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Synthesis of Fenthion Sulfoxide and Fenoxon Sulfoxide Enantiomers: Effect of Sulfur Chirality on Acetylcholinesterase Activity

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Earlier reports have demonstrated that recombinant flavin-containing monooxygenase 1 (FMO1) catalyzes the oxidation of the organophosphate pesticide fenthion to (+)-fenthion sulfoxide in a stereoselective fashion. In order to elucidate the absolute configuration of the sulfoxide metabolite produced, we established an efficient synthesis of both enantiomers of fenthion sulfoxide, which were transformed into chiral fenoxon sulfoxides using a two-step protocol. The use of chiral oxidants, namely, *N*-(phenylsulfonyl)(3,3-dichlorocamphoryl) oxaziridines, afforded enantioenriched fenthion sulfoxides with high ee (>82%) from the parent sulfide. Single recrystallizations afforded chiral fenthion sulfoxides with >99% ee, measured by chiral HPLC analysis. The absolute configuration of the (+)-sulfoxide generated from fenthion metabolism by FMO1 was determined to be (*R*)-(+)-fenthion sulfoxide, confirmed by X-ray crystallographic analysis of the (*S*)-(−)-antipode. Inhibition of human recombinant (hrAChE) and electric eel (eeAChE) acetylcholinesterase were assayed with fenthion, fenoxon, and the racemates and enantiomers of fenthion sulfoxide and fenoxon sulfoxide. Results revealed stereoselective inhibition with (*R*)-(+)-fenoxon sulfoxide when compared with that of (*S*)-(−)-fenoxon sulfoxide (IC_{50} of 6.9 and 6.5 μM vs 230 and 111 μM in hrAChE and eeAChE, respectively). Fenthion sulfoxide (R or S enantiomers) did not present anti-AChE properties. Although the stereoselective sulfoxidation of fenthion to (*R*)-(+)-fenthion sulfoxide by FMO represents a detoxification pathway, the results of this study support the notion that subsequent oxidative desulfuration of (*R*)-(+)-fenthion sulfoxide (*in vivo*) may represent a critical bioactivation pathway, resulting in the production of (*R*)-(+)-fenoxon sulfoxide, a potent AChE inhibitor.

Introduction

Organophosphate (OP) insecticides are a structurally diverse class of compounds that have virtually replaced the environmentally persistent organochlorine pesticides and represent the largest group of insecticides marketed worldwide (*1*, *2*). OP insecticides exert their principal biological effect by phosphorylation of the enzyme acetylcholinesterase (AChE), resulting in subsequent accumulation of acetylcholine and continuous stimulation of the nervous system (*3*). Fenthion (*O*,*O*-dimethyl-*O*-[4-(methylthio)-*m*-tolyl] phosphorothioate) (**1**) is a broad-spectrum insecticide with contact, stomach, and respiratory actions used to control insect and bird pests (*4*, *5*) and is classified by the U.S. Environmental Protection Agency as a restricted use pesticide because of its toxic effects in birds, reptiles, and fish. Upon uptake by organisms, fenthion undergoes oxidative metabolism to primary and secondary metabolites, with either enhanced or reduced potency to AChE, under the mediation of cytochrome P450 (P450) and flavin-containing monooxygenases (FMO). Two major pathways include oxidative desulfuration of the phosphorothioate and sulfoxidation of the thioether group (*6*−*9*). *In vitro* and *in vivo* studies demonstrated that fenthion is biotransformed to fenthion sulfoxide (**2**) and fenoxon (**3**) in liver microsomes of fish and rats (*7*−*9*). Residue analyses in animals and plants indicate the formation of several principal metabolites and include fenthion sulfoxide (**2**), fenoxon (**3**), fenoxon sulfoxide (**4**), and the corresponding sulfones (*10*−*12*). Fenthion also experiences nonenzymatic transformation including photodegradation to the sulfoxide, a relatively stable oxidation product in the environment (*13*). Fenoxon sulfoxide is also susceptible to nonenzymatic hydrolysis, representing a key detoxification mechanism (*14*).

