H-4,5,6,7); MS, m/z (relative intensity) 425 ($M^{\bullet+}$, 56), 259 (87), 199 (35), 168 (52), 167 (37), 166 (21), 157 (71), 139 (95), 97 (100).

3-α-D-Arabinofuranosylbenzothiazole-2-thione (15). After the general deblocking procedure was performed on 0.3 g (0.71 mmol) of **13**, the crude material obtained was precipitated from methanol–water to give 0.17 g (80%) of pure **15**: mp 130 °C; UV (MeOH) λ_{max} 325 nm (20.3), 241 (12.0), 227 (12.9); ^{1}H NMR (Me_2SO-d_6) δ 3.3–4.5 (m, 5 H, H-2′,3′,4′,5′,5″), 5.2 (br s, 3 H, OH-2′,3′,5′), 6.30 (d, 1 H, H-1′, $J_{1',2'}$ = 10 Hz), 7.5–8.1 (m, 4 H, H-4,5,6,7); MS, m/z (relative intensity) 299 ($M^{\bullet+}$, 20) 196 (16), 168 (82), 167 (100), 166 (20), 151 (31), 149 (36), 148 (29), 135 (25), 133 (25), 132 (20), 115 (16), 97 (20).

BIOLOGICAL METHODS

Fusarium strains, growth media, and experimental protocols were the same as previously reported (Bounaga, 1980).

The test compounds were first dissolved in ethanol and then added to the growth medium in such a way as to get final concentrations of 0.5, 1.0, and 1.5 mM.

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Phosphorylating Intermediates in the Peracid Oxidation of Phosphorothionates, Phosphorothiolates, and Phosphorodithioates

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Oxidation of *O,O*-diethyl phenyl phosphorothionate, phosphorothiolate, and phosphorodithioate with excess *m*-chloroperoxybenzoic acid in aprotic solvents yields primarily diethyl phenyl phosphate, diethylphosphoryl benzenesulfonate and diethylphosphoryl benzenethiosulfonate, respectively, but in methanol the major product in each case is diethyl methyl phosphate. The intermediate phosphorylating agents react more readily with methanol and *n*-propyl alcohol than with isopropyl or *tert*-butyl alcohol. ^{31}P NMR spectra for reactions run at −50 to −20 °C show minor signals 27–34 ppm upfield from the starting materials appropriate for the transient three-membered ring phosphoxathiiranes from the phosphorothionate and phosphorodithioate and the phosphorylsulfenate (formed via the phosphorothiolate *S*-oxide) from the phosphorothiolate. The phosphoxathiiranes and the phosphorothiolate *S*-oxide or their rearrangement products formed on biooxidation of related thiophosphorus toxicants are candidate phosphorylating agents, possibly contributing to their activity as acetylcholinesterase inhibitors and to their detoxification.

Bioactivation of thiophosphorus insecticides to potent phosphorylating agents for acetylcholinesterase (AChE) is proposed to involve cytochrome P450 mediated oxidation of phosphorothionates and phosphorodithioates to the corresponding phosphates and phosphorothiolates (Eto, 1974; Neal, 1980) and of some phosphorothiolates to their *S*-oxide derivatives (Wing et al., 1983, 1984). These bioxidation reactions also cleave phosphorus–sulfur bonds to form a variety of detoxification products (Eto, 1974). Peracid oxidation may be a biomimetic model for these activations of phosphorothionates (Bellet and Casida, 1974;

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Field et al., 1983), phosphorothiolates (Segall and Casida, 1981, 1982), and phosphorodithioates (McBain et al., 1971b; Miyamoto and Yamamoto, 1977). Direct spectroscopic observation of activated intermediates formed on S-oxidation are reported for a phosphorothionate (Field et al., 1983) and a phosphorothiolate (Thompson et al., 1984). The activation products of phosphorothiolates on oxidation with peracids react quickly with aliphatic alcohols and may be one of the most potent types of phosphorylating agents known (Segall and Casida, 1983a).

This study uses ^{31}P NMR to examine the oxidation of *O,O*-diethyl *O*-phenyl phosphorothionate (1) and the corresponding *S*-phenyl phosphorothiolate (2) and phosphorodithioate (3) with a peracid and an oxaziridine in aprotic solvents to seek activation products and in various alcohols to examine possible phosphorylation reactions.

