	$(S)_P$	61	107-108 (0.11)	1.5823		−107.87 (*c* 7.63)
X = O	8	Racemic	87		1.5719		
R = SSC_6H_5	8a	$(S)_P$	92		1.5731	−32.30	−68.27 (*c* 4.70)
X = O	9	Racemic	93		1.6076		
R = SSC_6H_4-*p*-Br	9a	$(S)_P$	94		1.6068	−65.50	−138.38 (*c* 8.20)[f]

[a] In cyclohexane solvent unless specified. [b] Melting point. [c] In methanol solvent. [d] A change in sign of rotation from the neat measurements also was observed in other solvents. [e] Rotation of melted material was measured. [f] In carbon tetrachloride solvent.

methoxyethane–ether solvent (1:1) at 0 °C, in general accordance with the procedure of Menn and Szabo (1965).

Racemic and enantiomeric *O*-ethyl *S*-phenyl ethylphosphonothioate (**6**, **6a**, and **6b**) and *O*-ethyl *S*-*p*-bromophenyl ethylphosphonothioate (**7** and **7a**) were prepared by the reaction between the corresponding aryldiazonium chloride and sodium salt of *O*-ethyl ethylphosphonothioic acid (**1**, **1a**, or **1b**) in ether–water (1:1) at 0 °C. Each product was purified on a Silicar CC7 column eluted with a mixed solvent consisting of equal volumes of ethyl acetate–ether–benzene–hexane. Fractions of 2 mL each were collected, and the product found in fractions 60–120 was combined and distilled in a microdistillation apparatus. A sample of **6a** gave the following elemental analysis: calcd for $C_{10}H_{15}O_2PS$: C, 52.16; H, 6.57; found: C, 52.05; H, 6.48. In addition to elemental analysis, the structures of this and other key compounds were confirmed by ^1H NMR, infrared, and mass spectral analysis.

Racemic and optically active phenyl (**8**, **8a**) and *p*-bromophenyl ethyl(ethoxy)phosphinyl disulfide (**9**, **9b**) were prepared according to the method of Schrader and Lorenz (1962) from the arylsulfenyl chloride and the acids **1** and **1a** in carbon tetrachloride at 5 °C. The individual products were purified on a Silicar CC7 column using ether–benzene (2:3) as the eluting solvent. Compounds **8** and **9a** gave the following elemental analysis: calcd for $C_{10}H_{15}O_2PS_2$ (**8**): C, 45.79; H, 5.76; found: C, 46.27; H, 6.01; calcd for $C_{10}H_{14}BrO_2PS_2$ (**9a**): C, 35.20; H, 4.14; found: C, 35.06; H, 4.09. The products were obtained in greater than 90% yield.

Synthesis of Enantiomeric ^{35}S-Labeled Fonofos (4a and 4b). Enantiomers of ^{35}S-labeled fonofos (**4a** and **4b**) were prepared by the same method used for nonradioactive isomers. To a stirred solution of sodium methoxide (64.8 mg, 1.20 mmol) in 5 mL of anhydrous methanol were added dropwise at 0 °C 3 mL of methanol containing 61.1 mg of [^{35}S]benzenethiol (Searle-Amersham, 20 mCi/mmol) and 55.6 mg of diluent benzenethiol (total of 1.09 mmol) under an argon atmosphere. The solution was divided into two equal portions, each used for the preparation of the individual isomers. The methanol solvent was removed under a gentle stream of argon, 5 mL of anhydrous dimethoxyethane was added, followed by dropwise addition of an excess of enantiomeric chloridothioate (**2a** or **2b**) in 3 mL of ether. The mixture was stirred at 0 °C for 30 min and at room temperature for an additional 5 h. After filtration, the mixture was concentrated under argon, dissolved in 5 mL of benzene, washed with water, and dried over sodium sulfate. The product was purified by column chromatography using Silicar CC7 (1 × 10 cm) and chloroform–hexane (2:3) as the solvent. Seventy fractions (10 mL each) eluted from the column were monitored for radioactivity by liquid scintillation counting (Beckman

LS-230 spectrometer), and the fractions containing [^{35}S]fonofos (fractions 26–57) were combined and concentrated. TLC analysis of ^{35}S-labeled **4a** on silica gel HLF_{254} plates (Analtech) using hexane–chloroform (3:2) solvent (Kalbfeld et al., 1968) gave a single spot (R_f 0.36) which was identical with an authentic sample of fonofos. TLC analysis of ^{35}S-labeled **4b**, however, showed another spot attributable to diphenyl disulfide (R_f 0.61) which was subsequently removed by preparative TLC using the same composition plates and developing solvent. Chemical and radioactive purity of >99% fonofos was established by both autoradiography of TLC plates (Eastman no-screen x-ray film) and by gas–liquid chromatography (Varian Model 1400 gas chromatograph equipped with an alkaline flame ionization detector). A glass column, 6 × $^1/_4$ in., packed with 5% (w/w) OV-17 on Gas-Chrom Q (80–100 mesh) was used. Temperatures of the injection port, column, and detector were 245, 235, and 260 °C, respectively. Flow rates of nitrogen carrier gas, hydrogen, and air were adjusted to 40, 34, and 210 mL/min, respectively. Samples were injected in redistilled ethyl acetate. Under the above conditions, the retention time of fonofos (**4**, **4a**, and **4b**) was 11.25 min and fonofos oxon (**7**, **7a**, and **7b**) was 8.75 min.

