Resolution and Stereoselective Action of Sulprofos and Related *S*-Propyl Phosphorothiolates

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The enantiomers of sulprofos [*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate] and its phosphorothiolate analogue were prepared by acid-catalyzed ethanolysis of the diastereoisomers of *N*-[(2*S*)-2-(carboxyethyl)pyrrolidinyl] *S*-propyl phosphoramidodithioate and the corresponding phosphoramidothiolate, respectively. Peracid oxidation converted the phosphorodithioate and phosphorothiolate to the sulfone derivative of sulprofos oxon. Analogous procedures were used to resolve profenofos [*O*-(4-bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothiolate] and its *O*-methyl and *O*-pentachlorophenyl analogues. The (+)-isomers are most toxic to house flies in the sulprofos series and the (−)-isomers in the profenofos series. The chiral specificity is reversed in each case relative to potency as inhibitors of house fly head acetylcholinesterase (AChE) activity. Most of these phosphorothiolates are activated to more potent inhibitors of electric eel AChE on coincubation with a mouse liver microsomal oxidase system. (+)-Profenofos and (−)-sulprofos oxon sulfone are the only exceptions, undergoing stereoselective detoxification when unwashed microsomes are used.

O-Ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate (sulprofos or Bolstar) and *O*-(4-bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothiolate (profenofos or Curacron) are used as foliar insecticides to control lepidopterous larvae and other pests of cotton and vegetables (Worthing, 1979). They are active on pest strains resistant to other organophosphorus insecticides and their mammalian toxicity is relatively low. Sulprofos undergoes metabolic activation by thiono and thioether sulfur oxidation (Bull and Ivie, 1976; Bull et al., 1976; Ivie et al., 1976), and profenofos is also bioactivated, probably by phosphorothiolate oxidation (Wing et al., 1983, 1984). Oxidation with *m*-chloroperoxybenzoic acid (MCPBA) serves as a biomimetic model for some but not all of these metabolic oxidations (Bellet and Casida, 1974: Segall and Casida, 1982), e.g., it yields the oxon sulfone of sulprofos but the phosphorothiolate *S*-oxide of profenofos is only a transitory intermediate (Segall and Casida, 1982). Microsomal oxidase activation is stereoselective for the enantiomers of profenofos; i.e., the more toxic (−)-isomer is activated 34-fold and the less toxic (+)-isomer is deactivated 2-fold as inhibitors of electric eel acetylcholinesterase (AChE) (Wing et al., 1983).

Studies with the enantiomers of profenfos have helped define the importance of stereochemical features in its metabolism and action (Casida, 1984). Extension of these findings to other *S*-propyl phosphorothiolates and phosphorodithioates requires their resolution. This paper describes the resolution, biological activity, and oxidative activation or detoxification of the enantiomers of sulprofos and its oxidized derivatives (sulprofos oxon and sulprofos oxon sulfone) and of profenofos and its *O*-methyl and *O*-pentachlorophenyl analogues.

MATERIALS AND METHODS

Spectroscopy. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker WM-300 wide-bore spectrometer at 300.13 MHz (^{1}H) or 121.50 MHz (^{31}P, with broad band decoupling) for samples dissolved in $CDCl_3$ or acetone-d_6, with chemical shifts related to internal tetramethylsilane (^{1}H) or external 1% trimethyl phosphate in $CDCl_3$ or acetone-d_6 (^{31}P), reporting the ^{31}P NMR chemical shifts (ppm) as positive when downfield of trimethyl phosphate. Mass spectrometry (MS) utilized a Hewlett-Packard 5985 system with ionization by electron impact (EI, 70 eV) or chemical ionization (CI, 230 eV) with methane. Masses are given for molecular ($M^{+\cdot}$) or quasi-molecular [$(M + 1)^+$] ions. Absorbance in the visible region was recorded on a Perkin-Elmer 576 ST spectrophotometer. Optical rotations were measured at 25 °C with a cell path length of 100 mm (capacity 1 mL) by using a Perkin-Elmer 241 polarimeter.

Chromatography. Thin-layer chromatography (TLC) for analysis involved silica gel 60 F_{254} chromatoplates with 0.25-mm gel thickness and product visualization with UV light or I_2 vapor, often after a preliminary spray with $PdCl_2$ solution (0.5% in 1 N HCl). High-pressure liquid chromatography (HPLC) for preparative isolations utilized a μPorasil column (30 × 0.78 cm) or Hibar column (LiChrosorb Si 60, 7 μM, 25 × 1 cm) with a Waters Associates liquid chromatograph and absorbancy detector at 254 nm.

Chemicals. Structures and names or designations for the phosphorodithioates (**1–3**), phosphorothiolates (**4–10**), phosphoramidodithioate (**11**) and phosphoramidothiolates (**12–14**) examined are given in Figure 1. The suffixes **a** and **b** for the amidate designations denote their sequential order of elution on HPLC whereas with the insecticides (e.g., **1a** or **1b**) they designate the enantiomer derived from acid alcoholysis of the corresponding amidate (e.g., **11a** or **11b**, respectively).

