Resolution and Biological Activity of the Chiral Isomers of *O*-(4-Bromo-2-chlorophenyl) *O*-Ethyl *S*-Propyl Phosphorothioate (Profenofos Insecticide)

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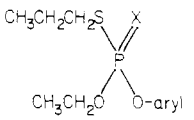
The individual diastereoisomers of *O*-(4-bromo-2-chlorophenyl) *N*-[(2*S*)-2-(carboxyethyl)pyrrolidinyl] *S*-propyl phosphorothioamidate, resolved by HPLC on μPorasil with hexane–ethyl acetate, 4:1, are converted with inversion of configuration to the chiral isomers of profenofos, obtained in 20–30% yield on treatment with 1 M sulfuric acid in ethanol at 55 °C for 48 h. The enantiomeric purity of the resolved profenofos antipodes was established by ^{1}H NMR with the chiral shift reagent tris[3-[(heptafluoropropyl)hydroxymethylene]-(+)-camphorato]europium (III). (−)-Profenofos is 4–23-fold more toxic than (+)-profenofos to mosquito and cabbage looper larvae, housefly adults, and mice. In contrast the (+) isomer is 2–48-fold more potent than the (−) isomer as an in vitro inhibitor of bovine erythrocyte and housefly head acetylcholinesterase, horse serum butyrylcholinesterase, and mouse liver "pyrethroid esterase". Both chiral isomers of profenofos are potent synergists for the toxicity of malathion and a pyrethroid to mice.

Several *O*-aryl *O*-ethyl *S*-propyl phosphorothioates and phosphorodithioates are important insecticides with high potency for insects, including strains resistant to earlier organophosphorus insecticides, and with good residual activity and low acute toxicity to mammals (Buholzer, 1975; Drabek and Flück, 1979; Kudamatsu et al., 1978). Four compounds of this type are



compound	X	aryl
profenofos (1)	O	2-Cl,4-Br-C_6H_3
RH 218	O	2,4,6-Cl_3-C_6H_2
prothiophos	S	2,4-Cl_2-C_6H_3
sulprofos	S	4-CH_3S-C_6H_4

The insecticidal activity of these phosphorothioates may be a function of factors other than their anticholinesterase activity (El-Sebae et al., 1980; Hart and O'Brien, 1976). Profenofos and sulprofos are potent inhibitors of pyrethroid esterase(s) in mouse liver and synergists for the toxicity of malathion and fenvalerate to mice (Gaughan et al., 1980).

Chiral phosphorus esters play an important role in understanding the action of organophosphorus toxicants. Chiral enantiomers differ greatly in their acute toxicity, delayed neurotoxicity, antiesterase activity, ease of aging of phosphorylated acetylcholinesterase, and rate of metabolism (Allahyari et al., 1980; Eto, 1974; Ohkawa et al., 1978).

This paper describes the first resolution of an *O*-alkyl *O*-aryl *S*-propyl phosphorothioate by using profenofos as an example (Figure 1). It also compares the biological activity of the chiral isomers of profenofos.

MATERIALS AND METHODS

General. NMR spectra were obtained for samples in $CDCl_3$ solutions under the following conditions: ^{1}H NMR, 90 MHz Perkin-Elmer R32B spectrometer or 250-MHz UCB-250 (University of California, Berkeley, Chemistry Department) instrument with chemical shifts related to internal tetramethylsilane; ^{31}P NMR, UCB-180 spectrometer operated at 72.9 MHz reporting chemical shifts (ppm) as positive when downfield of external 1% trimethyl phosphate in $CDCl_3$ [$\delta(^{31}P) = 0$]; d = doublet, t = triplet, q = quartet, dd = double doublet, dt = double triplet, dq = double quartet, m = multiplet, and brm = broad multiplet. CI-MS were run with the Finnigan 3200 spectrometer with methane as the reagent gas. Quasimolecular ions (M + 1) are reported for the lowest mass in the isotope cluster. Optical rotations were measured at 20 ± 2 °C with a cell path length of 100 mm (capacity ~ 2 mL) by using a Perkin-Elmer 241 polarimeter.

HPLC utilized a μPorasil column (30 × 0.39 cm for analysis and 30 × 0.78 cm for preparative isolations) with a Waters Associates liquid chromatograph and absorbance detector at 254 nm. TLC was accomplished by using silica gel F-254 chromatoplates with product detection by exposing to I_2 vapor, often after a preliminary spray with $PdCl_2$ solution.

Chemicals. Chemicals were from the following sources: profenofos analytical standard (CIBA-Geigy); 4-bromo-2-chlorophenol, propyl iodide, (1*R*,2*S*)-2-(methylamino)-1-phenylpropan-1-ol [(−)-ephedrine], and tris[3-[(heptafluoropropyl)hydroxymethylene]-(+)-camphorato]europium (III) [Eu(hfc)$_3$] (Aldrich); *O*-ethyl phosphorodichloridothioate (Aldrich) was distilled before use; *S*-propyl phosphorodichloridothioate (**2**) [prepared according to Bayer and Hurt (1977); $\delta(^{31}P) = 33.0$]; L-proline ethyl ester, from treatment of L-proline hydrochloride (Sigma) with thionyl chloride and ethanol [general procedure of Patel and Price (1965); see also Koizumi et al. (1977)], was distilled immediately before use.