Determination of Absolute Configuration. Colorless crystals of **2a**, mp 104.5 °C, $[\alpha]^{24}_D$ −12.75° (methanol), were obtained by slow evaporation of a pentyl acetate solution. (Anal. Calcd for $C_{12}H_{22}NO_2PS$: C, 52.37; H, 7.76. Found: C, 52.34; H, 7.85.) The crystal used for x-ray analysis had dimensions 0.10 × 0.45 × 0.10 mm. Precession and Weissenberg photographs showed monoclinic symmetry and systematic extinctions $0k0$ with $k = 2n + 1$. Since the molecule was chiral, the space group was uniquely determined as $P2_1$. The cell constants, a = 11.500 (13), b = 6.691 (7), c = 11.327 (13) Å, and β = 115.08 (3)°, were determined from a least-squares fit of 12 carefully centered reflections measured on a Picker automatic diffractometer (Mo K$\bar{\alpha}$, λ = 0.71069 Å). The density, 1.15 (1) g cm^{-3}, measured by flotation in hexane/carbon tetrachloride, agrees with the value 1.158 g cm^{-3} calculated for two molecules of $C_{12}H_{22}NO_2PS$ in the unit cell.

Three-dimensional intensity data were collected on the above diffractometer, Mo K$\bar{\alpha}$ radiation made monochromatic by Bragg reflection from a graphite crystal, scanning reflections in the 2θ–θ mode at a scan rate of 1°/min and a scan range of 2 + 0.69 tan θ, and taking background counts of 10 s at each end of the scan. Of 688 unique reflections having 2θ values less than 37°, 632 remained after rejection of those with intensities less than 1.5σ. The function σ is defined as $\sigma(I) = [p + (t/20)^2B + (0.045I)^2]^{1/2}$ where p is the peak count, t is the time spent scanning the peak in seconds, B is the background count, and I is the integrated intensity. The reflections were put on a common scale using the intensities of three standards (collected every 75 reflections) and corrected for Lorentz–polarization effects. No changes were observed in the intensities of the standards over the time of data collection.

The structure was solved by the heavy-atom method, successive cycles of Fourier synthesis, and subsequently full-matrix least-squares refinement minimizing the function $\Sigma w(|F_o| - |F_c|)^2$ where $w = 4F^2/L^2\sigma(I)$ and L is the reciprocal Lorentz–polarization factor. Atomic scattering powers were taken from a standard compilation (Ibers, 1962). Sulfur and phosphorus atoms were refined anisotropically, whereas other atoms were refined isotropically. The final residual R was 10.5%, where $R = \Sigma(|F_o| - |F_c|)/\Sigma|F_o|$. The weighted R, defined as wR, = $[\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|)^2]$ was 12.9%, and χ (the standard deviation of an observation of unit weight) was 4.22. All hydrogen atoms were observed on the difference Fourier map, but not included in the refinement. Final atomic coordinates together with other x-ray data are available (see Supplementary Material).

A colorless crystal of **5a**, suitable for x-ray analysis, was obtained by slow evaporation of a methanol–water (9.5:0.5) solution at 5 °C. Precession (Mo K$\bar{\alpha}$) and Weissenberg (Cu K$\bar{\alpha}$) photographs showed triclinic symmetry. Because of the optically active nature of the molecule, the space group was determined as $P1$. The unit cell constants a = 7.187 (8), b = 11.780 (12), c = 9.523 (10) Å, α = 113.94 (4), β = 84.61 (3), and γ = 105.13 (3)°, were determined as described above for **2a**. The density, 1.52 (1) g cm^{-3}, measured by flotation in carbon tetrachloride–chloroform, agrees with 1.519, the value calculated for two molecules of $C_{10}H_{14}BrOPS_2$ in the unit cell. There are two independent molecules in an asymmetric unit.

Three-dimensional intensity data were collected as described above for **2a**. During the period of data collection the room temperature was maintained at 16 °C. At the end of the data collection the intensities of three standards, measured every 50 reflections, showed a decrease of about 20% of their initial values. The data were scaled according to the standards and corrected for Lorentz–polarization effects. Of 1218 unique reflections collected, 1039 having intensities greater than 1.5σ were retained for use in subsequent calculations.

The structure was solved by the heavy-atom method. The positions of the two bromine atoms were deduced from a three-dimensional Patterson function. After improving the coordinates of the two bromine atoms by two cycles of least-squares refinement, an electron density map was calculated from which the locations of the remaining nonhydrogen atoms were ascertained. Seven cycles of least-squares refinement with isotropic thermal parameters followed by four additional cycles with bromine, sulfur, and phosphorus refined anisotropically yielded R = 0.067 and wR = 0.087. The atomic scattering power of bromine was corrected for the real and imaginary parts of the anomalous dispersion (Templeton, 1962). Scattering factors used were those of Ibers (1962). Bromine, sulfur, and phosphorus atoms were refined anisotropically whereas other atoms were refined isotropically. All hydrogen atoms were observed on the Fourier difference map but were not included in the refinement. The absolute configuration of the (−)-enantiomer of **5a** was ascertained from the anomalous dispersion effects of bromine, sulfur, and phosphorus atoms. The final wR, based on F, was 7.8% for the correct configuration and 8.7% for the enantiomeric configuration (Hamilton, 1965). The (−)-enantiomer **5a** was therefore assigned the configuration $(S)_P$. χ for the correct configuration was 2.62 and R = 6.1%. The final atomic parameters and observed and calculated structure amplitudes are available (see Supplementary Material).