MATERIALS AND METHODS

Spectroscopy. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded with the Bruker WM-300 spectrometer at 300.13 MHz (^{1}H), 75.47 MHz (^{13}C, with broad-band decoupling), or 121.50 MHz (^{31}P, with broad-band decoupling) for solutions in acetone-d_6, benzene-d_6, and chloroform-*d* or as specified. ^{1}H and ^{13}C chemical shifts are related to internal tetramethylsilane and ^{31}P chemical shifts to internal trimethyl phosphate. Phosphorus signals are negative if upfield relative to the reference. Chemical ionization mass spectrometry (CI-MS) used methane in the Hewlett-Packard 5985 system operated at 230 eV.

Chromatography. Column chromatography utilized Florisil (80–100 mesh) and sequential developments with hexane, methylene chloride, and acetonitrile or mixtures thereof. Thin-layer chromatography (TLC) involved silica gel 60 F_{254} chromatoplates with 0.25-mm gel thickness for analysis and 1.0-mm gel thickness for preparative isolations and product detection with ultraviolet light or iodine vapor. Phosphorus compounds were visualized by spraying with 4-(*p*-nitrobenzyl)pyridine (1% w/v in acetone) and then heating at 110 °C for 15 min followed by a spray of tetraethylenepentamine (1% w/v in acetone) (Bellet and Casida, 1974).

Organophosphorus Compounds. Structures of the compounds considered are given in Table I along with their ^{31}P NMR chemical shifts. Complete ^{1}H and ^{13}C NMR and CI-MS data for the compounds are given in the supplementary material.

Thiophosphorus compounds 1–3 and phosphates 4, 10–13, *O,O*-dimethyl *O*-ethyl phosphate (δ −1.16) and *O,O*-dimethyl *O*-phenyl phosphate (δ −6.27) were prepared according to the general procedures of Schrader (1963) and purified by column chromatography. Compounds 5 and 22 were obtained by treating phenol or methanol, respectively, with (*O,O*-diethylphosphoryl)sulfenyl chloride (from reaction of triethyl phosphorothionate with sulfuryl chloride; not isolated) according to Borecka et al. (1974). The crude materials were ~90% pure, but their instability precluded chromatography. Compound 7 was prepared according to Schrader and Lorenz (1959) from benzenesulfenyl chloride and equimolar phosphorothioic acid 19 with 1.1 equiv of triethylamine in benzene at 0 °C followed by column chromatography; it was also obtained from thiophenol and (*O,O*-diethylphosphoryl)sulfenyl chloride (as above) by the same procedure. Phosphoryl sulfonate 6 was prepared in ~90% purity (used for spectroscopy) by treating 2 with 4 equiv of MCPBA in dry ethanol-free chloroform at 0 °C (Segall and Casida, 1982), cooling to −20 °C, and filtration to remove most (~70%) of the *m*-chlorobenzoic acid (MCBA), followed by rapid extraction with cold (<5 °C) 10% aqueous solutions of $NaHSO_3$, $NaHCO_3$, and then water, finally drying the chloroform solution over $CaCl_2$, and evaporating the solvent. Compounds 8 and 9 were obtained as MCPBA oxidation products but could not be isolated. Sulfinate 8 was the transient intermediate (up to 80% of the phosphorus compounds), and sulfonate 9 was the final product (~90%) on treating 7 with excess MCPBA in acetone.

Pyrophosphate 14 from Chem Service (Westchester, PA) was purified by column chromatography. Thiopyrophosphate 15 was synthesized according to Michalski et al. (1974). Unknown 16 comprised up to 30% of the phosphorus compounds on treatment of 1 with excess MCPBA in *tert*-butyl alcohol, but it could not be isolated. Phosphoryl disulfide 17 was obtained by hydrogen peroxide oxidation of thioic acid 19 according to Metzger (1962).