Synthesis of Intermediates. *O-(4-Bromo-2-chlorophenyl) N-[(2S)-2-(Carboxyethyl)pyrrolidinyl] S-Propyl Phosphoroamidothioate* (**3a + 3b**). A solution of L-proline ethyl ester (1.5 g) and triethylamine (2.5 g) in dry benzene (50 mL) was added dropwise to a cooled (10 °C) solution of **2** (1.93 g) in dry benzene (50 mL). Stirring was continued for 4 h, followed by dropwise addition of a solution of 4-bromo-2-chlorophenol (2.1 g) in dry benzene (25 mL). The mixture was refluxed for 2 h, cooled to room temperature, filtered, washed with H_2O (2 × 100 mL), and dried over anhydrous $MgSO_4$, and the solvent was evaporated to leave a pale yellow viscous oil (3.5 g). After column chromatography (silica; benzene–ethyl acetate, 9:1), the mixture of **3a + 3b** was obtained (2.8 g) as a colorless

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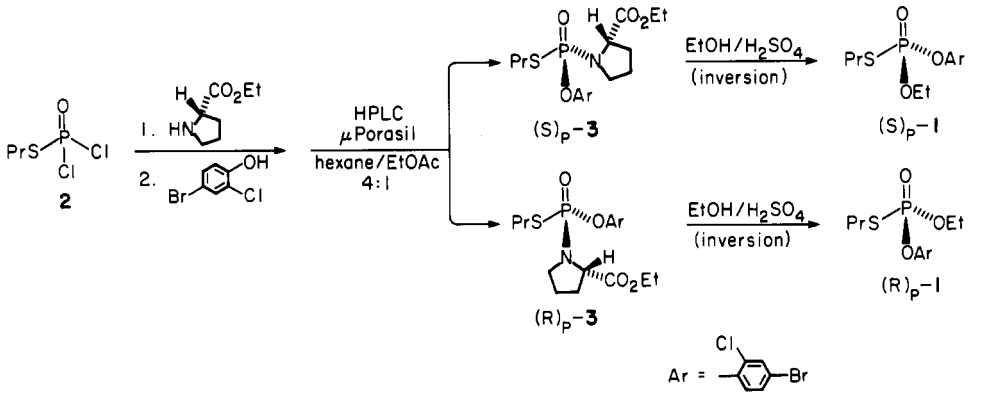


Figure 1. Resolution of chiral isomers of profenofos (1).

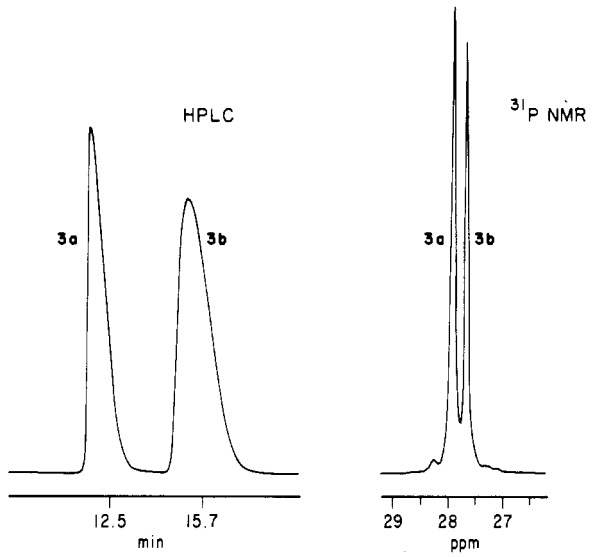


Figure 2. HPLC chromatogram (μPorasil; hexane–ethyl acetate, 4:1) and ^{31}P NMR spectrum (^1H decoupled; deuteriochloroform) of diastereomers **3a** and **3b** of *O*-(4-bromo-2-chlorophenyl) *N*-[(2*S*)-2-(carboxyethyl)pyrrolidinyl] *S*-propyl phosphoroamidothioate (**3**).

viscous oil: CI-MS 470 (M + 1); ^{31}P NMR (^1H decoupled) revealed two sharp singlets (δ +27.7 and +27.9) in a 1:1 ratio (Figure 2). Examination of many TLC and column chromatography systems revealed no method suitable to separate epimers **3a** and **3b**. However, analytical HPLC indicated equal amounts of two components (Figure 2).