Sulprofos (**1**) and its oxidized derivatives (**2–6**) were provided by Mobay Chemical Co. (Kansas City, MO) and profenofos (**7**) by CIBA-Geigy Corp. (Greensboro, NC). Preparation of methyl profenofos (**8**), the methyl and ethyl esters of the pentachloro derivative (**9** and **10**), and the four amidates (**11–14**) is described below. NMR and CI-MS data for these compounds are given in Table I.

Synthetic procedures for L-proline ethyl ester (used without distillation) and *S*-propyl phosphorodichloridothioate were referred to earlier (Leader and Casida, 1982). The latter compound was treated with excess phosphorus pentasulfide in xylene at reflux for 3 h followed by filtration and fractional distillation to obtain *S*-propyl phosphorodichloridodithioate (bp 78–80 °C/1.0 mmHg; δ^{31}P + 69 ppm, $CDCl_3$). The chiral shift reagent tris[3-

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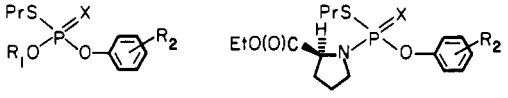
Table I. NMR and CI-MS Data for Sulprofos, Profenofos, Related Insecticides, and Amidate Intermediates for Their Resolution

$CH_3CH_2CH_2SP(X)(OAr\text{-}R)Y^{a}CH_2CH_3$

compound			a	b	c	d	e			CI-MS, $(M+1)^+$ (rel intensity)
			NMR,[b] δ [$CDCl_3$ (acetone-d_6)]							
no.	X	R	^{31}P	H_a	H_b	H_c	H_d	H_e	H_{Ar}	H_R	
1	S	4-SMe	91.7 (90.7)	0.95	1.65	2.85	4.20	1.35	7.08–7.22	2.40	323 (100)
2	S	4-S(O)Me	91.3 (90.5)	0.99	1.69	2.93	4.27	1.41	7.40, 7.64	2.72	339 (100)
3	S	4-$S(O_2)$Me	91.1 (90.2)	0.99	1.70	2.94	4.28	1.42	7.43, 7.93	3.05	355 (100)
4	O	4-SMe	22.9 (21.7)	0.97	1.67	2.84	4.28	1.40	7.17–7.26	2.46	307 (100)
5	O	4-S(O)Me	23.0 (21.8)	0.96	1.68	2.85	4.29	1.40	7.40, 7.62	2.70	323 (100)
6	O	4-$S(O_2)$Me	23.1 (21.8)	0.99	1.72	2.92	4.32	1.46	7.48, 7.98	3.08	339 (100)
7	O	2-Cl,4-Br	23.4	0.96[c]	1.68[c]	2.89[c]	4.31[c]	1.41[c]	7.32–7.56[c]		373 (76)
8	O	2-CI,4-Br	25.2	0.95	1.70	2.90	3.92 (OCH_3)		7.30–7.60		358 (7)[d]
9	O	Cl_5	24.2	1.02	1.75	3.05	4.40	1.45			431 (65)
10	O	Cl_5	26.0	1.00	1.78	3.05	3.98 (OCH_3)				417
11a	S	4-SMe	85.9	1.00	1.72	2.92	4.17	1.27	7.15–7.25	2.48	419 (12)[d]
11b	S	4-SMe	86.6	0.98	1.65	2.88	4.18	1.28	7.15–7.25	2.46	419[d]
12a	O	4-SMe	27.1	0.95	1.65	2.88	4.14	1.24	7.11–7.20	2.43	403 (78)[d]
12b	O	4-SMe	26.9	0.93	1.62	2.79	4.16	1.25	7.15–7.22	2.45	403[d]
13a	O	2-Cl,4-Br	27.9	0.95[c]	1.67[c]	2.91[c]	4.14[c]	1.24[c]	7.30–7.52[c]		470 (72)
13b	O	2-Cl,4-Br	27.7	0.94[c]	1.63[c]	2.83[c]	4.15[c]	1.24[c]	7.31–7.54[c]		470
14a	O	Cl_5	30.3	1.02	1.78	2.90 3.07	4.13	1.22			529
14b	O	Cl_5		0.95	1.65	2.85	4.15	1.25			529

[a] Y = O for 1–10 and *N*-[(2*S*)-2-carboxypyrrolidinyl] for **11a–14b**. [b] Proline for **11a–11b**, respectively: NCH 4.55, 4.48, 4.35, 4.38, 4.43,[c] 4.44,[c] 4.48, and 4.52; NCH_2 3.45–3.70, 3.40–3.65, 3.35–3.50, 3.38–3.55, 3.47–3.52, 3.46–3.52, 3.45–3.65, and 3.50–3.70; $NCHCH_2CH_2CH_2$ 1.85–2.30[c]. [c] Reported for **7**, **13a**, and **13b**, respectively: H_a 0.97, 0.97 and 0.95; H_b 1.70, 1.69, and 1.67; H_c 2.90, 2.93, and 2.85; H_d 4.33, 4.16, and 4.17; H_e 1.43, 1.21, and 1.24; H_{Ar} 7.3–7.5; NCH 4.45 and 4.46; CH_2 1.6–2.2 (Leader and Casida, 1982). [d] EI-MS, M^+.