Peracid Oxidation. The enantiomers of fonofos (**4a** or **4b**) were treated with *m*-chloroperoxybenzoic acid in dichloromethane as previously described (Wustner et al., 1972; McBain et al., 1971a). **4a** or **4b** were fortified with approximately 1% of the corresponding ^{35}S-labeled materials to facilitate separation and identification of the products and to determine the fate of the thiol sulfur atom. The reaction mixture was washed with carbonate buffer (pH 8.0), dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The residue was taken up in 3.0 mL of benzene and 1-μL aliquots were subjected to two-dimensional TLC analysis using silica gel HLF_{254} plates (0.25 mm, Analtech) developed, in turn, with

Table II. R_f Values for the Various Products Obtained from the Oxidation of Fonofos and Its Enantiomers on Silica Gel HLF_{254} Plates

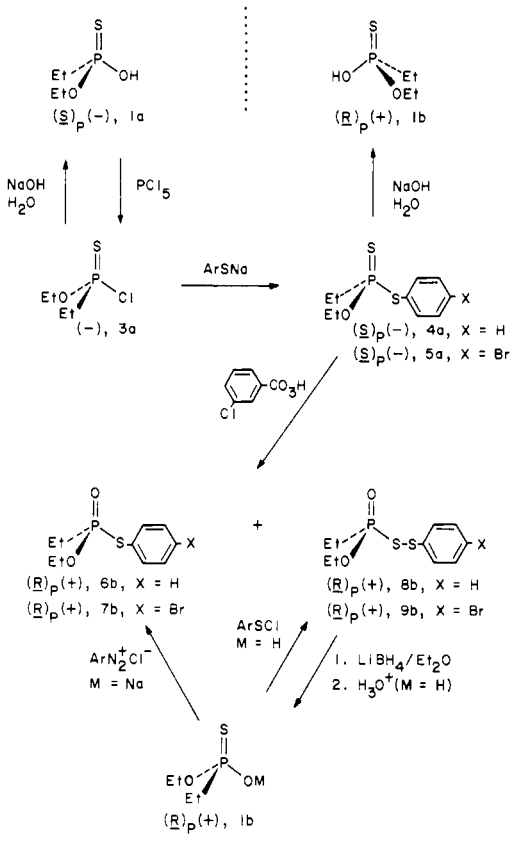
Compound	R_f values: Ether–hexane (4:1)	Hexane–chloroform (3:2)
Fonofos (4)	0.73	0.36
Fonofos oxon (7)	0.26	0.03
Phenyl ethyl(ethoxy)-phosphinyl disulfide (8)	0.33	0.03
Diphenyl disulfide	0.77	0.61
Diphenyl disulfide oxide	0.65	0.13

hexane–chloroform (3:2) and ether–hexane (4:1). R_f values of the various oxidation products in the two solvent systems are given in Table II. The products were located by scanning the plates with a Berthold Varian Aerograph Model 6000-10 thin-layer radioscanner equipped with a dot printer and verified by autoradiography. Individual spots were collected and radioactivity was quantified by scintillation counting. The relative yields of products were: 45% unreacted fonofos, 15% fonofos oxon (**6**), 23% phenyl ethyl(ethoxy)phosphinyl disulfide (**8**), 12.5% diphenyl disulfide, and 4.5% diphenyl disulfide oxide. A large sample of fonofos oxon was repurified by preparative TLC using GF_{254} plates developed with ether–hexane (4:1). Chemical and radiochemical purity were established by autoradiography of the TLC plate and by gas–liquid chromatography as previously described. Pure phenyl ethyl(ethoxy)phosphinyl disulfide was purified by taking a portion of the reaction mixture and subjecting it to column chromatography using a Silicar CC7 silica gel column (1 × 10 cm) and ether–hexane (5:1) developing solvent.

In order to determine the fate of the sulfur atoms in the oxidation of fonofos to phenyl ethyl(ethoxy)phosphinyl disulfide, **8b**, obtained from the peroxy acid oxidation of [^{35}S]phenyl fonofos (**4a**), was spotted on a TLC plate and allowed to stand for 2 h prior to development with hexane–chloroform (3:2). Analysis of the plate by radioscanning and scintillation counting showed that 22.2% of the applied radioactivity was associated with diphenyl disulfide, the remaining 77.8% with unchanged **8**. In addition, another nonradioactive product was observed under ultraviolet light at the origin which was identified as *O*-ethyl ethylphosphonothioic acid (**1**) by cochromatography with a sample of independently synthesized **1** (Wustner et al., 1972).

Reduction with Lithium Borohydride. Cleavage of the sulfur–sulfur bond in phenyl (**8**) or *p*-bromophenyl ethyl(ethoxy)phosphinyl disulfide (**9**) to *O*-ethyl ethylphosphonothioic acid (**1**) and the corresponding arylthiol was carried out with lithium borohydride in ether at room temperature using slightly more than an equivalent amount of borohydride reagent (Brown and Subba Rao, 1956). After decomposition of excess borohydride with water and separation of the two phases, the ether layer was extracted with water and the aqueous phases were combined. Careful acidification to pH 3.8 with 6 N hydrochloric acid liberated the arylthiol which was extracted into ether. The remaining aqueous phase was chilled in ice, acidified to pH 1 with hydrochloric acid, and saturated with sodium chloride, and the acid **1** was extracted into benzene and dried. Microdistillation at 65 °C (0.8 mm) gave the product in 85% yield, identical with separately synthesized *O*-ethyl ethylphosphonothioic acid. The same procedure was used for the enantiomers of **8** and **9**.

Scheme I



Alkaline Hydrolysis. Racemic and the (−)-enantiomers of **3**, **4**, and **5** (**3a**, **4a**, and **5a**) were hydrolyzed in dioxane–water (1:1) containing 5 equiv of sodium hydroxide. The reaction mixture containing **4** or **5** (and enantiomers) was heated at reflux for 8 h while that containing **3** was refluxed for 3 h. The reaction mixture was concentrated to near dryness, water was added, and the aqueous mixture was washed with ether. The principal products, *O*-ethyl ethylphosphonothioic acid and arylthiol, were isolated from the aqueous solution in the same manner as that described for the lithium borohydride reduction.