O-(4-Bromo-2-chlorophenyl) S-Propyl Phosphorothioate (**4**). A solution of 4-bromo-2-chlorophenol (0.38 g) and triethylamine (0.2 g) in benzene (10 mL) was added dropwise to a solution of **2** (0.35 g) in benzene (15 mL). The reaction mixture was stirred for 2 h at room temperature and filtered, most of the benzene was evaporated under reduced pressure, and the residue dissolved in 0.1 N NaOH. After extraction with ether, the aqueous phase was acidified to pH 1 and extracted again with ether. The latter ether extract was dried over $MgSO_4$ and evaporated to leave a colorless oil (0.25 g): CI-MS 345 (M + 1); ^1H NMR δ 0.95 (t, 3 H, CH_3CH), 1.70 (5 lines, 2 H, CCH_2C), 2.95 (5 lines, 2 H, SCH_2), 7.3–7.6 (m, 3 H, aromatic); ^{31}P NMR δ + 21.9 (t, J_{P-H} = 13.6 Hz).

O-(4-Bromo-2-chlorophenyl) O-Ethyl Phosphorothioate (**5**). A solution of 4-bromo-2-chlorophenol (10.4 g) and triethylamine (5.5 g) in dry benzene (100 mL) was added dropwise to a stirred solution of *O*-ethyl phosphorodichloridothioate in dry benzene (100 mL). The mixture was stirred for 24 h, triethylamine hydrochloride was filtered off, the benzene solution was washed with H_2O and saturated NaCl solution and dried over anhydrous $MgSO_4$, and the solvent was evaporated to leave crude *O*-(4-bromo-2-chlorophenyl) *O*-ethyl phosphorochloridothioate as a colorless oil (16.0 g). This phosphorochloridothioate was dissolved in 1,4-dioxane (100 mL) and added to a solution of KOH (4.9 g) in dioxane–H_2O (1:1; 100 mL). After the solution was stirred for 24 h at room temperature, the dioxane was removed under reduced pressure, and the alkaline aqueous solution was extracted with benzene and acidified with concentrated HCl to pH 1. The acidic mixture was reextracted with benzene, and the benzene was dried over anhydrous $MgSO_4$ and evaporated to leave a viscous colorless oil (9.0 g): CI-MS 331 (M + 1); ^1H NMR δ 1.37 (t, 3 H, CH_3CH_2), 4.28 (m, 2 H, CH_2CH_3), 7.29–7.54 (m, 3 H, aromatic), 8.48 (s, 1 H, O*H*, exchangeable with D_2O); dicyclohexylammonium salt, mp 170–171 °C (from ethyl acetate).

(2R,4S,5R)-2-(4-Bromo-2-chlorophenoxy)-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine-2-thione (**7a**) *and Its 2S,4S,5R Isomer* (**7b**). A solution of sodium 4-bromo-2-chlorophenoxide (prepared from 1.05 g of 4-bromo-2-chlorophenol and sodium methoxide) in dry acetonitrile (50 mL) was added dropwise to a solution of 1.32 g of (2*S*,4*S*,5*R*)-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine-2-thione (Cooper et al., 1977) (**6a**; mp 125–128 °C) in dry acetonitrile (50 mL). The mixture was stirred for 24 h at room temperature, filtered, and concentrated under reduced pressure. The residue was dissolved in chloroform (100 mL), washed with H_2O (50 mL) and dried, and the solvent was removed to leave a pale yellow viscous oil (1.6 g). After column chromatography (silica; chloroform), the pure oxazaphospholidinethione **7a** was obtained as a viscous oil (70% yield): CI-MS 432 (M + 1); ^1H NMR δ 0.80 (d, 3 H, CH_3CH), 2.86 (d, J_{P-H} = 12.4 Hz, 3 H, NCH_3), 3.65 (m, 1 H, $CHCH_3$), 5.70 (dd, J = ~7 Hz, J_{P-H} = ~3 Hz, 1 H, C*H*Ph), 7.20–7.55 (m, 8 H, aromatic); ^{31}P NMR δ + 75.6 (6 lines).

The 2*S*,4*S*,5*R* isomer **7b** was prepared as with **7a**, but starting with **6b** (the 2*R*,4*S*,5*R* isomer of **6a**; mp 58 °C), in 65% yield as a viscous oil: CI-MS 432 (M + 1); ^1H NMR δ 0.82 (d, 3 H, CH_3CH), 2.84 (d, J_{P-H} = 12.8 Hz, 3 H, NCH_3), 3.80 (m, 1 H, $CHCH_3$), 5.70 (m, 1 H, C*H*Ph), 7.20–7.55 (m, 8 H, aromatic); ^{31}P NMR δ +75.5 (6 lines).

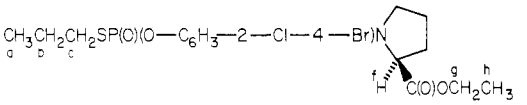
Acid-Catalyzed Alcoholysis of 3. Suitable conditions for maximum yield and purity were examined under the following conditions with ^{31}P NMR and TLC (hexane–ethyl acetate, 4:1) monitoring: anhydrous HCl–ethanol (0.15, 1, and 5 M), H_2SO_4–ethanol (0.15 and 1 M), *p*-toluenesulfonic acid–ethanol (1 M), and trifluoroacetic acid–ethanol (1 M); HCl–methanol (1 M) and H_2SO_4–methanol (1 M); alcoholysis at 25, 55, and 70 °C and for periods of several hours to 4 days.