O,O-Diethyl phosphorus acids 18 and 19 were prepared by hydrolysis of the corresponding chloridates with NaOH (>5 equiv) in aqueous dioxane with refluxing for 5 h, followed by cooling, washing with ethyl ether, acidification of the aqueous phase and extraction with ether, drying the ether solution, and solvent evaporation. *O*-Ethyl *O*-phenyl phosphorus acids 20 and 21 were obtained from *O*-ethyl phosphorodichloridate and the corresponding thiono compound, respectively, on reaction with phenol followed by alkaline hydrolysis and acidification by the general procedure of Leader and Casida (1982). Phosphoric carboxylic anhydride 23 was obtained by a procedure based on Segall and Casida (1982) by dropwise addition of *m*-chlorobenzoyl

Table I. ^{31}Phosphorus Nuclear Magnetic Resonance Chemical Shifts for *O,O*-Diethyl *O*-Phenyl Phosphorothionate and the Corresponding *S*-Phenyl Phosphorothiolate and Phosphorodithioate and Their Derivatives (R = EtO, Ph = Phenyl)

compound		$\delta(^{31}P)$	
no.	structure[a]	$CDCl_3$	$(CD_3)_2CO$
	Starting Materials		
1	$R_2P(S)OPh$	60.88	60.68
2	$R_2P(O)SPh$	20.31	18.92
3	$R_2P(S)SPh$	85.65	84.64
	Oxidation/Rearrangement Products		
4	$R_2P(O)OPh$	−8.66	−8.74
5	$R_2P(O)SOPh$	19.09	17.82
6	$R_2P(O)OS(O_2)Ph$	−15.42	−15.63
7	$R_2P(O)SSPh$	20.15	18.37
8	$R_2P(O)SS(O)Ph$	11.21	10.25
9	$R_2P(O)SS(O_2)Ph$	6.35	5.42
	Phosphorylated Alcohols		
10	$R_2P(O)OMe$	−2.20	−2.24
11	$R_2P(O)OPr$-*n*	−3.21	−3.27
12	$R_2P(O)OPr$-*i*	−5.02	−5.06
13	$R_2P(O)OBu$-*t*	−7.98	−8.02
	Bisphosphorus Compounds		
14	$[R_2P(O)]_2O$	−15.45	−15.67
15	$[R_2P(O)]_2S$	13.67	12.61
16	$[R_2P(O)S]_2SO_2$	5.75	5.58
17	$[R_2P(O)S]_2$	18.80	18.92
	Phosphorus Acids		
18	$R_2P(O)OH$	−1.76	−3.28
19	$R_2P(S)OH$	61.29	63.39
20	$R(PhO)P(O)OH$	−7.41	−8.06
21	$R(PhO)P(S)OH$	56.63	58.71
	Others		
22	$R_2P(O)SOMe$	21.72	20.59
23	$R_2P(O)OC(O)C_6H_4Cl$-3	−10.28	−10.98

[a] Authentic standards except for unstable compounds 8, 9, and 16. Structures proposed for 8 and 9 are based on their reactions and NMR chemical shifts. The tentative structure indicated for 16 is based primarily on an NMR chemical shift similar to that of 9.

Table II. Three Types of Phosphorus-Containing Products on Oxidation of *O,O*-Diethyl *O*-Phenyl Phosphorothionate and the Corresponding *S*-Phenyl Phosphorothiolate and Phosphorodithioate with *m*-Chloroperoxybenzoic Acid in the Presence of Aliphatic Alcohols at 20 °C (R = EtO, Ph = Phenyl)

	product composition,[b] %		
alcohol reactant[a]	phosphorylated alcohols	oxidn/ rearrangement products	bisphosphorus compds
	$R_2P(S)OPh$ (1) + MCPBA (1:4)		
	10–13	4	16
none	0	100	0
methyl alcohol	60	40	0[c]
n-propyl alcohol	20	70–75	5–10[c]
isopropyl alcohol	5–10	75–85	10–15[c]
tert-butyl alcohol	<0.5	70–75	25–30[c]
	$R_2P(O)SPh$ (2) + MCPBA (1:4)		
	10–13	6	14
none	0	85	15[d]
methyl alcohol	95	0	5
n-propyl alcohol	95	0	5
isopropyl alcohol	25	70	5
tert-butyl alcohol	0	85	15
	$R_2P(S)SPh$ (3) + MCPBA (1:7)		
	10–12	9	15
none	0	85	15[e]
methyl alcohol	60–65	35–40	0
n-propyl alcohol	25–30	70–75	0
isopropyl alcohol	<5	<95	0