no.	name or designation	R_1	R_2	X
1	sulprofos	Et	4-SMe	S
2	sulprofos sulfoxide	Et	4-S(O)Me	S
3	sulprofos sulfone	Et	4-$S(O_2)$Me	S
4	sulprofos oxon	Et	4-SMe	O
5	sulprofos oxon sulfoxide	Et	4-S(O)Me	O
6	sulprofos oxon sulfone	Et	4-$S(O_2)$Me	O
7	profenofos	Et	2-Cl,4-Br	O
8	methyl profenofos	Me	2-Cl,4-Br	O
9	pentachloro profenofos	Et	Cl_5	O
10	methyl pentachloro profenofos	Me	Cl_5	O
11	sulprofos amidate	Et	4-SMe	S
12	sulprofos oxon amidate	Et	4-SMe	O
13	profenofos amidate	Et	2-Cl,4-Br	O
14	pentachloro profenofos amidate	Et	Cl_5	O

Figure 1. Structures and names or designations for sulprofos, profenofos, related insecticides, and amidate intermediates for their resolution.

(heptafluoro-*n*-propylhydroxymethylene)-(+)-camphorato]europium (III) [Eu(hfc)$_3$] was from Aldrich Chemical Co. (Milwaukee, WI). MCPBA (commercial, 85% assay) (Aldrich) was purified to >99% by the procedure of Fieser and Fieser (1967). All other materials were obtained from commercial sources.

Synthesis of Diastereoisomers of Intermediate Phosphoramidodithioates (11a and 11b) and Phosphoramidothiolates (12a–14a and 12b–14b) (Figure 2). To prepare phosphorodithioate 11, 4-(methylthio)phenol (1.4 g, 0.01 mol) was added to a solution of sodium methoxide [prepared from sodium (0.23 g, 0.01 mol) in methanol (20 mL)] and the mixture was evaporated to dryness. The residue was dissolved in toluene–acetonitrile (4:1) (60 mL) and added to *S*-propyl phosphorodichloridodithioate (2.09 g, 0.01 mol) in dry benzene (100 mL) over 30 min. The mixture was warmed to reflux for 10 min, and then a solution of L-proline ethyl ester (1.4 g, 0.01 mol) and triethylamine (1.2 g) in dry benzene (70 mL) was added dropwise over 15 min. After refluxing for 2 h, the solvent was evaporated. The residue was dissolved in chloroform, then washed with saturated NaCl aqueous solution, and dried over anhydrous $MgSO_4$. The solvent was evaporated to leave a pale yellow viscous oil (3.3 g). After column chromatography (silica; hexane–ethyl acetate, 19:1), the mixture of **11a + 11b** was obtained (2.5 g) as a colorless viscous oil: ^{31}P NMR revealed two sharp singlets [δ 85.9 and 86.6 ($CDCl_3$) for **11a** and **11b**, respectively] in a 1:1 ratio. Preparative HPLC (μPorasil or LiChrosorb; hexane–ethyl acetate, 39:1) gave diastereoisomers **11a** and **11b** eluting in that sequence with base-line separation [for analogous separation, see Leader and Casida (1982)].

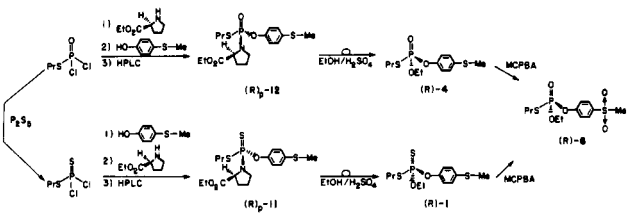


Figure 2. Resolution of enantiomers of sulprofos, sulprofos oxon, and sulprofos oxon sulfone. The resolution sequence is shown for only one of the enantiomers.

To obtain phosphorothiolate **12**, a solution of L-proline ethyl ester (1.4 g, 0.01 mol) and triethylamine (2.5 g) in dry benzene (50 mL) was added dropwise with stirring to a cooled (10 °C) solution of *S*-propyl phosphorodichloridothiolate [1.93 g, 0.01 mol; bp 66–68 °C/1.5 mmHg; δ ^{31}P + 33 ($CDCl_3$)] in dry benzene (50 mL). Stirring was continued for 4 h and then a solution of 4-(methylthio)-phenol (1.4 g, 0.01 mol) in dry benzene (25 mL) was added dropwise. The mixture was refluxed for 2 h, cooled to 25 °C, filtered, washed with saturated NaCl (2 × 30 mL), and dried over anhydrous $MgSO_4$, and the solvent was evaporated to leave a pale yellow viscous oil (2.5 g). After column chromatography (silica; benzene–ethyl acetate, 9:1), **12** was obtained (2.0 g) as a colorless viscous oil. Preparative HPLC (LiChrosorb; hexane–ethyl acetate, 3:1) gave diastereoisomers **12a** and **12b** in sequence with base-line separation.

Phosphoramidothiolates **13a** and **13b** were prepared as previously described (Leader and Casida, 1982). Pentachlorophenyl compound **14** was made in an analogous manner, with easy separation of the diastereoisomers (**14a**,

mp 142–144 °C; **14b**, oil) by column chromatography (24 × 300 mm) on silica with a gradient of hexane to hexane–ethyl acetate (4:1).