Optical Purity. When stereospecificity was evaluated, the pure epimer **3a** or **3b** was used for alcoholysis at 4

Table I. Optical Rotation and NMR Data for (+)- and (−)-Profenofos (1) and Their Phosphorothioamidate Precursors (3)

$CH_3CH_2CH_2SP(O)(O\text{-}C_6H_3\text{-}2\text{-}Cl\text{-}4\text{-}Br)OCH_2CH_3$
a b c d e

compound	$[\alpha]^{20}_D$,[a] deg	NMR[b] ^{31}P		H_a	H_b	H_c	H_d	H_e
profenofos, 1								
(−)-1 from 3a	−15.3	δ:	+23.4	0.97	1.70	2.90	4.33	1.43
(+)-1 from 3b	+15.6	mult.:	7[c]	t	6	10[d]	dq[e]	dt[f]



compound	$[\alpha]^{20}_D$,[a] deg	NMR[b] ^{31}P		H_a	H_b	H_c	H_f	H_g	H_h
phosphorothioamidate 3									
3a	−39.5	δ:	+27.9	0.97	1.69	2.93	4.45	4.16	1.21
		mult.:	m	t	6	5	7	q	t
3b	−51.9	δ:	+27.7	0.95	1.67	2.85	4.46	4.17	1.24
		mult.:	m	t	6	5	7	q	t

[a] 1 in deuteriochloroform; 3 in ethanol. [b] ^1H spectra with a 250-MHz spectrometer. Aromatic protons 7.3–7.5 (m). Proline $-(CH_2)_3$ = 1.6–2.2 (br m). [c] Multiplicity expressed as number of lines. [d] Diastereotopic proton: J_{H-H} = 2.0 Hz; J_{P-H} = 9.3 Hz. [e] J_{P-H} = 9.0 Hz. [f] $^4J_{P-H}$ = 0.9 Hz.

mmol in 5 mL of the alcohol–acid solution. The ^1H NMR spectrum of (±)-profenofos [2% in deuteriochloroform with an equal weight of Eu(hfc)$_3$] shows a pair of triplets for the $-SCH_2CH_2CH_3$ resonance (Figure 3), whereas one sharp triplet is expected for each pure enantiomer. These conditions were selected for the maximum shift difference for the two indicated methyl triplets of each antipode and permitted detection of 5% of the minor isomer.

Resolution of the Chiral Isomers of Profenofos. Resolution was achieved by a procedure based on Koizumi et al. (1977, 1978a) which consists of three steps (Figure 1): reacting **2** with the easily available L-proline ethyl ester as a chiral reagent to form the phosphoroamidothioate diastereomeric mixture **3** described above; separation of the diastereoisomers of **3**; acid-catalyzed alcoholysis to give the desired chiral phosphorus esters, i.e., $(R)_P$-1 and $(S)_P$-1.

The individual chiral isomers of **3** were separated with base-line resolution on preparative HPLC with up to 80 mg for each injection; they are designated **3a** and **3b** according to their order of elution (Figure 2). Optical rotation and NMR data for **3a** and **3b** are given in Table I.

Acid-catalyzed ethanolysis of **3a** gave (−)-profenofos and of **3b** gave (+)-profenofos, each in 20–30% yield. **3a** or **3b** (1.0 g) in 1 M H_2SO_4–absolute ethanol (50 mL) was protected from moisture and held at 55 °C with stirring for 48 h. ^{31}P NMR revealed three major compounds: unreacted **3a** or **3b**, profenofos (^{31}P data in Table I), and phosphorothioate **4** [$\delta(^{31}P)$ + 20 ± 1 (t, J_{P-H} = 13.6 Hz)] identified by comparison with an authentic synthetic sample. After rapid removal of most of the ethanol at <40 °C under reduced pressure, the residue was dissolved in chloroform and the organic phase was washed with H_2O, cold 0.1 N NaOH, and finally concentrated NaCl solution. Unreacted **3a** or **3b** (300–350 mg) and the profenofos enantiomer (180–220 mg) were separated by preparative HPLC (μPorasil; hexane–ethyl acetate, 4:1; profenofos elutes completely before **3**). The two profenofos enantiomers gave essentially the same optical rotation with opposite signs and had identical ^{31}P and ^1H NMR spectra (Table I); each gave CI-MS 374 (M + 1). Only one sharp triplet was observed for the $-SCH_2CH_2CH_3$ resonance of either the (−)- or (+)-profenofos–Eu(hfc)$_3$ mixture (parts A and B of Figure 4), thereby substantiating the high enantiomeric purity of the resolved antipodes.