Because of the formation of an asymmetric sulfur center, enzyme-catalyzed sulfoxidation can produce an enantiomeric enrichment of fenthion sulfoxide or fenoxon sulfoxide (*15*). Stereoselective oxidations by P450 and FMO have long been recognized in other organophosphates (*16*). Similar to other organophosphates, fenthion was predominantly converted into (+)-fenthion sulfoxide when incubated with human tissue microsomes (*6*). However, information regarding the biological activity and/or toxicity of the enantiomers as well as other metabolites of fenthion is scarce. Furnes and Schlenk reported that (+)- and (−)-fenthion sulfoxides had similar potency toward AChE inhibition, which was less than the potency of fenthion, indicating detoxification (*6*). In contrast, the LD_{50} of fenthion sulfoxide (125 mg/kg) is nearly half that of fenthion (220 mg/kg) (*17*). Therefore, the objective of this study was to evaluate

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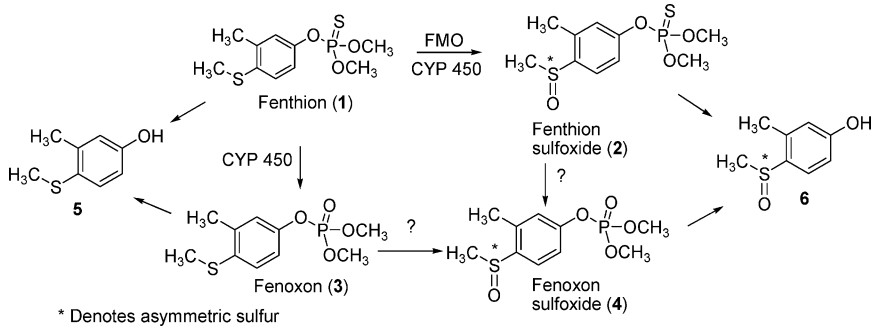
Scheme 1. Metabolism of Fenthion



the relative anti-AChE potency of fenthion, fenoxon, fenthion sulfoxide, and fenoxon sulfoxide (the latter two as racemates and enantiomers). The results obtained from our study support the contributions of stereoselective FMO-catalyzed sulfoxidation, antecedent to a bioactivation pathway that may play a significant role in the toxicity of thioether organophosphates.

Experimental Procedures

Fenthion (98%), fenoxon (100 μg/mL in isooctane), and *rac*-fenthion sulfoxide (97%) were purchased from Chem Service (West Chester, PA). All other chemicals and reagents for the synthesis of compounds used in this study were purchased from Sigma-Aldrich (St. Louis, MO). Thin-layer chromatography was performed on Merck silica gel 60 F_{254} plates. Column chromatography was performed using standard grade Sorbent Technologies silica gel with a particle size of 32−63 μm. Mass spectral data were acquired using a Waters ZQ LCMS system (ESI+ mode). Elemental analysis was performed using a Perkin-Elmer Series II 2400 CHNS/O Elemental Analyzer. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker Avance DXR 400, using $CDCl_3$ as the solvent. Optical rotations were measured using a Rudolph Autopol IV polarimeter and were recorded at 25 °C. HPLC analysis was performed using a Waters DeltaPrep 4000 system (analytical mode) equipped with a Rheodyne 7725I injector and a Waters 2487 Dual Wavelength Absorbance Detector. Enantiomeric purity was established by HPLC analysis, using a Regis (*R, R*)-Whelk O1 chiral stationary phase HPLC column eluting with hexane/isopropanol/dichloromethane (60:30:10) as the solvent system at a flow rate of 1.0 mL/min at λ = 240 nm.

Synthesis of Camphorylsulfonyl Oxaziridines. Enantiomerically pure (3′*S*,2*R*)-(−)-*N*-(phenylsulfonyl) (3,3-dichlorocamphoryl) oxaziridine (**(−)-7**) and (3′*R*,2*S*)-(+)-*N*-(phenylsulfonyl) (3,3-dichlorocamphoryl) oxaziridine (**(+)-7**)) were prepared in four steps from (1*R*)-(+)-camphor and (1*S*)-(−)-camphor, respectively, following the reported literature procedure (*18*).

General Procedure for the Oxidation of Sulfides to Sulfoxides (18). In a round-bottom flask equipped with a magnetic stirring bar and argon inlet was placed 1 equiv of oxaziridine (**7**) in CCl_4, followed by the addition of 1.1 equiv of fenthion (**1**) in CCl_4. The contents were stirred at room temperature, and progress of the reaction was monitored by TLC (80% CH_2Cl_2/*n*-pentane followed by 100% ethyl acetate). The appearance of corresponding camphorimine (reduced oxaziridine) indicated the progress of the reaction. After the reaction was complete (4 h), the solvent was evaporated, and the sulfoxide was isolated by subjecting the residue to flash column chromatography (silica gel G, standard grade) and eluting with 100% ethyl acetate.