Synthesis of Enantiomers of Sulprofos (1a and 1b) and Its Oxon (4a and 4b) and Oxon Sulfone Derivatives (6a and 6b) (Figure 2). Phosphorodithioates **1a** and **1b** were obtained by acid ethanolysis of **11a** and **11b** (100 mg) with 1 M H_2SO_4 in absolute ethanol (5 mL) by stirring for 40 h at 75 °C with protection from moisture. After rapid removal of most of the ethanol at 25 °C under reduced pressure, the residue was dissolved in chloroform and the organic phase was washed with cold 0.1 M NaOH and saturated NaCl solution. **1a** or **1b** (20 mg, 26%) and unreacted **11a** or **11b** (50 mg, 50%) were separated by preparative HPLC (μPorasil; hexane–ethyl acetate, 40:1: **1a** or **1b** eluted completely before **11a** or **11b**).

Phosphorothiolates **4a** and **4b** were obtained as above but from **12a** and **12b** (100 mg) on stirring for 12 h at 60 °C. The yields were ~20% with ~60% recovery of starting material using HPLC on μPorasil with hexane–ethyl acetate, 3:1.

Phosphorothiolates **6a** and **6b** were prepared by two procedures. In the first case, a solution of MCPBA (54 mg, 0.5 mmol) in acetone (5 mL) was added slowly to a solution of **1a** or **1b** (32 mg, 0.1 mmol) in acetone (5 mL). After being stirred for 1 h at 25 °C, activated KF (1 h, 100 °C/0.1 torr; 0.7 mmol) was added, and the mixture in acetone was stirred for 1 h at 25 °C to complete precipitation of MCPBA and *m*-chlorobenzoic acid (Camps et al., 1981). HPLC (μPorasil; hexane–ethyl acetate, 4:1) of the filtrate gave **6a** or **6b** in ~10% yield. By the second procedure, MCPBA (26 mg, 0.15 mmol) in acetone (5 mL) was added to **4a** or **4b** (15 mg, 0.05 mmol) in acetone (5 mL) to obtain **6a** or **6b** in ~60% yield.

Synthesis of Methyl Profenofos (8), Pentachloro Profenofos (9) and Its Methyl Analogue (10), and Enantiomers of Profenfos (7a, 7b) and Related Compounds (8a, 8b, 9a, and 9b). To prepare **8–10**, a solution of 4-bromo-2-chlorophenol (2.1 g, 0.01 mol) [or pentachlorophenol (2.67 g, 0.01 mol)] and triethylamine (1.0 g) in dry benzene (50 mL) was added dropwise with stirring to a cooled (10 °C) solution of *S*-propyl phosphorodichloridothiolate (1.93 g, 0.01 mol) in dry benzene (50 mL). Stirring was continued for 4 h then a solution of ethanol (4.6 g, 0.01 mol) [or methanol (3.2 g, 0.01 mol)] in dry benzene (25 mL) was added dropwise. The mixture was stirred at 25 °C for 18 h, and then filtered. The filtrate was washed with 5% NaOH (2 × 50 mL) (for **8**) or 5% Na_2CO_3 (2 × 100 mL) (for **9** and **10**) and dried ($MgSO_4$). Evaporation left a pale yellow viscous oil that was purified by HPLC (μPorasil; hexane–ethyl acetate, 5:1) to give **8** (0.72 g, 20%), **9** (0.42 g, 10%; mp 66–68 °C), or **10** (0.63 g, 15%; mp 45–47 °C).

Phosphorothiolates **7a** and **7b** were prepared from **13a** and **13b** by the procedure of Leader and Casida (1982) in ~20% yield with 1 M H_2SO_4 in ethanol at 55 °C for 48 h. The *O*-methyl analogues **8a** and **8b** were obtained in the same way in ~50% yield from **13a** and **13b** but with 1 M H_2SO_4 in methanol for 12 h at 55 °C. The pentachloro compounds **9a** and **9b** were made by alcoholysis of amidates **14a** and **14b** in 0.5 M H_2SO_4 in ethanol at 60 °C for 48 h. Purification of **9a** and **9b** by HPLC (μPorasil; hexane–ethyl acetate, 17:3) gave overall yields of ~3%.

Bioassays. Insecticidal activity was determined by topically applying the test compound in acetone (0.5 μL) to the abdomen of adult female houseflies (*Musca domestica* L.; SCR strain; ~20 mg each). Each 24-h LD_{50} value is the mean of two to four experiments.

Anti-AChE activity was determined with enzymes from electric eel (Simga Chemical Co., St. Louis, MO) and housefly head homogenate. The enzyme (4 units of electric eel or one house fly head equivalent) was incubated with a candidate inhibitor (added in 5 μL of acetone) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (0.76 μmol) in 50 mM phosphate, pH 7.5, buffer (3 mL) for 15 min at 25 °C. The reaction was started by addition of the substrate (20 μL of 75 mM acetylthiocholine iodide in 50 mM phosphate, pH 7.5, buffer). The increase in absorbance at 412 nm was continuously recorded against a water blank and the change in absorbance per 30 s calculated (O'Brien, 1968). Values for 50% inhibition concentrations (I_{50}) are the means of two or three experiments.