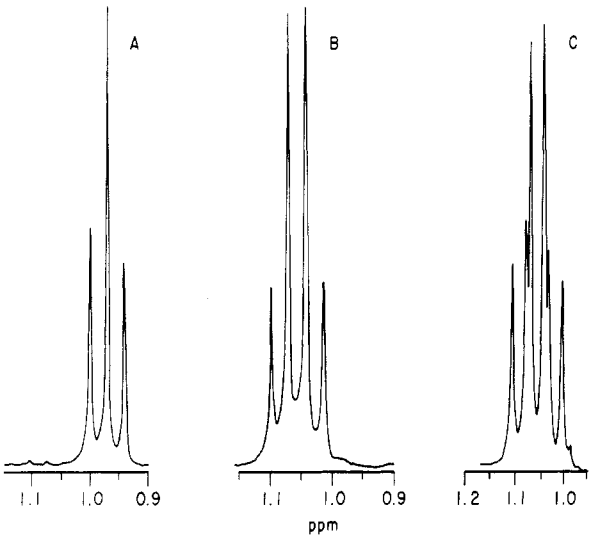


Figure 3. Effect of Eu(hfc)$_3$ on the $-SCH_2CH_2CH_3$ resonance of (±)-profenofos (^1H NMR; 250 MHz; deuteriochloroform). Profenofos alone (A) and profenofos–Eu(hfc)$_3$ in ratios of 2:1 (B) and 1:1 (C).

Bioassays. Four esterase sources were used: bovine erythrocyte acetylcholinesterase and horse serum butyrylcholinesterase (Sigma); housefly head acetylcholinesterase and mouse liver "pyrethroid esterase" (15000*g* supernatant fractions of housefly head and mouse liver homogenates, respectively). Cholinesterase assays were made by a modified Ellman procedure (O'Brien, 1968), incubating the profenofos isomer (added in 5 μL of acetone) and enzyme in 1 mL of pH 7.5 50 mM phosphate buffer for 15 min at 25 °C before adding acetyl- or butyrylthiocholine. Pyrethroid esterase assays (Gaughan et al., 1980) involved incubating the inhibitor and enzyme in pH 7.4 50 mM phosphate buffer for 20 min at 37 °C prior to adding (1*R*)-*trans*-[*carbonyl*]-^{14}C]permethrin.

LD_{50} determinations involved mice and three insect species. Male albino mice (8–10 g) were administered the test compound by the intraperitoneal (ip) route with 25 μL of methoxytriglycol (MTG) as the carrier vehicle. Adult female houseflies (*Musca domestica* L.; SCR strain; ~20 mg each) and cabbage looper larvae (*Trichoplusia ni*

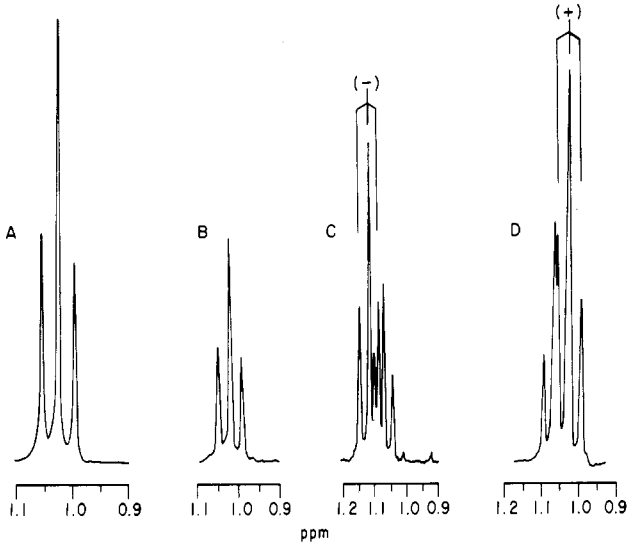


Figure 4. ^1H NMR spectra [250 MHz; deuteriochloroform; Eu(hfc)$_3$] of $-SCH_2CH_2CH_3$ resonance of resolved and partially resolved profenofos. (A) (-)-Profenofos: $[\alpha]_D$ -15.3° from **3a** with 1 M H_2SO_4 in ethanol. (B) (+)-Profenofos: $[\alpha]_D$ +15.6° from **3b** with 1 M H_2SO_4 in ethanol. (C) (±)-Profenofos: $[\alpha]_D$ +5.8° from **3a** with 5 M HCl in ethanol. (D) (±)-Profenofos: $[\alpha]_D$ +11.9° from **3b** with 5 M HCl in ethanol.

Hubner; third instar; ~90 mg each) were treated topically on the abdomen with the toxicant in 0.5 μL of acetone. Mosquito larvae (*Culex tarsalis* Coquillett; fourth instar) were held in groups of three in 5 mL of distilled water to which the toxicant was added in 10 μL of methanol. Mortality determinations were made at 3 h (mosquito larvae) or 24 h (other cases).

For synergized toxicity studies, male albino mice (~20 g) were administered the profenofos isomer at 7.5 mg/kg followed 1 h later by malathion at 10 mg/kg or the pyrethroid (1*R*)-*trans*-ethanomethrin [5-benzyl-3-furylmethyl (1*R*)-*trans*-3-(cyclopentylidenemethyl)-2,2-dimethylcyclopropanecarboxylate] (Roussel-Uclaf) at 20 mg/kg by using MTG (25 μL) as the carrier vehicle for each ip treatment. Mortality was recorded at 24 h with 28 mice per group in the malathion series and 22 in the ethanomethrin series.