(*S*)-(−)-Fenthion Sulfoxide ((*S*)-(−)-2). Fenthion (**1**; 0.088 mmol; 24.4 mg) in 2 mL of CCl_4 was reacted with **(−)-7** (0.079 mmol; 30 mg) in 2 mL of CCl_4. The workup and purification following the general procedure described above yielded **(*S*)-(−)-2** (21 mg, 81% yield, 82% ee). Slow recrystallization of this sample from a CH_2Cl_2/*n*-pentane solution yielded **(*S*)-(−)-2** (99.5% ee as determined by chiral HPLC analysis). Mp 61−62 °C; LCMS (positive ion mode, cone voltage: 36): *m*/*z* $[MNa]^+$ 317 ($C_{10}H_{15}O_4PS_2Na$); $[\alpha]^{21}_D$: −123 (*c* = 1.0, $CHCl_3$). ^{1}H NMR 500 MHz ($CDCl_3$) δ 2.39 (s, 3H, C*H*$_3$-Ar), 2.71(s, 3H, C*H*$_3$-SO), 3.90 (d, 6H, 2 × O−C*H*$_3$), 7.05 (s, 1H, Ar-*H*), 7.27 (t, 1H, Ar-*H*, *J* = 8.5 Hz), 7.96 (d, 1H, Ar-*H*, *J* = 8.5 Hz); ^{13}C NMR 125 MHz ($CDCl_3$) δ 18.52, 42.58, 55.51, 55.56, 119.86, 119.89, 122.80, 122.84, 124.97, 135.93, 140.60, 152.19, 152.24. Anal. Calcd for $C_{10}H_{15}O_4PS_2$: C, 40.81; H, 5.14. Found: C, 40.55; H, 4.65. HPLC analysis (see Experimental Procedures): t_r = 11.4 min. X-ray analysis (Figure 1) established absolute configuration as (*S*)-(−)-fenthion sulfoxide (**(*S*)-(−)-2**).

(*R*)-(+)-Fenthion Sulfoxide ((*R*)-(+)-2). 20 mg; 79% yield; 82% ee. Slow recrystallization of this sample from a CH_2Cl_2/*n*-pentane solution yielded **(*R*)-(+)-2** (99.5% ee as determined by chiral HPLC analysis). Mp 61−62 °C; LCMS (positive ion mode, cone voltage: 36): *m*/*z* $[MNa]^+$ 317 ($C_{10}H_{15}O_4PS_2Na$); $[\alpha]^{21}_D$: +123 (c 1.0, $CHCl_3$). Anal. Calcd for $C_{10}H_{15}O_4PS_2$: C, 40.81; H, 5.14. Found: C, 40.58; H, 4.91. Optical purities of **(*R*)-(+)-(2)** was determined using a Regis (*R, R*)-Whelk O1 HPLC column eluting with hexane/isopropanol/dichloromethane (60:30:10) as the solvent system at a flow rate of 1.0 mL/min at λ = 240 nm. t_r = 9.75 min.

Synthesis of 3-Methyl-4-methylsulphinylphenol (6) from Fenthion Sulfoxide (2) by Base Hydrolysis. General Procedure (14). Each enantiomer of fenthion sulfoxide (**2**) was added to a 1 N NaOH/ethanol solution (ethanol:H_2O, 1:1), and the mixture was stirred for 8 h at 50−60 °C. After complete consumption of the starting material, the solvent was evaporated, and an aqueous solution of 2.5 N HCl was added to neutralize the mixture. The mixture was diluted with saturated aqueous NaCl and extracted with ethyl acetate. The organic layer was washed with water, dried over sodium sulfate, and solvent evaporated to afford 3-methyl-4-methylsulphinylphenol (**6**) as a white solid. Crystallization of the solid was effected by ethyl acetate and hexane.