[a] Molar ratio of 20:1 for the alcohol and thiophosphorus compound. MCPBA level selected for complete reaction. [b] The tabulated results are for acetone but essentially identical findings are obtained with benzene or chloroform. Minor products in footnotes *c–e* are not included in these normalized tabulations. [c] Additional products in the alcohol reaction mixtures are **5**, **15**, and **17** totaling 1–5% yield. [d] Compound **18** appears in yields of 2% in acetone and 1% in benzene or chloroform but is not detected in the alcohols. [e] A compound with a ^{31}P NMR chemical shift in the region of those for **5** and **17** appears in 3–4% yield in acetone, benzene, or chloroform but is not detected in the alcohols.

chloride to an equimolar solution of the dicyclohexylamine salt of **18** in dry ethyl ether at 0 °C followed by filtration to remove the amine hydrochloride and solvent evaporation. It was examined directly since it decomposed on attempted chromatography.

Oxidants. MCPBA (commercial, 85%) was upgraded (99%) by removing MCBA on extracting a dichloromethane solution with a pH 7.5 buffer (Fieser and Fieser, 1967). 2-(Phenylsulfonyl)-3-(4-nitrophenyl)oxaziridine (referred to as the oxaziridine) was synthesized according to Davis and Stringer (1982).

Oxidation Reactions. Phosphorothioates **1–3** were reacted with MCPBA or the oxaziridine in acetone (10% acetone-d_6), benzene (10% benzene-d_6), or chloroform-*d* at room temperature or as specified, using 10–20 mg of the phosphorothioate with appropriate equivalents of the oxidant in an NMR tube, monitoring the reaction course by ^{31}P NMR. Reactions at low temperature starting at −50 °C were employed to seek possible intermediates in the MCPBA oxidation of **1–3** in acetone. In some cases the reaction mixture in chloroform was cooled to −30 °C and decanted to remove the precipitated MCBA, thereby facilitating CI-MS and TLC.

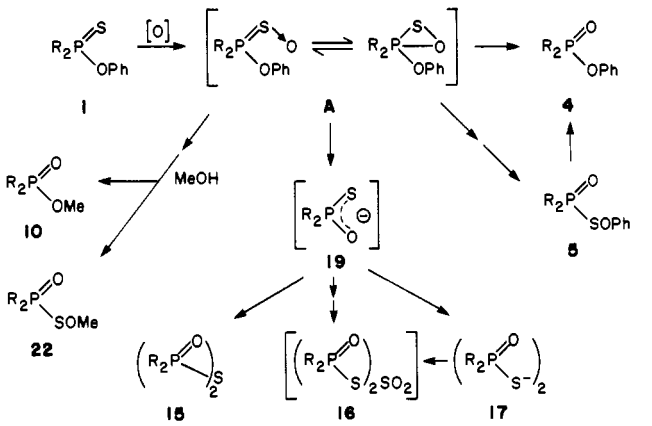

Figure 1. Transformations of *O,O*-diethyl *O*-phenyl phosphorothionate (**1**) upon oxidation with *m*-chloroperoxybenzoic acid in aprotic solvents alone or containing methanol. There are other possible rearrangement products of A and **5** in addition to those shown. Compound **16** is not identified but is considered to be a thiosulfonate such as the structure shown (R = EtO, Ph = phenyl).

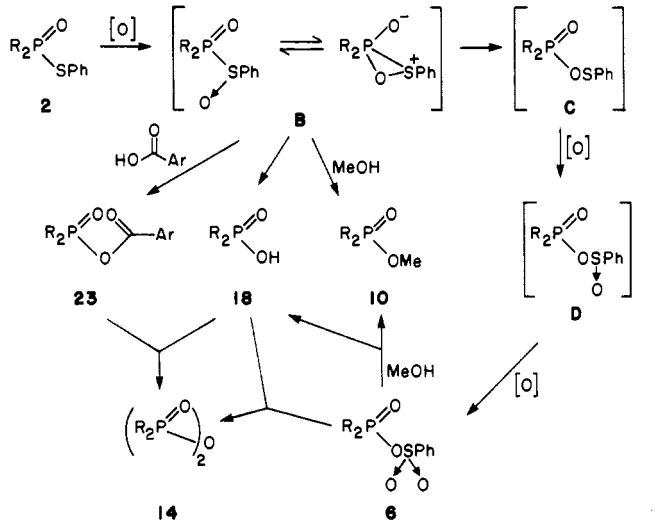

Figure 2. Transformations of *O,O*-diethyl *S*-phenyl phosphorothiolate (**2**) upon oxidation with *m*-chloroperoxybenzoic acid in aprotic solvents alone or containing methanol. Compound **23** can originate not only from B but also from other phosphorylating agents (R = EtO, Ph = phenyl).