RESULTS AND DISCUSSION

Acid-Catalyzed Alcoholysis. Resolution of the chiral isomers of profenofos is preferably carried out under acidic conditions to minimize racemization (Hall and Inch, 1979b). Acyclic phosphoroamidates readily undergo acidic alcoholysis at the P–N bond with predominant inversion of configuration. The mechanism involves protonation of the nitrogen atom followed by a bimolecular direct displacement at the phosphorus (A-S_N2-P mechanism or A-2 associative mechanism) (Garrison and Boozer, 1968; Kobayashi et al., 1979; Koizumi et al., 1974). However, there are exceptions in which the stereochemistry depends on the nature of the substituents on the phosphorus and the reaction conditions. Alcoholysis of chiral *O,S*-dialkyl phosphorothioamidates at high acid concentration involves up to 86% retention of configuration (Hall and Inch, 1977), and there is also low stereospecificity for some BF_3-catalyzed solvolyses (Koizumi et al., 1978b). These results have been interpreted in terms of an A-1 dissociative mechanism, of a double-inversion mechanism, and by formation of a pentacoordinated intermediate followed by pseudorotation (Hall and Inch, 1979b; Harger, 1977; Koizumi et al., 1974; Modro and Graham, 1981). In light of the many possible factors determining the ultimate stereochemical purity, it was appropriate to examine the acid-catalyzed alcoholysis of **3** in considerable detail.

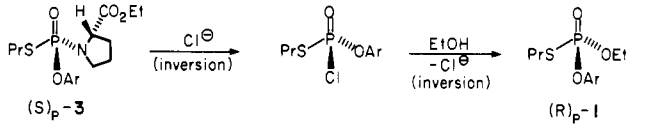


Figure 5. Overall retention of configuration by a double-inversion mechanism due to chloride ion participation on solvolysis of $(S)_P$-**3** in ethanol–HCl. Ar = 4-bromo-2-chlorophenyl.

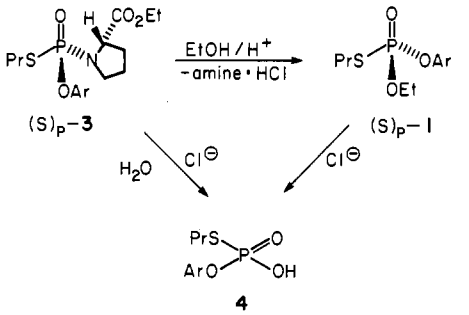


Figure 6. Formation of *O*-(4-bromo-2-chlorophenyl) *S*-propyl phosphorothioate (**4**) as a byproduct on solvolysis of $(S)_P$-**3** in acidic ethanol with a trace of moisture. Ar = 4-bromo-2-chlorophenyl.

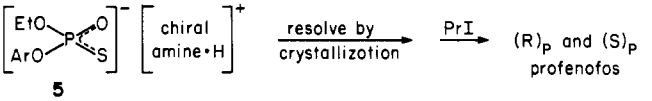


Figure 7. Diastereomeric salt mixture approach to resolution of chiral isomers of profenofos. Ar = 4-bromo-2-chlorophenyl.

Profenofos enantiomers of high optical purity although in relatively low yield (~20%) were obtained by treating **3a** or **3b** with 1 M H_2SO_4 in ethanol at 55 °C for 48 h. Above 70 °c the yield is decreased due to decomposition of either the starting amidate or the product. Ethanolysis proceeds rapidly with 5 M HCl even at 25 °C, but heating is required at 1 M or lower concentrations of H_2SO_4 or HCl. Acid-catalyzed methanolysis of **3a** and **3b** is much faster than ethanolysis using either H_2SO_4 or HCl, and the yields of the methyl ester analogue are much higher than those of the ethyl ester. Acid-catalyzed ethanolysis of **3a** or **3b** with 5 M HCl gives very poor stereospecificity (parts C and D of Figure 4). This observation is consistent with a recent report (Harger, 1979) that in HCl-catalyzed solvolysis of phosphoroamidates there is competitive participation of chloride ion as a nucleophile, leading to double inversion and therefore retention of configuration at the phosphorus atom (Figure 5). This is not a problem in the H_2SO_4-catalyzed solvolysis. Preliminary studies indicated that *p*-toluenesulfonic acid offered no advantage over H_2SO_4 and trifluoroacetic acid did not catalyze the ethanolysis.

Significant amounts of phosphorothioate **4** appear as a byproduct on ethanolysis of **3a** or **3b**, particularly with HCl. This phosphorothioic acid is presumably formed either by dealkylation of the ester generated (Hall and Inch, 1979b) or by deamination of the starting material, in each case catalyzed by the liberated amine hydrochloride (Figure 6). Theoretically, acid **4** can be esterified by excess ethanol in the acidic reaction mixture, forming racemic profenofos. However, this does not occur as no profenofos is generated on holding **4** in 1 M H_2SO_4 in ethanol for 24 h at 70 °C; in addition, no racemic profenofos is formed on ethanolysis of **3a** or **3b** with H_2SO_4. These observations also rule out the possibility of racemization due to exchange reactions between the profenofos enantiomer and excess ethanol.