(*S*)-(−)-3-Methyl-4-methylsulphinylphenol ((*S*)-(−)-6). Yield: 36 mg (95%); Mp: 129−130 °C. LCMS (positive ion mode, cone voltage: 36): *m*/*z* 193 ($C_8H_{10}O_2S_1Na$); $[\alpha]^{21}_D$: −215 (c 1.0, $CHCl_3$). ^{1}H NMR 500 MHz ($CDCl_3$) δ 2.33 (s, 3H, C*H*$_3$-Ar), 2.75 (s, 3H, C*H*$_3$-S-), 6.71 (s, 1H, Ar-*H*), 6.93 (d, 1H, Ar-*H*, *J* = 7.5 Hz), 7.74 (d, 1H, Ar-*H*, *J* = 8.5 Hz). ^{13}C NMR 125 MHz ($CDCl_3$) δ 18.62, 42.98, 115.03, 117.79, 125.24, 131.88, 136.76, 159.75.

(*R*)-(+)-3-Methyl-4-methylsulphinylphenol ((*R*)-(+)-6). Yield: 28 mg (88%); Mp 130−131 °C. LCMS (positive ion mode, cone voltage: 36): 193 ($C_8H_{10}O_2S_1Na$); $[\alpha]^{21}_D$: +212 (c 1.0, $CHCl_3$).

Synthesis of Fenoxon Sulfoxides (4). In a flame-dried small round-bottom flask equipped with a magnetic stirring bar and argon inlet was placed 1 equiv of **6** dissolved in dry acetone (8 mL), anhydrous Na_2CO_3 (200 mg), and 1.2 equiv of dimethyl phosphorochloride. The reaction mixture was refluxed at 60−70 °C for 8 h. After acetone was removed under reduced pressure, water was added to the residue and the product extracted successively with chloroform (3 × 25 mL). The organic layer was washed with water and dried over anhydrous sodium sulfate, and the solvent was evaporated to yield a yellow residue. Purification by silica gel chromatography (mobile phase; methanol/ethyl acetate (8:92)) afforded fenoxon sulfoxide (**4**) as a white solid. Crystals were obtained from a single recrystallization from ethyl acetate/hexane.

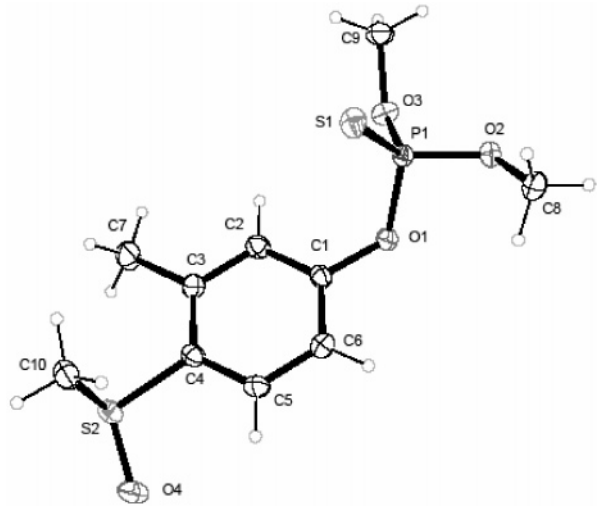


Figure 1. ORTEP view of **(*S*)-(−)-2**, with the atom-numbering scheme with displacement ellipsoids drawn at the 50% probability level.

(*S*)-(−)-Fenoxon sulfoxide ((*S*)-(−)-4). The reaction of **(*S*)-(−)-6** (0.188 mmol; 32 mg) with dimethyl phosphorochloride (0.226 mmol; 32.64 mg; 24.4 μL) yielded 22 mg (44%) of **(*S*)-(−)-4**; mp 82−83 °C. LCMS (positive ion mode, cone voltage: 33): *m*/*z* 301 ($C_{10}H_{15}O_5PSNa$); $[\alpha]^{21}_D$: −106 (c 1.0, $CHCl_3$). ^{1}H NMR 500 MHz ($CDCl_3$) δ 2.34 (s, 3H, C*H*$_3$-Ar), 2.65 (s, 3H, C*H*$_3$-SO), 3.86(d, 6H, 2x O−C*H*$_3$,), 7.07 (s, 1H, Ar−H), 7.25 (t, 1H, Ar−H, J = 8 Hz), 7.91 (d, 1H, Ar−H, J = 8.5 Hz). ^{13}C NMR 125 MHz ($CDCl_3$) δ 18.45, 42.46, 55.26, 55.31, 118.88, 118.92, 121.82, 121.86, 125.11, 136.18, 140.25, 152.12, 152.17. Anal. Calcd for $C_{10}H_{15}O_5PS$: C, 43.16; H, 5.43. Found: C, 42.39; H, 5.35. HPLC analysis: Regis (*R, R*)-Whelk O1 chiral stationary phase HPLC column eluting with a hexane/isopropanol/dichloromethane (60:30:10) mobile phase at a flow rate of 1.5 mL/min at λ = 240 nm. t_r = 18.2 min.