Microsomal oxidase activation or detoxification was measured by the procedure of Wing et al. (1983). The microsomes were prepared from livers of male albino mice at 20% (w/v) fresh tissue weight equivalent in sodium phosphate buffer (50 mM, pH 7.4). The microsomal fraction was considered to be the 100000*g* pellet on recentrifugation for 60 min of the 10000*g* (20 min) supernatant fraction. This was used either directly or following resuspension in buffer and resedimentation by centrifugation; these preparations are referred to as microsomes and washed microsomes, respectively. The soluble fraction was purified by passage through Sephadex G-50–80. Protein levels for the microsome and soluble fractions were determined by the method of Lowry et al. (1951). The coupled microsomal oxidase–AChE assay involved coincubation of the following components in 50 mM phosphate, pH 7.5, buffer (2.5 mL): AChE (4 units of electric eel), microsomes (0 or 1 mg of protein), soluble fraction (0, 0.5, or 1.0 mg of protein), reduced nicotinamide adenine dinucleotide phosphate (NADPH) (0 or 2 μmol), and the thiophosphorus compound (0–10 μmol). After 30-min incubation at 37 °C in a 25-mL Erlenmeyer flask with shaking in air, a 50-μL aliquot was removed for the determination of residual AChE activity. Inhibition is expressed relative to incubations with no organophosphorus compound. The data are means of three experiments.

RESULTS AND DISCUSSION

Resolution of Sulprofos, Sulprofos Oxon, and Related Compounds. The resolutions were achieved by preparing the phosphoramidodithioate (**11**) or phosphoramidothiolates (**12–14**), following by HPLC separation of the diastereoisomeric pair and acid alcoholysis with presumed inversion of configuration (Koizumi et al., 1977, 1978; Leader and Casida, 1982), a reaction scheme indicated in Figure 2 for phosphorodithioate **1** and phosphorothiolate **4**. The earlier methods were adequate to prepare phosphoramidothiolates **12–14**, but a modification was required for phosphoramidodithioate **11**. *S*-Propyl phosphorodichloridothioate was converted to the dithioate with phosphorus pentasulfide. The order of reaction suitable for **12–14** was reversed to obtain **11**. Initial coupling in the phosphorodithioate series was not successful with ethyl proline in the presence of triethylamine (due to the low reactivity of the phosphorodichloridothiolate), but fortunately it was satisfactory via the sodium salt of the phenoxide. The diastereoisomers of the amidates were separated with base-line resolution in each case by preparative HPLC with loadings of up to 20 mg for each injection except for **11** where 10 mg was the limiting amount. The most easily separated diastereoisomers were those of **14**: **14a** and **14b** were obtained pure by direct chromatography on a silica column.

Ethanolysis is faster with the phosphoramidothiolates (**12–14**) than the phosphoramidodithioate (**11**). The low

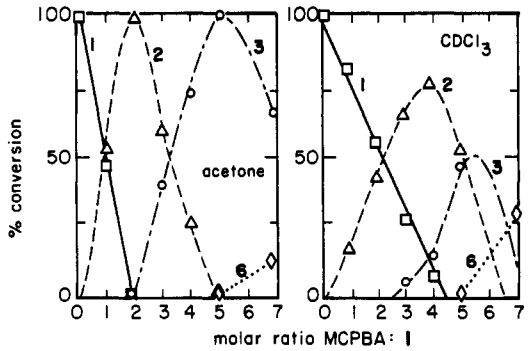


Figure 3. Reactions of sulprofos with various molar ratios of *m*-chloroperoxybenzoic acid (MCPBA) in acetone or chloroform. Sulprofos was at 1 mg/mL. The ratio of 1 to its oxidation products 2, 3, and 6 was based on relative ^{31}P NMR peak areas. ^{31}P NMR was measured by inserting a 5-mm tube containing $CDCl_3$ (for lock) into a 20-mm NMR tube when acetone was used as the solvent.

Table II. Optical Rotation Data for Enantiomers of Sulprofos, Its Oxidized Derivatives, and Related Compounds

	$(R_1O)(PrS)P(X)OAr R_2$			$[\alpha]^{20}_D$ (*c*, $CHCl_3$)	
no.	R_1	R_2	X	isomer **a**	isomer **b**
1	Et	4-SMe	S	+14.0 (0.6)[a]	−13.3 (0.8)[a]
4	Et	4-SMe	O	+4.5 (0.2)[a]	−4.8 (0.1)[a]
6	Et	4-S(O_2)Me	O	+3.9 (0.3)	−4.4 (0.3)
7	Et	2-Cl,4-Br	O	−15.8 (0.3)[a,b]	+15.9 (0.6)[a,b]
8	Me	2-Cl,4-Br	O	−18.9 (0.2)[a]	+19.3 (0.3)[a]
9	Et	Cl_5	O	−0.9 (0.4)[a]	+0.9 (0.5)

[a] $[\alpha]^{20}_D$ (*c*, $CHCl_3$) values for the amidate intermediates were as follows: **11a**, −50.9 (0.2); **11b**, −28.6 (0.1); **12a**, −44.4 (0.4); **12b**, −23.5 (0.3); **13a**, −29.9 (0.3); **13b**, −46.9 (0.1) (reported −39.5 and −51.9, respectively, in ethanol; Leader and Casida, 1982); **14a**, −64.5 (0.2). [b] Reported: −15.3 and +15.6 for **7a** and **7b**, respectively (Leader and Casida, 1982).

yield of the pentachloro derivative **9** was due in large part to competing cleavage of pentachlorophenoxide. Methanolysis proceeds faster and in higher yield than ethanolysis (**8** vs. **7**; confirmed also by conversion of **14** to **10**). ^{31}P NMR monitoring revealed less formation of byproducts by a milder procedure for ethanolysis involving replacement of the H_2SO_4 by an ion-exchange resin (Dowex AG 50W-X8, 100–200 mesh, H^+ form; dried by azeotropic distillation of benzene); however, a mixture of 100 mg of **13** and 300 mg of resin in 5 mL ethanol required more than 1 week at 60 °C for optimal conversion.