Other Resolution Procedures Examined. The classical approach shown in Figure 7 was unsuccessful due to an inability to obtain crystalline salt mixtures with chiral

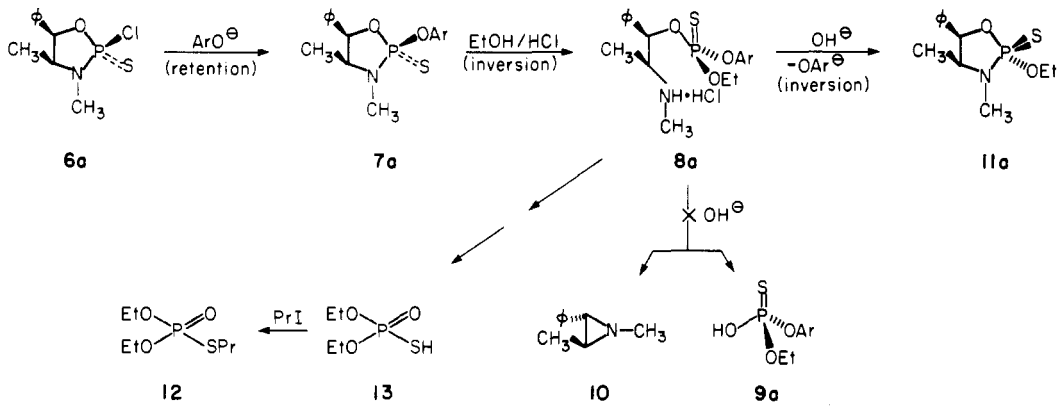


Figure 8. Cyclic phosphorothionoamidate approach to resolution of chiral isomers of profenofos. Ar = 4-bromo-2-chlorophenyl.

Table II. Biological Activity of the Chiral Isomers of Profenofos

assay	isomer (+)	isomer (−)
Esterase Inhibition in Vitro, I_{50}, M × 10^{-7}		
acetylcholinesterase		
bovine erythrocyte	6.2	140
housefly head	1.6	3.1
butyrylcholinesterase		
horse serum	0.13	6.3
pyrethroid esterase		
mouse liver	0.019	0.095
Toxicity to Mice and Insects, LD_{50}		
mice, mg/kg	~1000	44
housefly adults, μg/g	23	6
cabbage looper larvae, μg/g	56	13
mosquito larvae, ppb	270	22
Synergized Mouse Toxicity at 7.5 mg/kg, Percent Mortality		
malathion at 10 mg/kg	75	50
(1*R*)-*trans*-ethanomethrin at 20 mg/kg	36	45

amines such as (−)- and (+)-α-phenylethylamine, brucine, and quinine.

The cyclic phosphorothionoamidate approach (Figure 8; illustrated with only one of the isomers, **6a**) was based on the method of Cooper et al. (1977). Aryl ester **7a** with anhydrous HCl in ethanol undergoes P–N bond cleavage exclusively to form **8a** with inversion of configuration at phosphorus. However, addition of NaOH solution, which is essential for nucleophilic attack by nitrogen at the benzylic carbon, does not generate the expected phosphorothioate **9a** and the *trans*-aziridine **10**. Although no profenofos was detected (TLC; ^{31}P NMR) after the basic reaction mixture of **8a** was treated with propyl iodide, three other products were isolated by chromatography and identified as 4-bromo-2-chlorophenol, oxazaphospholidinethione **11a** (formed via **8a**), and phosphorothioate **12**. The major reaction of **7a** to give 4-bromo-2-chlorophenol and **11a** [$(R)_P$ stereochemical assignment based on comparison with the standard from synthesis; see also Cooper et al. (1977)] involves nucleophilic attack of nitrogen at the phosphorus leading to inversion (Figure 8). Formation of **11a** and the phenol is favored over **9a** plus **10**, perhaps because the former reaction involves an intramolecular cyclization to form a stable five-membered ring and 4-bromo-2-chlorophenoxide is a good leaving group under the required basic conditions. *S*-Propyl ester **12** may be formed on alkylation of phosphorothioic acid **13** by propyl iodide.

Absolute Configuration of (+)- and (−)-Profenofos. Earlier observations on more than 40 chiral compounds with known configurations at phosphorus led to the following generalization: when $Eu(hfc)_3$ significantly alters the chemical shift of signals associated with protons of the largest group according to the sequence rule, then the enantiomer with the lower field signals has the *R* configuration and the one at the higher field the *S* configuration (Cooper et al., 1977; Hall and Inch, 1979a). Careful examination of the 250-MHz ^1H NMR spectra of various mixtures of **3a** and **3b** with $Eu(hfc)_3$ revealed that the $-SCH_2CH_2CH_3$ protons resonate at a lower field with **3a** than with **3b**. These relationships suggest a tentative assignment of **3a** as *R* and of **3b** as *S* (Figure 1). (−)-Profenofos is derived from **3a** and (+)-profenofos from **3b** (Table I), in each case with inversion (Figure 1). Accordingly, (−)-profenofos should be *R* and (+)-profenofos should be *S* (Figure 1). The Hall and Inch generalization is once again in support of this assignment; i.e., the $-SCH_2CH_2CH_3$ protons resonate at lower field with (−)-profenofos than with (+)-profenofos (Figure 4). Although all findings are consistent with the assignments of $(R)_P$-(−)-profenofos and $(S)_P$-(+)-profenofos, these conclusions must be considered as tentative since they are based only on NMR evidence.