(*R*)-(+)-Fenoxon Sulfoxide ((*R*)-(+)-4). The reaction of **(*R*)-(+)-6** (0.165 mmol; 28 mg) with dimethyl phosphorochloride (0.198 mmol; 29 mg; 21.3 μL) yielded 28 mg (52%) of **(*S*)-(−)-4**. Mp (crystals): 85−86 °C. LCMS (positive ion mode, cone voltage: 33): 301 ($C_{10}H_{15}O_5P_1S_1Na$); $[\alpha]^{21}_D$: +108 (c 1.0, $CHCl_3$). Anal. Calcd for $C_{10}H_{15}O_5PS$: C, 43.16; H, 5.43. Found: C, 42.83; H, 4.91. HPLC analysis: Regis (*R, R*)-Whelk O1 chiral stationary phase HPLC column eluting with a hexane/isopropanol/dichloromethane (60:30:10) mobile phase at a flow rate of 1.5 mL/min at λ = 240 nm. t_r = 16.4 min.

Crystal Structure Determination. The structure of **(*S*)-(−)-2** was determined using data collected at T = 110 K with MoKα radiation on a Nonius KappaCCD diffractometer. Crystal data: $C_{10}H_{15}O_4PS_2$, orthorhombic space group $P2_12_12_1$; a = 5.898(3), b = 10.188(4), c = 22.776(8) Å; V = 1368.6(10) Å^3; Z = 4; R = 0.027 for 2658 observed data of 2765 unique data having θ_{max} = 28.7°. The absolute configuration was determined by refinement of the Flack parameter (*19*) to a value of −0.01(7), versus 1.01(7) for the alternate enantiomer, using 712 Friedel pairs. The crystallographic data are available in CIF format in Supporting Information.

AChE Inhibition Assay. Electric eel AChE (eeAChE, EC 3.1.1.7, Type V-S, Sigma, lyophilized powder) and human AChE (hrAChE, EC 3.1.1.7, recombinant, Sigma, lyophilized powder) were dissolved in distilled deionized water with a concentration of 10 U/mL and stored at −80 °C until use. AChE activities were determined using a method described by Ellman et al. (*20*) and adapted to a microplate assay. The assay included a buffer blank, a solvent blank, and increasing concentrations of each chemical with three replicates. The reaction was carried out in each well of a 96-well microplate with 200 μL of DTNB/0.05 M Tris buffer at pH 7.5 at 25 °C. Initially, 5 μL of 10 U/mL human or eel AChE was preincubated with 5 μL of each of 10 concentrations of tested chemicals dissolved in ethanol for 30 min, followed by addition of 20 μL of acetylthiocholine iodide (20 mM). The remaining AChE activities were measured immediately at a 6 s interval for 2 min at 405 nm wavelength on a Molecular Device microplate reader (Sunnyvale, CA) and analyzed using the software package SOFTmax Pro (Molecular Devices, Sunnyvale, CA). The final concentrations in the 230 μL reaction volume for the chemicals ranged from 1.95 to 1000 μM (except for fenoxon, which was 0.1295 to 49.68 μM), which were doubled from low to high. The half-maximal inhibitory concentration (IC_{50}) was calculated using TOXCALC 5.0 software (Tidepool Scientific Software, McKinleyville, CA) on the basis of likelihood-max probit regression analysis and the method. The dissociation constant for the binding of the inhibitor to the enzyme, *K*i, was calculated using the equation of Cheng and Prusoff (*21*).