RESULTS

Synthesis and Determination of Absolute Configuration. The various reactions used in the synthesis of compounds relevant to this study are indicated in Scheme I for one of the enantiomers. Physical constants and values of observed (neat) and specific rotations for all racemic and enantiomeric materials which were prepared are presented in Table I.

The absolute configuration of the starting material, i.e., enantiomeric *O*-ethyl ethylphosphonothioic acid (**1a** or **1b**), was established by x-ray diffraction analysis of the (−)-α-phenethylammonium salt of the acid (**2a**), obtained after repeated crystallizations. The acid (**1a**), regenerated from the batch of crystals of **2a** used for x-ray analysis, showed an improved value for observed rotation, α^{24}_D −16.32° (neat) (compare with values in Table I). The absolute configuration of the acid **1a** was established as $(S)_P$ by relation to the known configuration of the (*S*)-(−)-α-phenethylammonium ion (Leithe, 1931; Watson and Youngston, 1945; Cahn et al., 1956). A stereoscopic view of **2a** (both anion and cation) is shown in Figure 1 (top). The quaternary ammonium protons were found to be hydrogen bonded to three neighboring ions, one on the sulfur (N–H–S distance = 3.29 Å) and two on the oxygen

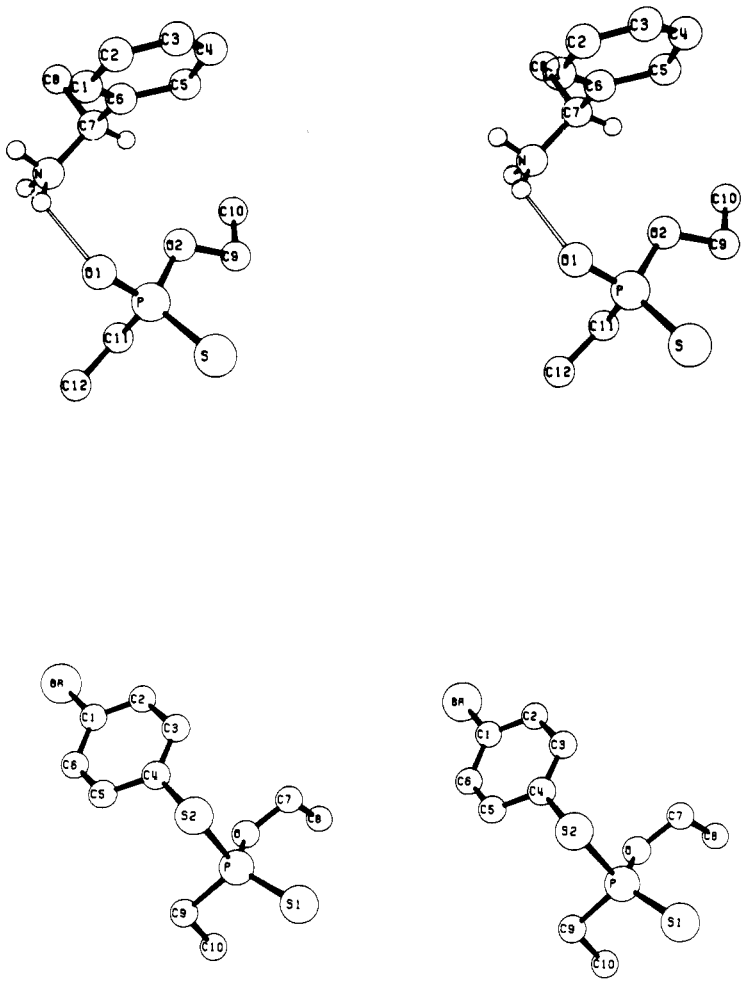


Figure 1. Stereoscopic view of $(S)_P$-(−)-α-phenylethylammonium *O*-ethyl ethylphosphonothioate (**2a**). The double line indicates hydrogen bond. Bottom: Stereoscopic view of $(S)_P$-(−)-*O*-ethyl *S*-*p*-bromophenyl ethylphosphonodithioate (**5a**) in the unit cell.

atoms (N–H–O distances = 2.78 and 2.77 Å) of two different molecules. One of the N–H---O bonds is shown in Figure 1. Since the phosphonothioic acid **1a** is $(S)_P$, its enantiomer **1b** is $(R)_P$.

Treatment of **1a** and **1b** with phosphorus pentachloride in carbon tetrachloride afforded (−)- and (+)-*O*-ethyl ethylphosphonochloridothioate, respectively. This reaction probably proceeded with a small amount of racemization since treatment of the chloridothioate **3a** with sodium hydroxide in aqueous dioxane resulted in **1a** of reduced optical rotation. Values for the observed rotation of several different preparations of **3a** from **1a** and the acid **1a** regenerated by alkaline hydrolysis of **3a** are presented in Table III. Thus, optical retention for the two-reaction cycle, **1a** to **3a** to **1a** (see Scheme I), ranged from 83 to 93.4%. Since general correspondence between optical rotation of **3a** and regenerated **1a** is observed, racemization probably occurred during the preparation of **3a**. Further, since the conversion of **3a** to **1a** is believed to take place with inversion (Mikolajczyk, 1967), the formation of **3a** from **1a** by treatment with phosphorus pentachloride also occurs with inversion.