Oxidation of Enantiomers of Sulprofos and Sulprofos Oxon to Sulprofos Oxon Sulfone. The oxidation products of sulprofos are readily distinguished by ^{31}P NMR (Table I), providing a convenient method to monitor the oxidation reactions. The principal oxidation sequence with MCPBA is **1** → **2** → **3** → **6** with rapid thioether sulfur oxidation relative to phosphorothionate sulfur oxidation, ultimately yielding **6** in yields of 10–30% (based on NMR; Figure 3) or 10% (based on isolation). The reaction was faster and smaller amounts of byproducts were formed in acetone than in chloroform. The amount of **6** formed on MCPBA oxidation of **1** was sufficient for the determination of optical purity with chiral shift reagent but not for preparative purposes. Improved yields (~60%) of **6a** and **6b** were obtained on MCPBA oxidation of **4a** and **4b** due to the facile thioether sulfur oxidation.

Determination of Optical Purity. Enantiomers **a** and **b** of **1**, **4**, and **6–9** gave essentially the same optical rotation but with opposite signs (Table II). ^1H NMR using Eu(hfc)$_3$ allowed a direct estimate of the optical purity of

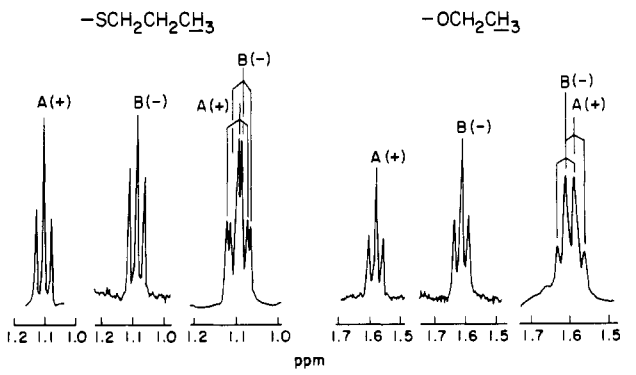


Figure 4. ^1H NMR spectra [300 MHz; $CDCl_3$; optimal 1:1 (w/w) ratio of Eu(hfc)$_3$ to **6a** or **6b**] of $-SCH_2CH_2CH_3$ and $-OCH_2CH_3$ resonances of resolved (+)- and (−)-sulprofos oxon sulfone.

compounds containing a phosphoryl oxygen (P=O), as illustrated in Figure 4 for **6a** and **6b**. Each enantiomer of **4** and **6–9** exhibited a sharp triplet for each $-CH_3$ group of the P–O–Et and P–S–Pr substituents, indicating an optical purity of >95%. This NMR method, however, is limited to P=O compounds since the P=S sulfur is not a sufficiently good Lewis base to establish an equilibrium complex with Eu(hfc)$_3$ (Hall and Inch, 1979). Accordingly, **1a** and **1b** were oxidized to **6a** and **6b** as above before the shift reagent was used. The enantiomeric purity of (+)-**6a** and (−)-**6b** are indicated in Figure 4. The ^1H NMR chemical shift with Eu(hfc)$_3$ of the $-SCH_2CH_2CH_3$ protons appears at lower field with **6a** derived from **1a** than with **6b** derived from **1b**. The shift of these protons also appears at lower field with **4a** than with **4b**, establishing that the chirality at the phosphorus atom of **1a** and **1b** is retained on peracid oxidation to **6a** and **6b**, respectively.

The effect of Eu(hfc)$_3$ on the resonances of the $-SCH_2CH_2CH_3$ and $-OCH_2CH_3$ (when present) protons of the enantiomers **7–9** is consistent with the assignments of the *R* configuration for (−)-profenofos and related compounds and the *S* configuration for (+)-profenofos and its analogues (Copper et al., 1977; Hall and Inch, 1979; Leader and Casida, 1982). On the same basis (−)-sulprofos and its oxidation products are assigned as *S* and (+)-sulprofos and its oxidation products as *R*. These are obviously tentative conclusions since they are based only on circumstantial NMR evidence.