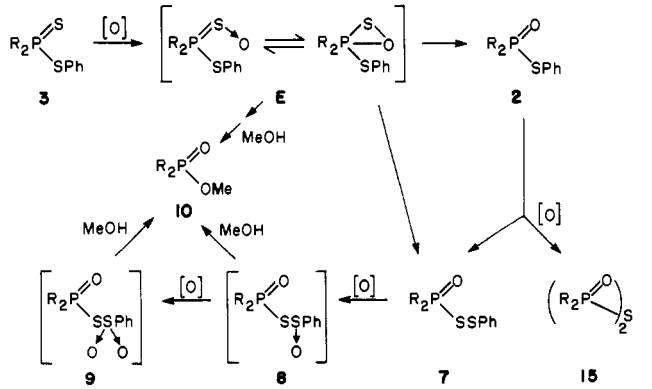

Figure 3. Transformations of *O,O*-diethyl *S*-phenyl phosphorodithioate (**3**) upon oxidation with *m*-chloroperoxybenzoic acid in aprotic solvents alone or containing methanol. There are also other possible rearrangement products of E in addition to those shown (R = EtO, Ph = phenyl).

RESULTS

The major products formed on MCPBA oxidation of **1–3** are shown in Figures 1–3 and quantitated in Table II for

Table III. ^{31}Phosphorus Nuclear Magnetic Resonance Chemical Shifts for Intermediates in the Oxidation of *O,O*-Diethyl *O*-Phenyl Phosphorothionate and the Corresponding *S*-Phenyl Phosphorothiolate and Phosphorodithioate with *m*-Chloroperoxybenzoic Acid in Acetone at −50 to −20 °C (R = C_2H_5O, Ph = Phenyl)

	δ(^{31}P)		
reactant[a]	parent	intermed	diff
$R_2P(S)OPh$ (1)	60.68	26.08	34.60
$R_2P(O)SPh$ (2)	18.92	−8.71[b]	27.63[b]
$R_2P(S)SPh$ (3)	84.64	57.52	27.12

[a] MCPBA used at 4:1 with 1 and 3:1 with 2 and 3. [b] A second intermediate appears at δ −31.74 for a difference of 50.66.

reactions in aprotic solvents and with aliphatic alcohols. Transitory intermediates are considered in Table III.

***O,O*-Diethyl *O*-Phenyl Phosphorothionate (1).** Phosphorothionate 1 reacts completely with 1.1–4 mol equiv of MCPBA in acetone, benzene, or chloroform within 5 min at 20 °C to yield exclusively phosphate 4. With 1, MCPBA, and methanol at a 1:1.1:20 molar ratio in benzene at 20 °C, the reaction proceeds much more slowly and is complete only after about 7 h, yielding ~25% unreacted 1, 30% 4, 35–45% 10, and 5–10% of other products including *O,O*-dimethyl *O*-ethyl phosphate (probably from ester exchange), phosphorylsulfenates 5 and 22, and a new compound (16). Neither compound 4 nor 5 reacts with methanol at 20 °C to yield 10. Although not identified, compound 16 is tentatively shown as a bis(thiosulfonate) because its chemical shift is very similar to that of 9, suggesting the $(EtO)_2P(O)SSO_2-$ moiety, and it does not incorporate the alcohol residue since its chemical shift is practically independent of the alcohol used. On comparing aliphatic alcohols, the proportion of 10–13 from phosphorylation decreases and of 4 and 16 increases in the sequence of methyl, *n*-propyl, isopropyl, and *tert*-butyl alcohol. Three minor products in the alcohol reaction mixtures are 5, 15, and 17.