Reaction between the enantiomeric *O*-ethyl ethylphosphonochloridothioate (**3a** and **3b**) and the sodium salt of benzenethiol or *p*-bromobenzenethiol resulted in the corresponding enantiomers of fonofos (**4a** and **4b**) and the *p*-bromo analogues (**5a** and **5b**). The absolute configuration of the (−)-rotating isomer **5a** [α^{24}_D −257.90° (neat, after melting)] was determined by x-ray analysis and established as $(S)_P$. A stereoscopic view of one of the two

Table III. Values for the Observed Optical Rotations ($\alpha_D{}^{24}$) in Degrees Arc of $(S)_P$-(−)-*O*-Ethyl Ethylphosphonothioic Acid (1a), (−)-Chloridothioate (3a), and 1a Regenerated by Alkaline Hydrolysis of 3a

α^{24}_D, original acid 1a	α^{24}_D, chloridothioate 3a	α^{24}_D, regenerated acid 1a	% optical retention
−15.34	−79.25	−12.62	83.5
−15.34	−79.25	−12.48	83.0
−15.34	−81.70	−13.26	85.0
−15.34	−81.70	−13.44	86.0
−15.34	−84.31	−14.26	92.3
−15.34	−84.31	−14.38	93.4

molecules in the unit cell is included in Figure 1 (bottom). The other molecule is not included since the two molecules have identical configuration and chirality. It should be pointed out that the two molecules in the unit cell are related to each other by a pseudo center of symmetry. Fortunately, the nature of the molecular arrangement in the crystal was such that the heavy atoms in the two molecules in the unit cell deviated slightly from being centrosymmetrical, and this allowed the determination of the absolute configuration of **5a**. Since **5a** is $(S)_P$, **5b** is $(R)_P$.

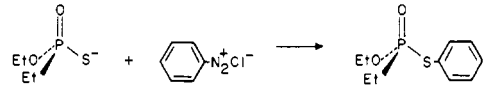
The configurations of the fonofos enantiomers (**4a** and **4b**) were assigned on the basis of their chemical and structural similarity to the *p*-bromo analogues (**5a** and **5b**). For example, both **4a** and **5a** were prepared from (−)-*O*-ethyl ethylphosphonochloridothioate, which in turn was synthesized from $(S)_P$-(−)-*O*-ethyl ethylphosphonothioic acid (**1a**). Further, alkaline hydrolysis of **4a** and **5a** both

returned $(R)_P$-(+)-*O*-ethyl ethylphosphonothioic acid (**1b**), the expected isomer from three inversion reactions. Rotations (neat, degrees arc) for the various products in the three-reaction cycle involving the preparation and alkaline hydrolysis of fonofos (**4a**) and the *p*-bromo analogue (**5a**) are given as follows:

	1a	3a	4a	1b
α_D^{24}	−14.50°	−77.71°	−149.20°	+11.43°
	1a	3a	5a	1b
α_D^{24}	−14.94°	−82.50°	−257.99°	+14.65°

Based on the rotations of the starting acid **1a** and the recovered acid **1b**, the three-reaction cycle involving fonofos proceeded with about 21% racemization. The reaction cycle was significantly cleaner with the *p*-bromo analogue where only 2% racemization was observed. The formation of **1b** from either **4a** or **5a** upon reaction with sodium hydroxide demonstrates unequivocally that nucleophilic displacement (S_N2P) by hydroxide ion at the phosphorus atom takes place with inversion.

The enantiomeric oxons of fonofos (**6a** and **6b**) and the corresponding *p*-bromo analogue (**7a**) were prepared by the reaction between the sodium salt of **1a** or **1b** and the respective aryl diazonium chloride. This reaction undoubtedly proceeds by reaction between the aryl carbonium ion and the anion of **1a** or **1b** and bonds connected to the phosphorus atom are not involved. Therefore, the configurations of the oxon should be identical with that of the acid. Since the configuration of the acid **1a** by x-ray



1a $(S)_P$ 6a $(S)_P$

analysis was determined to be $(S)_P$, **6a** and **7a** are $(S)_P$, and **7b** is $(R)_P$. The observed rotation, α^{24}_D, of **1a** used in the synthesis of the oxons **6a** and **7a** was −14.45°. Assuming that the observed rotation of optically pure **1a** is −16.32° (observed rotation of the purified sample of **1a** used for x-ray analysis) and the arylation reaction proceeds with complete optical retention, the optical purity of **6a** and **7a** (see Table I) was estimated to be about 94%.

The configurations of (−)-phenyl ethyl(ethoxy)phosphinyl disulfide (**8a**) and the corresponding *p*-bromo analogue (**9a**) were assigned by two independent methods. The first was based on their synthesis from the appropriate arylsulfenyl chloride and the acid **1a** (see Scheme I). As in the case of the synthesis of the oxons, the chiral phosphorus atom is not involved in this reaction and **8a** and **9a** should have the same configuration as **1a**. Second, lithium borohydride reduction of **8a** or **9a**, resulting in cleavage of the S–S bond, returned **1a** with greater than 99% optical retention in both cases. Observed rotations (neat, degrees arc) of products involved in this reaction cycle are given as follows:

	1a	8a	1a
α_D^{24}	−14.50°	−32.30°	−14.40°
	1a	9a	1a
α_D^{24}	−14.50°	−65.50°	−14.45°

These reactions show that the configurations of **8a** and **9a** are identical with **1a** and, therefore, they are $(S)_P$. Based on an α^{24}_D value of −16.32° for optically pure **1a**, the optical purity of **8a** and **9a** was approximately 94%.