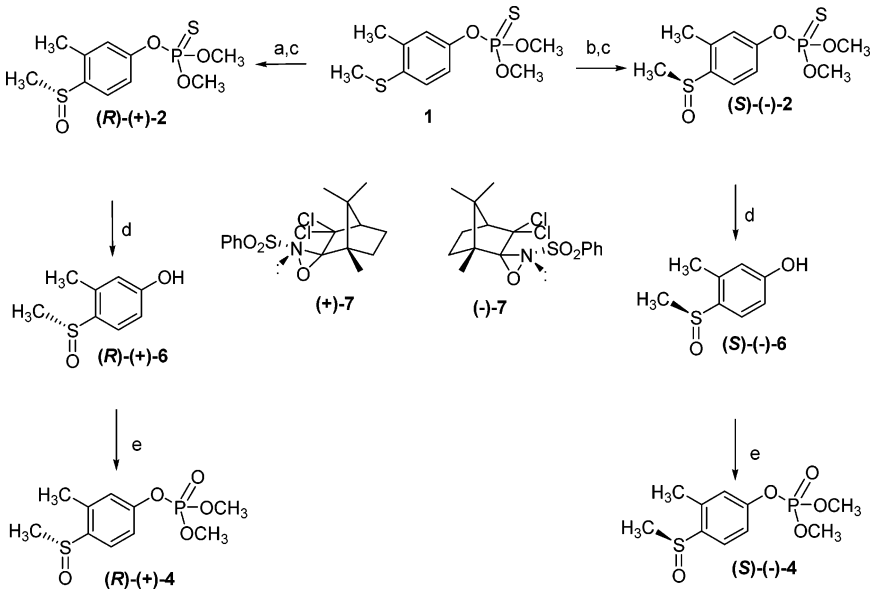
$$Ki = IC_{50}/1 + Km.$$

Results

Chemistry. In order to examine the effects of fenthion sulfoxide and fenoxon sulfoxide configuration on AChE activity, synthetic methods for the construction of enantiopure sulfoxides were first explored. Although a number of reagents have been developed as useful chiral oxidants, enantiopure *N*-sulfonyloxaziridines have surfaced as valuable reagents for the enantioselective oxidation of sulfides to sulfoxides with high and predictable stereoselectivities (*18*). One particular class of chiral oxidants, *N*-(phenylsulfonyl)(3,3-dichlorocamphoryl) oxaziridines (**7**), function as robust and general reagents for the synthesis of sulfoxides derived from sulfides. Because the configuration of the oxaziridine ring controls the stereochemistry of the resulting product, both enantiomers of the sulfoxides could be easily prepared by using either **(+)-** or **(−)-7**. On the basis of the active-site model proposed by Davis, fenthion should serve as an excellent substrate for chiral oxidation by **7**, with high ee's predicted on the basis of the large group side difference (GSD) of the substituents directly bonded to the sulfur atom (aromatic vs methyl). The synthesis of the individual oxaziridine enantiomers **7** was performed using a four-step protocol initially reported by Davis et al. (*18*). Analytical data of the oxaziridine reagents (mp, NMR, MS, and optical rotation) were in complete agreement with literature reported values. Fenthion (**1**) was reacted initially with an equimolar quantity of oxaziridine **(−)-7** in CCl_4 at ambient temperature, and the reaction progress was monitored by both thin-layer chromatography and LC-MS. After the reaction was complete (4 h), the solvent was evaporated and the residue purified by column chromatography. The enantiomeric purity of the sulfoxide produced was determined using a chiral Pirkle HPLC column, which revealed approximately 82% ee. Although the enantiomeric purity was not sufficient to carry forward to biological analysis, a single recrystallization from CH_2Cl_2/*n*-pentane enriched the sample to >99% ee, as measured by HPLC analysis. Suitable crystals of this sample were submitted for single-crystal X-ray analysis, and the absolute configuration of the sulfoxide derived from **(−)-7** oxaziridine was determined to be **(*S*)-(−)-2** (Figure 1).

Similarly, **(+)-7** oxaziridine was used to prepare (*R*)-(+)-fenthion sulfoxide with >99.5% ee, following chromatography and recrystallization. Each fenthion sulfoxide enantiomer was further subjected to a hydrolysis reaction (*14*) for cleavage of the thiophosphate group, leading to the production of phenols **(*R*)-(+)-6** and **(*S*)-(−)-6**. This mild method of hydrolysis reduced the probability of sulfoxide racemization, and optical rotation measurements of the phenol products confirmed the chiral integrity of the sulfoxides produced. It has been established that certain diaryl, alkyl aryl, and dialkyl sulfoxides can racemize but only at a significant rate above 200 °C (*22*).