Biological Activity of (+)- and (−)-Profenofos. The (+) isomer is most potent as an in vitro esterase inhibitor and the (−) isomer as a toxicant with four examples in each case (Table II). The differential potency with the esterases varies from 2-fold (housefly head acetylcholinesterase) to 48-fold (horse serum butyrylcholinesterase), in the latter case supporting the enantiomeric purity of the resolved compounds. The least sensitive enzyme as assayed is acetylcholinesterase, and the most sensitive is the pyrethroid esterase. Toxicity studies establish a 23-fold potency difference with mice and a 4–12-fold potency difference with the insects. Synergism investigations with mice indicate that the (+) isomer is more potent with malathion but little if any isomer difference exists with (1*R*)-*trans*-ethanomethrin.

The biological activity of (+)-profenofos may be related to its potency as a direct-acting esterase inhibitor. The higher toxicity of (−)-profenofos despite its lower potency as an in vitro esterase inhibitor suggests that this chiral isomer may undergo metabolic activation (e.g., by sulfoxidation; Segall and Casida, 1981).

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Fate of Trichlorfon in Buffer and Soluble Fraction (105000*g*) from Cow and Chicken Liver Homogenates

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The fate of trichlorfon in pH 7.4 buffer and the soluble fractions from cow and chicken liver homogenates prepared in the same buffer was studied. In pH 7.4 buffer, trichlorfon was readily converted into dichlorvos. With the enzyme systems, the insecticide was metabolized into both dichlorvos and desmethyltrichlorfon. The rate of degradation of trichlorfon in enzyme systems was greater than that in buffer. Cow liver soluble fraction metabolized trichlorfon at a slightly higher rate than chicken liver preparation. A modified analytical technique for the detection of important metabolites is also described.

Trichlorfon, (2,2,2-trichloro-1-hydroxyethyl)phosphonic acid dimethyl ester, is registered under different trade names, Neguvon, Dipterex, Dylox, etc., as an insecticide for the control of both endo- and ectoparasites of domestic animals.

It is well documented that trichlorfon rearranges via dehydrochlorination in slightly acidic, neutral, or alkaline media to yield a highly potent insecticide, dichlorvos (DDVP) (Barthel et al., 1955; Lorenz et al., 1955; Mattson et al., 1955). The rate of dehydrochlorination is pH dependent—i.e., the higher the pH, the greater the rate of formation of dichlorvos (Miyamoto, 1959; Metcalf et al., 1959; Aksnes and Yuksekisik, 1974).

Arthur and Casida (1957) and Hassan et al. (1965) suggested that trichlorfon is a direct inhibitor of acetylcholinesterase, while Metcalf et al. (1959) and Miyamoto (1959) held the view that the compound is itself a poor inhibitor and owes its insecticidal properties to the formation of the strong inhibitor dichlorvos. Miyamoto (1959) also concluded that the formation of dichlorvos in different biological systems was not necessarily the result of dehydrochlorinating enzyme(s) but could occur spontaneously under normal physiological conditions.

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The present studies were undertaken to obtain information on the in vitro metabolism of trichlorfon in poultry and farm animals. This report records the data on the effects of buffer and chicken and cow liver soluble enzymes on the degradation of trichlorfon. The paper also provides a modified method for the detection of important trichlorfon metabolites in biological samples.

EXPERIMENTAL SECTION

Materials. Glass-distilled pesticide-grade solvents were used as received. Trichlorfon (I) and dichlorvos (II) were prepared by following published procedures (Barthel et al., 1955; Lorenz et al., 1955). *O*-Desmethyltrichlorfon (III) and desmethyldichlorvos (IV) were obtained by refluxing the parent compound with equimolar amounts of sodium iodide in methyl ethyl ketone (Schneider and Fischer, 1977). Diazomethane was prepared by treating *N*-nitroso-*N*-methylurea with cold aqueous 50% potassium hydroxide (Schultz et al., 1971). Silylating agents Tri-Sil and Tri-Sil Concentrate were purchased from Chromatographic Specialties, Ltd., Brockville, Ontario, Canada.

Enzyme Preparation. The soluble fraction (105000*g*) from livers of chicken and cow were prepared in ice-cold 0.134 M phosphate buffer (pH 7.4) in a glass–Teflon homogenizer as described earlier (Akhtar and Foster, 1977).

Rate of Degradation. The rate of degradation studies for trichlorfon were carried out at 37.5 °C for specified

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