Biological Activity. House fly toxicity in the sulprofos series (**1**, **4**, and **6**) is 2.5–20-fold higher for the (+)- than the (−)-isomers, whereas in the profenofos series (**7** and **8**) the (−)-isomer is 2.5–3.5-fold more toxic than the (+)-isomer (Table III). The house fly head anti-AChE activity has the opposite relationship, i.e., (−) is 2.1–7.7-fold more active than (+) in the sulprofos series and (+) is 1.7–5.7-fold more active than (−) in the profenfos series. In every case there is a reversal of chiral specificity for toxicity and anti-AChE activity. The magnitude of isomer difference is greatest for **6** relative to LD_{50} and I_{50}. Remarkably high house fly knockdown activity is found for **4a** and house fly anti-AChE activity for **6** [see also Bull et al. (1976)], especially **6b**. The probable metabolic conversion in house flies of **1a** and **1b** to **6a** and **6b**, respectively, greatly enhances their potency as AChE inhibitors with much less effect on their toxicity, suggesting that oxidation at the thiono and thioether sulfur centers activates the compounds for both inhibition of esterases and detoxification. Sulprofos oxon sulfone (**6**) appears to be very selective for house fly compared to electric eel AChE, i.e., I_{50} values of 0.0032 and 0.43 μM, respectively.

The *O*-ethyl and *O*-methyl esters are similar to each other in toxicity and anti-AChE activity with house flies

Table III. Biological Activity of the Enantiomers of Sulprofos and Profenofos and Related Compounds

	$R_1O(PrS)P(X)OAr-R_2$				AChE I_{50}, μM		house fly
no.	R_1	R_2	X	enantiomer	electric eel	house fly	LD_{50}, μg/g
			Sulprofos (1) and Its Oxidation Products (2–6)				
1a	Et	4-SMe	S	(+)	3.9	51	7
1b				(−)	2.0	24	26
1				(±)	3.5	30	13
2	Et	4-S(O)Me	S	(±)	3.9	10	10
3	Et	4-$S(O_2)$Me	S	(±)	4.0	13	9
4a	Et	4-SMe	O	(+)	4.5	12	4
4b				(−)	0.2	5	10
4				(±)	3.6	10	5
5	Et	4-S(O)Me	O	(±)	0.79	0.094	8
6a	Et	4-$S(O_2)$Me	O	(+)	0.64	0.0069	1
6b				(−)	0.03	0.0009	20
6				(±)	0.43	0.0032	7
			Profenofos (7) and Related Compounds (8–10)				
7a	Et	2-Cl,4-Br	O	(−)	1.30	0.19[a]	4[a]
7b				(+)	0.22	0.11[a]	10[a]
7				(±)	0.56	0.13	5
8a	Me	2-Cl,4-Br	O	(−)	0.42	0.27	2
8b				(+)	0.02	0.10	7
8				(±)	0.30	0.22	5
9a	Et	Cl_5	O	(−)	0.30	4.0	18
9b				(+)	0.26	0.7	35
9				(±)	0.28	1.2	23
10	Me	Cl_5	O	(±)	1.7	1.3	15

[a] Reported for 7a and 7b, respectively: I_{50} = 0.31 and 0.16 μM; LD_{50} = 6 and 23 μg/g (Leader and Casida, 1982).

Table IV. Oxidative Bioactivation or Detoxification as Acetylcholinesterase Inhibitors of the Enantiomers of Sulprofos and Profenofos and Related Compounds by Mouse Liver Microsomal Oxidases

	$R_1O(PrS)P(X)OAr-R_2$					AChE inhibition, %[a]	
no.	R_1	R_2	X	enantiomer	μM	control	oxidase
			Sulprofos (1) and Its Oxidation Products (2–6)				
1a	Et	4-SMe	S	(+)	250	0 ± 1	56 ± 2
1b				(−)	250	1 ± 1	34 ± 5
1				(±)	250	1 ± 3	43 ± 1
2	Et	4-S(O)Me	S	(±)	1000	7 ± 6	62 ± 0
3	Et	4-$S(O_2)$Me	S	(±)	1000	15 ± 5	57 ± 4
4a	Et	4-SMe	O	(+)	10	2 ± 0	98 ± 0
4b				(−)	10	7 ± 5	38 ± 4
4				(±)	10	5 ± 1	61 ± 2
5	Et	4-S(O)Me	O	(±)	5	43 ± 6	97 ± 1
6a	Et	4-$S(O_2)$Me	O	(+)	10	87 ± 1	95 ± 0
6b				(−)	10	90 ± 1	74 ± 1
6				(±)	10	89 ± 1	92 ± 0
			Profenofos (7) and Related Compounds (8–10)				
7a	Et	2-Cl,4-Br	O	(−)	10	59 ± 2	97 ± 0
7b				(+)	10	79 ± 2	68 ± 3
7				(±)	10	76 ± 4	92 ± 1
8a	Me	2-Cl,4-Br	O	(−)	25	66 ± 2	96 ± 1
8b				(+)	25	75 ± 2	99 ± 0
8				(±)	25	67 ± 7	99 ± 0
9a	Et	Cl_5	O	(−)	10	10 ± 8	99 ± 0
9b				(+)	10	17 ± 4	85 ± 3
9				(±)	10	13 ± 6	98 ± 1
10	Me	Cl_5	O	(±)	20	38 ± 3	99 ± 0

[a] Data are mean ± SE for three separate experiments.

in both the profenofos (7 vs. 8) and pentachloro series (9 vs. 10).