Compound 1 reacts very slowly with MCPBA (1:4) in acetone at −50 °C to give 4 and an equal amount of a new compound (referred to as A; see Discussion) (δ +26.08) with total product formation of 1–5%. When the reaction mixture is warmed to −30 °C, the amount of A remains nearly unchanged and of 4 greatly increases. At −20 °C A is no longer evident, and after the reaction mixture is brought to 20 °C, the products are in similar proportion to those formed on direct reaction at 20 °C. When methanol (20-fold molar excess) is present in the reaction mixture, the products at −50 °C are 4, 10, and A in decreasing amounts totaling 1–5% at 1 h, and at 20 °C they are 10 and 4 in a 60:40 ratio.

Reaction of 1 with the oxaziridine (1:4) in various solvents (acetone, benzene, chloroform, or acetone containing 20 mol equiv of methanol) yields only 4, requiring 2 h to complete the reaction. Ester-exchange product 10 is formed on addition of 2 equiv of an organic acid to the reaction mixture with methanol, with yields of 18–20% for acetic and benzoic acids and 35% for MCBA, the remainder being 4. Even more extensive phosphorylation occurs within 20 h when a solution of 1 and the oxaziridine (1:2) in chloroform–methanol is saturated with dry HCl, giving 30% 4, 65% *O,O*-dimethyl *O*-ethyl phosphate, and 5% *O,O*-dimethyl *O*-phenyl phosphate.

***O,O*-Diethyl *S*-Phenyl Phosphorothiolate (2).** Phosphorothiolate 2 in acetone, benzene, or chloroform is oxidized with at least 3 equiv of MCPBA within 1 h to give predominantly 6 with small amounts of 14, 18, and 23. Complete conversion (>99%) of the starting material requires 2.5–3 equiv of MCPBA. The product composition does not change even on decreasing the MCPBA ratio to 0.5 relative to 2. A 2–3-fold larger amount of 18 is found in acetone than in benzene or chloroform. Virtually the same findings on reactants and product composition are obtained with *O,O*-diethyl *S*-propyl phosphorothiolate, confirming an earlier study (Segall and Casida, 1983a). Reaction mixtures of 2, MCPBA, and methanol (1:4:20) in acetone give almost exclusively 10. The yield of phosphorylated alcohol (10–13) decreases and of 6 increases with the bulkiness of the alcohol moiety. Phosphoryl sulfonate 6 reacts with methanol in acetone to give 70% 18 and 30% 10, but in benzene or chloroform there is 20% 18 and 80% 10, an apparent solvent difference perhaps related to the water content. Compounds 6 and 14, with very similar NMR chemical shifts, can be differentiated by methanolysis because 6 reacts completely under conditions in which 14 is stable.

No transitory intermediates are evident by ^{31}P NMR examination of any of the above reaction mixtures at 20 °C. With 2 and MCPBA (1:3) in acetone at −50 °C, there are two new peaks (δ −31.74, −8.71) in a 35:65 ratio totaling 1–2% in amount. When the mixture was warmed to −30 °C, the δ −31.74 peak disappears and 6 emerges, giving a ratio for the δ −8.71 peak and 6 of about 70:30. On warming to −20 °C the δ −8.71 peak also disappears, giving ~5% 6. At 20 °C the starting material is gone to give 55% 6 and 45% 14.

The oxaziridine in 5-fold excess does not react with 2 in acetone, benzene, chloroform, or methanol at 20 °C within 48 h.

***O,O*-Diethyl *S*-Phenyl Phosphorodithioate (3).** Phosphorodithioate 3 and MCPBA (1:1.1 to 1:3) in acetone, benzene, or chloroform react within 30 min at 20 °C to give at least 95% of an equimolar mixture of 2 and 7. With a large excess of MCPBA (1:5), 3 disappears within 5 min and subsequently 7 reacts faster than 2. The concentration of 8 increases for the first 2 h of the reaction, at which time it is the major product, and it then decreases so that at 4 h 9 is the major product and 15 is next in amount. With 7 mol equiv of MCPBA the reaction is complete in 10 min, giving 9 and 15 in an 85:15 ratio, and no 8 is detected. Disulfide 7 reacts with MCPBA (1:3) via 8, yielding 9 as the only product. Compound 8 is considered to be the thiosulfinate and 9 the thiosulfonate based on their sequence of formation on oxidation of 3 and 7 and on their ^{31}P NMR chemical shifts. Quenching the reaction mixtures with methanol when 8 attains its highest concentration causes it to collapse almost instantly with formation of 10. Thiosulfonate 9 phosphorylates methanol very slowly, requiring 20 h for complete reaction. Phosphorodithioate 3 reacts almost completely with equimolar MCPBA in methanol within 90 min at 20 °C to give 45% 7, 30% 10, and 25% 2. Oxidation of 3 in the presence of excess alcohol gives ester-exchange products 10–12, decreasing in amount in the order methyl, *n*-propyl, and isopropyl alcohol along with increasing amounts of 9.