Oxidation with *m*-Chloroperoxybenzoic Acid. Oxidation of the enantiomers of fonofos (**4a** and **4b**) with *m*-chloroperoxybenzoic acid in dichloromethane gave fonofos oxon and phenyl ethyl(ethoxy)phosphinyl disulfide as the principal products. Other materials isolated were unreacted fonofos, diphenyl disulfide, and diphenyl disulfide oxide. Data for the specific rotations (cyclohexane) of the oxon and phosphinyl disulfide isolated from oxidation of **4a** and **4b** are presented in Table IV. From the sign and magnitude of the specific rotations of the oxon and disulfide isolated, it is apparent that $(S)_P$-fonofos (**4a**) was converted predominantly to $(R)_P$-fonofos oxon (**6b**) and $(R)_P$-phenyl ethyl(ethoxy)phosphinyl disulfide (**8b**). Conversely, $(R)_P$-fonofos (**4b**) was converted primarily to **6a** and **8a**.

Table IV. Specific Rotations in Cyclohexane (Degrees Arc) of the Chiral Products Obtained from Peracid Oxidation of Fonofos Enantiomers

	$[\alpha]^{24}_D$, peracid products	
Fonofos enantiomer[a]	Oxon	Disulfide
4a, S_P	+66.17 (*c* 0.141)	+55.40 (*c* 2.22)
4a, $(S)_P$	+66.32 (*c* 0.117)	+54.32 (*c* 1.81)
4b, $(R)_P$	−57.87 (*c* 1.30)	−51.86 (*c* 1.84)

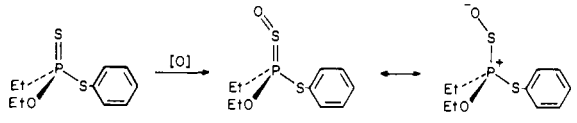
[a] $[\alpha]^{24}_D$ of synthesized 4a and 4b were +138.20 (*c* 0.302) and −114.56 (*c* 0.309), respectively.

An approximation of the stereospecificity of the peroxy acid oxidation of fonofos may be made from the specific rotations of the products and an estimate of the optical purity of the enantiomeric fonofos, synthesized oxon, and phosphinyl disulfide. For the study of the oxidation of $(S)_P$-fonofos (**4a**) a preparation with a neat observed rotation of −181.50° ($[\alpha]^{24}_D$ +138.20° in cyclohexane) was used. The optical purity of this sample was substantially greater than that used in the alkaline hydrolysis study, α^{24}_D (neat) −149.20°, which previously was found to give *O*-ethyl ethylphosphonothioic acid (**1b**) with an α^{24}_D of −11.43° upon treatment with sodium hydroxide. Assuming that the hydrolysis reaction proceeded with complete inversion and the α^{24}_D of optically pure **1b** was +16.32°, the optical purity of the **4a** used in the oxidation reaction was estimated to be 93%. The specific rotations, $[\alpha]^{24}_D$, for synthesized $(S)_P$-(**6a**) and $(R)_P$-oxon **6b** in cyclohexane solvent were −121.70° (*c* 0.636) and +112.64° (*c* 1.02), respectively, and that of $(S)_P$-disulfide (**8a**) was −68.27° (*c* 4.70). Since calculations for **6a** and **8a** gave estimated values for their optical purity of about 94%, the purity of **4a** ($[\alpha]^{24}_D$ −138.20°), **6a** ($[\alpha]^{24}_D$ −121.70°), and **8a** ($[\alpha]^{24}_D$ −68.27°) was assumed to be the same, i.e., about 93–94%. Based on these approximations, it is possible to estimate the degree of stereospecificity of the oxidation reaction by directly comparing the specific rotations of the oxon and phosphinyl disulfide obtained from the oxidation of **4a** with those of the synthesized materials. Thus, the oxidation of **4a** to **6b** was estimated to occur with about 77% retention of configuration and the oxidation of **4a** to **8b** with about 90% inversion.

DISCUSSION

The principal objective of this study was to determine the stereochemical course of the reaction between fonofos and *m*-chloroperoxybenzoic acid. The major products from this reaction were the corresponding fonofos oxon and phenyl ethyl(ethoxy)phosphinyl disulfide, an unusual oxidative rearrangement product. It was necessary, therefore, to establish the absolute configuration of these products and relate them to the absolute configuration of the starting fonofos isomer.

The conversion of $(S)_P$-fonofos (**4a**) to $(R)_P$-fonofos oxon (**6b**) by the action of *m*-chloroperoxybenzoic acid occurred predominantly with retention of configuration of the phosphorus atom. In contrast, oxidation to the rearrangement product, $(R)_P$-phenyl ethyl(ethoxy)phosphinyl disulfide (**8b**) took place with predominant inversion of configuration. In the case of the reaction leading to the oxon, the stereochemical course is identical with that observed previously for the oxidative desulfuration of resolved *l*-menthyl methylphenylphosphinothioate (Herriott, 1971). While it is possible that fonofos oxon and phenyl ethyl(ethoxy)phosphinyl disulfide are formed by two separate reactions between fonofos and *m*-chloroperoxybenzoic acid, a plausible mechanism implicating a single intermediate may be suggested which accounts for the two major products. The first step in the mechanism involves formation of an *S*-oxide intermediate. Closure



4a

of the *S*-oxide to the three-membered ring with subsequent loss of sulfur gives the oxon with retention of configuration.

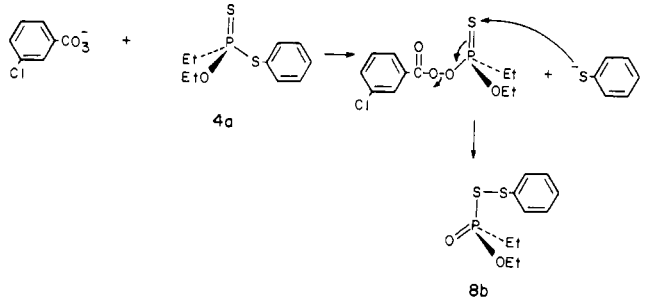
6b

This mechanism was suggested by Herriott (1971) to explain retention observed in the oxidation of a phosphinothioate ester. The same three-membered ring intermediate also may lead to inverted phenyl ethyl(ethoxy)phosphinyl disulfide as follows:

8b

Thus, the stereochemistry of both oxidation products may readily be accounted for by the three-membered ring intermediate. This intermediate is possible by considering hybridization of an sp^3 orbital with a d_{xy} orbital, giving two spd bonds at 71° and three sp^3 bonds (Gillespie, 1952). Hybridization of this type is compatible with the angular strain associated with a three-membered ring.