Scheme 2. Synthesis of Chiral Fenthion Sulfoxides and Fenoxon Sulfoxides[a]



[a] Reagents and conditions: (a) **(+)-7**, CCl_4, RT, 20 h (82%); (b) **(−)-7**, CCl_4 RT, 20 h (81%); (c) recrystallization (>99% ee); (d) 1 N NaOH, EtOH, 8 h, 55 °C (95%); (e) dimethyl phosphorochloride, Na_2CO_3, acetone, 65 °C, (50%).

Table 1. IC_{50} and *Ki* Values of Fenthion and Its Metabolites for Purified Electric Eel and Human Recombinant AChE

	eeAChE		hrAChE		
inhibitor	*Ki* (μM)	IC_{50} ± SD (μM)	*Ki* (μM)	IC_{50} (μM)	rat LD_{50} (mg/kg) (*17*)
Fenthion (**1**)	22.1 ± 3.0	114 ± 14	14.3 ± 2.3	80.7 ± 1.7	220
Fenoxon (**3**)	4.1 ± 0.6	21.0 ± 2.2	1.7 ± 0.3	9.7 ± 1.4	125
(*rac*) fenoxon sulfoxide (**4**)	1.7 ± 0.2	8.7 ± 0.7	4.3 ± 0.7	23.0 ± 0.7	50
(*R*)-(+)-fenoxon sulfoxide (**4**)	1.3 ± 0.2	6.5 ± 0.2	1.2 ± 0.2	6.9 ± 0.6	ND
(*S*)-(−)-fenoxon sulfoxide (**4**)	21.5 ± 3.0	111 ± 3	40.7 ± 6.4	230 ± 16	ND
(*rac*) fenthion sulfoxide (**2**)		>1000		>1000	125
(*R*)-(+)-fenthion sulfoxide (**2**)		>1000		>1000	ND
(*S*)-(−)-fenthion sulfoxide (**2**)		>1000		>1000	ND

[a] The values represent the mean ± SD; ND = not determined.

The phenols were reacted with dimethylphosphorochloride under mildly basic conditions to afford fenoxon sulfoxides **(*R*)-(+)-4** and **(*S*)-(−)-4**. Optical rotation measurements in conjunction with chiral HPLC analysis confirmed enantiomeric purity of >99.5% ee.

Inhibition of AChE Activities. The *Ki* and IC_{50} values for the inhibition of purified eel AChE by fenthion, fenoxon, its metabolic racemates, and enantiomers are shown in Table 1. The results demonstrated a strong enantioselectivity of fenoxon sulfoxide to AChE, where (*R*)-(+)-fenoxon sulfoxide was superior to (*S*)-(−)-fenoxon sulfoxide in inhibitory potency in both AChEs tested (IC_{50} values of 6.9 and 6.5 μM vs 230 and 111 μM in hrAChE and eeAChE, respectively). Compared to fenthion, (*R*)-(+)-fenoxon sulfoxide was about 12- and 14-fold more potent an anti-AChE inhibitor in hrAChE and eeAChE, respectively. Inhibitory potency of (*R*)-(+)-fenoxon sulfoxide was 3-fold greater than that of fenoxon in eeAChE, and it was very similar to that of hrAChE. Fenthion sulfoxide did not present anti-AChE effects, irrespective of stereochemistry.

Discussion

Previous results from our laboratory have documented the FMO1-catalzyed oxidation of fenthion, leading to the production of an enriched sulfoxide, characterized as the (+)-sulfoxide enantiomer (*6*). The X-ray analysis of one of the synthetic fenthion sulfoxide enantiomers ((*S*)-(−)-fenthion sulfoxide) allowed for the absolute configuration to be determined and compared to the (+)-metabolite produced from the FMO1 catalyzed reaction, now unambiguously characterized as (*R*)-(+)-fenthion sulfoxide. The synthetic conversion of the chiral fenthion sulfoxides to the chiral fenoxon sulfoxides provided a unique opportunity to evaluate the contribution of sulfoxide stereochemistry to biological activity, particularly against AChE. The conversion of fenthion to the sulfoxide metabolite does reduce the IC_{50} value relative to those of fenthion and fenoxon and is consistent with the observed toxicity in mammals. However, the contribution of sulfur stereochemistry to AChE inhibition was found to be minimal because no difference was observed between the fenthion sulfoxide isomers when tested independently against AChE. Similar results were observed previously when (+)- and (−)-fenthion sulfoxide enantiomers were examined (*6*).