Oxidative Bioactivation and Detoxification as AChE Inhibitors. The oxidase system increases the anti-AChE activity of each *S*-propyl phosphorothiolate and phosphorodithioate examined with two exceptions: **6b** and **7b** are detoxified (Table IV). The chiral specificity for the profenofos enantiomers was further examined by using washed microsomes. This showed an oxidative activation for both (+)-profenofos and (−)-profenofos (Table V). Addition of a small amount of soluble fraction does not greatly change the tendency for (−)-profenofos but shifts an activation into an effective detoxification system for (+)-profenofos. It therefore appears that both (+)-profenfos and (−)-profenofos undergo oxidative bioactivation but that a factor in the soluble fraction nullifies the activation of (+)-profenofos.

On incubation of *S*-propyl phosphorothiolates in the coupled AChE–microsomal oxidase assay, the microsomes alone generally reduce the anti-AChE activity (Tables III and IV) whereas the further addition of NADPH increases their AChE inhibitory potency (Table IV). The microsomal incubations result in major hydrolytic and oxidative ester cleavage reactions (Ohkawa, 1982; Wing et al., 1983, 1984) in addition to the activations measured here.

Chiral Specificity of Toxic Action. This study and our earlier reports (Wing et al., 1983, 1984; Glickman et al., 1984; Casida, 1984) reveal that the stereospecificity in

Table V. Oxidative Bioactivation or Detoxification as Acetylcholinesterase Inhibitors of the Enantiomers of Profenofos by Mouse Liver Microsomal Oxidases and Soluble Fraction

protein, mg		AChE inhibition, %	
microsome	soluble	−NADPH	+NADPH
	(−)-Profenofos (20 μM)		
0	0	89 ± 2	90 ± 3
1	0	11 ± 6	96 ± 1
1	0.5	46 ± 2	97 ± 1
1	1	49 ± 5	98 ± 2
0	1	59 ± 5	81 ± 3
	(+)-Profenofos (10 μM)		
0	0	78 ± 2	84 ± 2
1	0	45 ± 5	59 ± 4
1	0.5	42 ± 5	47 ± 4
1	1	57 ± 4	49 ± 8
0	1	65 ± 4	52 ± 7

the toxicity of the profenofos enantiomers is due to a combination of factors: intrinsic potency as AChE inhibitors; bioactivation, presumably by S-oxidation; detoxification of the parent compound and the activated intermediate; aging differences for AChE inhibited by the (+)- and (−)-isomers depending on the leaving group on direct reaction or after oxidase activation. Sulprofos has the additional feature of requiring three sequential oxidative activation steps to form the oxon sulfone. These studies illustrate the value of optically resolved phosphorus compounds in studies on insecticide mode of action (Ohkawa, 1982).

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Registry No. 1, 92642-31-4; 1a, 92642-32-5; 1b, 92642-33-6; 2, 92642-34-7; 3, 92642-35-8; 4, 92642-36-9; 4a, 92642-37-0; 4b, 92642-38-1; 5, 92642-39-2; 6, 92642-40-5; 6a, 92642-41-6; 6b, 92642-42-7; 7, 92760-41-3; 7a, 81116-98-5; 7b, 81123-19-5; 8, 92760-44-6; 8a, 92642-43-8; 8b, 92642-44-9; 9, 92760-45-7; 9a, 92642-45-0; 9b, 92642-46-1; 10, 92642-47-2; 11 (isomer I), 92642-48-3; 11 (isomer II), 92642-49-4; 12 (isomer I), 92642-50-7; 12 (isomer II), 92642-51-8; 13a, 92760-42-4; 13b, 92760-43-5; 14a, 92642-52-9; 14b, 92642-53-0; acetylcholinesterase, 9000-81-1; 4-(methylthio)phenol, 1073-72-9; *S*-propyl phosphorodichloridodithioate, 5390-61-4; L-proline ethyl ester, 5817-26-5; 4-bromo-2-chlorophenol, 3964-56-5; pentachlorophenol, 87-86-5.

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Photochemistry of Polychlorinated Phenoxyphenols. 2. Phototransformations of *m*-(Pentachlorophenoxy)-2,4,5,6-tetrachlorophenol

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The direct irradiation of *m*-(pentachlorophenoxy)-2,4,5,6-tetrachlorophenol (**1b**) in cyclohexane at 300 nm generates products resulting from reductive dechlorination (60%), ether cleavage (10%), and cyclization (1%). In contrast, photolysis in acetone at 300 nm provides 66% cyclization, 10% reductive dechlorination, and less than 2% ether cleavage. Irradiation of perchloro-*m*-phenoxyphenol (**1b**) in cyclohexane (300 nm) in the presence of *m*-methoxyacetophenone results in cyclization (7%), dechlorination (28%), and less than 1% ether cleavage. Photolysis of *m*-phenoxyphenol in acetone in the presence of the electron donor triethylamine at 300 nm leads to 54% dechlorination, 18% ring closure, and less than 1% ether cleavage. The mechanistic implications of these results are described.

Our interest in the phototransformations of the isomeric perchlorophenoxyphenols (**1a–c**) is based upon their presence as major impurities in pentachlorophenol (up to 15% present in the technical product) (Rappe and Nilsson, 1972; Jensen and Renberg, 1972; Nilsson and Renberg, 1974; Deinzer et al., 1978, 1979, 1981a), their absorption in the sunlight range (near 300 nm), and their potential for photocyclization to polychlorodibenzodioxins and

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