Although a three-membered ring intermediate may account for the stereochemistry of both products, it is possible that the disulfide (**8b**) is formed by a separate mechanism. Since inversion is observed, **8b** possibly may be formed by a recombination reaction after displacement of benzenethiolate ion by the peroxy acid. An inter-



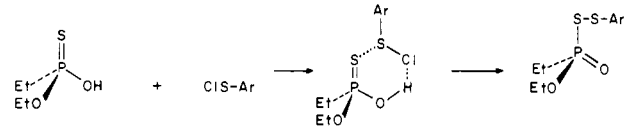
4a

8b

mediate similar to the type proposed for this mechanism also has been suggested to account for the inverted phosphinate observed in the *m*-chloroperoxybenzoic acid oxidation of a chiral phosphinothioate ester in the presence of a strong acid (Herriott, 1971).

In the two possible mechanisms suggested for the formation of phenyl ethyl(ethoxy)phosphinyl disulfide (**8**), migration of the *S*-phenyl moiety to the thiono sulfur atom is proposed. Although highly unlikely, it is possible also that the oxidative rearrangement reaction may occur by insertion of the thiono sulfur atom into the *S*-phenyl bond. This possibility, however, was ruled out by TLC examination of the decomposition products of phenyl ethyl(ethoxy)phosphinyl disulfide. Previous results (Wustner et al., 1972) showed that the disulfide (**8**) was unstable on TLC plates and decomposed slowly to form diphenyl disulfide and ethyl ethylphosphonothioic acid. When **8b**, obtained from the peroxy acid oxidation of [^{35}S]phenyl fonofos (**4a**), was spotted and allowed to stand on a TLC plate, only undecomposed **8b** and diphenyl disulfide were observed to possess radioactivity, indicating that the *S*-phenyl bond was not affected in the oxidative rearrangement reaction.

In addition to the oxidation reaction, the stereochemistry of the reaction between *O*-ethyl ethylphosphonothioic acid and arylsulfenyl chloride to give aryl ethyl(ethoxy)phosphinyl disulfide was clearly established by the results obtained from the two-reaction cycle **1a** to **8a** to **1a** or **1a** to **9a** to **1a**. This reaction cycle took place with greater than 99% optical retention and the reaction between **1a** and arylsulfenyl chloride may be conceived in terms of a nucleophilic displacement of the P=S sulfur atom on the sulfur atom of the arylsulfenyl halide, possibly through a cyclic intermediate as shown below. The results of this



two-reaction cycle also confirmed that S–S bond cleavage by lithium borohydride occurs with virtually no racemization of the phosphorus atom.

High stereospecificity was observed in the reaction between fonofos or the *p*-bromo analogue and hydroxide ion, resulting in the ethylphosphonothioic acid of inverted configuration relative to fonofos. This is in contrast to results by Reiff et al. (1971) who reported racemization for the alkaline hydrolysis of (−)-*O*-isopropyl *S*-phenyl methylphosphonodithioate. Establishment of the absolute configuration of the enantiomers of fonofos and ethyl ethylphosphonothioic acid proved unequivocally that the reaction between fonofos and hydroxide ion occurs with inversion. This reaction undoubtedly proceeds through a trigonal-bipyramidal intermediate as has been suggested for displacement reactions involving other organophosphorus esters (Hudson, 1965). The results of the two-reaction cycle, **1a** to **3a** to **1a**, also confirm an earlier report that the conversion of *O*-alkyl alkylphosphonothioic acid to the chloridothioate by the action of phosphorus pentachloride also takes place with inversion (Michalski and Mikolajczyk, 1966).

Supplementary Material Available: A listing of final atomic parameters and the observed and calculated structure amplitudes, interionic distances, and bond lengths and angles for the (−)-α-phenethylammonium salt of $(S)_P$-*O*-ethyl ethylphosphonothioic acid (**2a**) and $(S)_P$-*O*-ethyl *S*-*p*-bromophenyl ethylphosphonothioate (**5a**) (53 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

Aaron, H. S., Shryne, T. M., Miller, J. I., *J. Am. Chem. Soc.* **80**, 107 (1958).