Typically, oxon forms of phosphothionates are the most potent AChE inhibitors of organophosphate metabolites (*23*). However, in contrast to previous toxicity studies in rats, which showed the oxon and sulfoxide of fenthion to be equipotent (*17*), fenthion sulfoxide had no anti-AChE effect when tested *in vitro* with hrAChE and eeAChE. Similar results were recently observed with another thioether-containing organophosphate, disulfoton, where detoxification was observed following sulfoxide formation (*30*). Unfortunately, the investigators did not examine the potential oxon-sulfoxides to see whether a secondary oxidation (i.e., oxon formation) had any affect on AchE. Thus, fenthion sulfoxide may undergo further *in vivo* transformation to more toxic metabolites.

Although sulfoxidation has been hypothesized to be the initial step in the activation of fenthion (*16*), it appears that additional oxidative desulfuration by P450 significantly enhances the toxicity of fenthion. Consistent with rat studies, fenoxon

sulfoxide was significantly more toxic to rats than either fenoxon, fenthion sulfoxide, or fenthion (*16*). The IC_{50} of the racemic mixture of (*R*,*S*)-fenoxon sulfoxide for AChE inhibition was approximately 13- and 4-fold lower than that of fenthion in eeAChE and hrAChE, respectively. The enhanced potency of the fenoxon sulfoxide supports the results of Kim et al. (*24*), who demonstrated that the inhibition of AChE was significantly enhanced by oxidizing fenoxon to fenoxon sulfoxide, although no enantiomers were identified. Similar results were observed with phorate, where sulfoxidation only enhanced the IC_{50} of acetylcholinesterase 2-fold, but sulfoxidation of the phorate oxon enhanced potency more than 3000-fold (*25*). Unfortunately, the absolute stereochemistry of the enantiomers was likewise not examined in that study. Results from the current study, however, suggest that the inhibition of AChE by fenoxon sulfoxide compounds is stereoselective, with (*R*)-(+)-fenoxon sulfoxide being primarily responsible for inhibition.

The stereoselective inhibition of AChE has been observed in numerous organophosphate insecticides (*16*). However, most of these studies have focused on enantiomers resulting from a chiral phosphorus (*26*). Few studies have examined the sulfoxidation of thioether moieties, which tend to enhance the potency of organophosphates by providing a partial positive charge on the sulfur, allowing interaction with the anionic docking site of AChE. Stereoselective inhibition of AChE was also observed with enantiomers of the oxygenated carbamate, methiocarb sulfoxide with one enantiomer (uncharacterized optical rotation or absolute stereochemistry) being nearly 10-fold more potent than the other (*27*). In studies carried out in the German cockroach, the (*R*)-(+)-enantiomer of propaphos sulfoxide had more insecticidal activity than the (*S*)-(−)-enantiomer (*28*). These results mirror the results with fenoxon sulfoxide because propaphos already has an oxygenated phosphorus (equivalent to oxon).

FMO1 stereoselectively catalyzes the sulfoxidation of fenthion to (+)-fenthion sulfoxide (*6*), now established by this study as (*R*)-(+)-fenthion sulfoxide. Biotransformation of fenthion had been previously examined in two fish species, rats, and two cattle species using either *in vitro* or *in vivo* approaches (*7*, *8*, *29*). Fenthion sulfoxide appears to be the predominant initial metabolite, followed by a small percentage of fenoxon, fenthion sulfone, and fenoxon sulfoxide. These data indicate that the sulfoxide undergoes sequential oxidative desulfuration to the oxon sulfoxide. Preliminary studies in our laboratory have indicated the minimal formation of fenthion oxon sulfoxide following incubations of fenoxon with purified FMO1 (data not shown). Further studies are necessary to determine whether P450 catalyzes the oxidation of fenthion sulfoxide to fenoxon sulfoxide and whether this pathway is stereoselective.

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Supporting Information Available: Crystallographic information file (CIF) of (*S*)-(−)-fenthion sulfoxide. This material is available free of charge via the Internet at http://pubs.acs.org.

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