Bellet, E. M., Casida, J. E., *J. Agric. Food Chem.* **22**, 207 (1974).
Brown, H. C., Subba Rao, B. C., *J. Am. Chem. Soc.* **78**, 2582 (1956).
Cahn, R. S., Ingold, C. K., Prelog, V., *Experientia* **12**, 81 (1956).
Fahmy, M. A. H., Fukuto, T. R., *Tetrahedron Lett.* **41**, 4245 (1972).
Fenton, H. S. H., *J. Chem. Soc.* **65**, 899 (1894).
Fukuto, T. R., Metcalf, R. L., *Ann. N.Y. Acad. Sci.* **160**, 97 (1969).
Gillespie, R. J., *J. Chem. Soc.*, 1002 (1952).
Hamilton, W. H., *Acta Crystallogr.* **18**, 502 (1965).
Herriott, A. W., *J. Am. Chem. Soc.* **93**, 3304 (1971).
Hoffman, F. W., Kagan, B., Canfield, J. H., *J. Am. Chem. Soc.* **81**, 148 (1959).
Hudson, R. F., "Structure and Mechanism in Organo-Phosphorus Chemistry", Academic Press, London, 1965, p 342.
Ibers, J. A., in "International Tables for X-ray Crystallography", Vol. III, Table 3.3.1a, Kynoch Press, Birmingham, England, 1962, pp 202–209.
Kalbfeld, J., Gutman, A. D., Hermann, D. A., *J. Labelled Compd.* **4**, 367 (1968).
Leithe, W., *Chem. Ber.* **64**, 2827 (1931).
McBain, J. B., Yamamoto, I., Casida, J. E., *Life Sci.* **10**, 947 (1971a).
McBain, J. B., Yamamoto, I., Casida, J. E., *Life Sci.* **10**, 1311 (1971b).
Menn, J. J., Szabo, K., *J. Econ. Entomol.* **58**, 734 (1965).
Michalski, J., Mikolajczyk, M., *Chem. Ind. (London)*, 661 (1964).
Michalski, J., Mikolajczyk, M., *Tetrahedron* **22**, 3055 (1966).
Mikolajczyk, M., *Tetrahedron* **23**, 1543 (1967).
Ptashne, K. A., Neal, R. H., *Biochemistry* **11**, 3224 (1972).
Ptashne, K. A., Wolcott, R. M., Neal, R. H., *J. Pharmacol. Exp. Ther.* **179**, 380 (1971).
Reiff, L. P., Szafraniec, L. J., Aaron, H. S., *Chem. Commun.*, 366 (1971).
Schrader, G., Lorenz, W., German Patent 1 103 324 (Oct 20, 1959); *Chem. Abstr.* **56**, 5888c (1962).
Templeton, D. H., in "International Tables for X-ray Crystallography", Vol. III, Table 3.3.2c, Kynoch Press, Birmingham, England, 1962, pp 215–216.
Theilacker, W., Winkler, H. G., *Chem. Ber.* **87**, 690 (1954).
Udenfriend, S., Clarke, C. T., Axelrod, J., Brodie, B. B., *J. Biol. Chem.* **208**, 731 (1954).
Ullrich, V., *Angew. Chem., Int. Ed. Engl.* **11**, 702 (1972).
Watson, M. B., Youngston, G. W., *J. Chem. Soc.*, 2145 (1945).
Wilkinson, C. F., Brattsten, L. B., *Drug Metab. Rev.* **1**, 153 (1972).
Wustner, D. A., Desmarchelier, J., Fukuto, T. R., *Life Sci* **11**, 583 (1972).
Wustner, D. A., Fahmy, M. A. H., Fukuto, T. R., *Residue Rev.* **53**, 53 (1974).

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Radiosynthesis and Metabolism of the Insect Antijuvenile Hormone, Precocene II

Tomihisa Ohta, Ronald J. Kuhr,* and William S. Bowers

The antijuvenile hormone precocene II (6,7-dimethoxy-2,2-dimethylchromene) was radiosynthesized with carbon-14 in the 2-*gem*-dimethyl position. Injection of the labeled compound into nine insect species revealed a variation of at least 37-fold in metabolic rate. In vitro studies using cabbage looper and European corn borer gut and fat body tissue homogenates indicated that the metabolic pathway was primarily dependent on catalysis by a mixed-function oxidase system. The principal metabolite in each insect was 6,7-dimethoxy-2,2-dimethylchroman-3,4-diol which was readily conjugated in vivo. Other identified metabolites include 6,7-dimethoxy-2,2-dimethylchroman-3-ol and 3,4-epoxy-6,7-dimethoxy-2,2-dimethylchroman.

A recent bioassay of a lipid extract of *Ageratum houstonianum* revealed that the plant contains chemicals possessing insect antijuvenile hormone activity (Bowers et al., 1976). Isolation and characterization studies showed the active ingredients to be 7-methoxy-2,2-dimethylchromene (precocene I) and 6,7-dimethoxy-2,2-dimethylchromene (precocene II). These compounds are able to induce precocious metamorphosis, cause sterilization, and/or force diapause in certain insects, especially Heteropteran species. Thus, it is possible that such natural products or synthetic analogues could form the basis for development of a fourth generation of insecticide chemicals.

To facilitate structure optimization and mode-of-action studies, we have radiolabeled precocene II with carbon-14 in the 2-*gem*-dimethyl position and have initiated metabolism studies on several insect species. Below are reported synthetic procedures and potential metabolic pathways.

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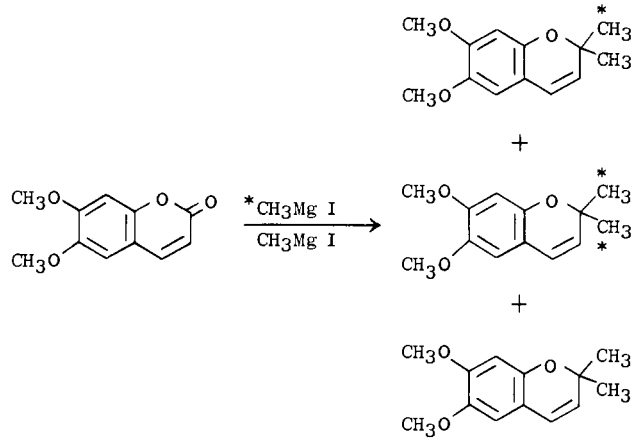
Scheme I



MATERIALS AND METHODS

Radiosynthesis of Precocene II. Unlabeled 6,7-dimethoxycoumarin was prepared from 3,4-dimethoxyphenol and 3-ethoxyacrylyl chloride according to the procedure of Crosby and Berthold (1962). The coumarin was