When a −50 °C reaction mixture of 3 and MCPBA (1:3) in acetone is warmed, the first products (~1–2% yield) evident at −30 °C are 30% 7, 40% 23, and 30% of a new compound (referred to as E; see Discussion) (δ +57.52). When warmed to −20 °C the products (5%) consist of 30% 7, 35% 23, and 35% E. At −10 °C the products (10%) are equimolar 7 and 23 and no E. At 0 °C with 20% reaction, phosphorothiolate 2 emerges (15%) with 60% 7 and 25% 23. At 10 °C with 90-min overall warming time, the starting material is gone with 15% 2, 70% 7, and 15% 23. Finally, after 12 h at 20 °C, disulfide 7 is gone and the products are 25% 2, 65% 9, and 10% 23.

The oxaziridine (4 equiv) oxidizes 3 exclusively to 2 within 8 h in acetone, benzene, chloroform, or methanol at 20 °C.

DISCUSSION

Figures 1–3 give the proposed transformations for *O,O*-diethyl phenyl phosphorothionate, phosphorothiolate, and phosphorodithioate, respectively, upon oxidation with MCPBA in aprotic solvents alone or containing methanol. Each type of thiophosphorus compound yields an initial oxidation product(s) that readily phosphorylates methanol either by direct means or after rearrangement. In each case ^{31}P NMR spectra for reactions run at −50 to −20 °C show signals appropriate for the transient phosphoxathiiranes or phosphorylsulfenates with chemical shifts 27–34 ppm upfield from the starting materials. Oxidation of the thiono, thiolo, and dithio compounds therefore appears to involve similar structural changes about the phosphorus atom. Solubility limitations of the oxaziridine precluded attempts with this oxidant to directly observe an activated intermediate at low temperature. Product variations in the oxidation/rearrangement reactions with oxidant or temperature may have synthetic implications in selective transformations of thiophosphorus compounds.

The thionate sulfur is oxidized much easier than the thiolate sulfur as reported earlier for phosphorodithioates (Bellet and Casida, 1974; Segall and Casida, 1982) and dithiocarbamates (Segall and Casida, 1983b) and confirmed here with 1–3. 1 reacts with both MCPBA and the oxaziridine in aprotic solvents at 25 °C to give phosphate 4 exclusively. A transient compound, observed at −50 °C in acetone, has a chemical shift (δ +26.08 related to trimethyl phosphate) equivalent to an earlier report (δ +29.8 related to phosphoric acid) (Field et al., 1983) and appropriate for the three-membered ring "phosphorus oxythionate" or "phosphoxathiirane" intermediate (shown as A) proposed earlier (McBain et al., 1971a; Ptashne et al., 1971; Fukuto, 1978).

Phosphoxathiirane A, or a rearrangement product thereof other than 5, efficiently phosphorylates methanol, but its reactivity with higher alcohols depends on their nucleophilicity and steric features. This phosphorylating agent is weaker than the one(s) derived from the corresponding phosphorothiolate. It undergoes kinetically controlled rearrangement reactions to give only 4 in aprotic solvents but leading to both 4 and 16 in higher alcohols. A minor product, 5, is formed from A by phenoxide migration to sulfur. The sulfur incorporated on the conversion of 17 or 19 to 16 probably comes from the desulfuration of A in forming 4. The activated intermediate in the oxaziridine reaction gives only phosphate 4 even in methanol, unless an acid is present to facilitate phosphorylation, for instance by protonation of the phenoxy oxygen.

Oxidation of phosphorothiolate 2 by MCPBA gives 6 probably via B–D (Segall and Casida, 1981, 1982). Two thermolabile intermediates evident at −50 °C but not at −20 °C have ^{31}P NMR signals at δ −31.74 and −8.71 compared with δ +18.92 for 2, indicating replacement of the P–S bond by a P–O bond. The δ −31.74 signal at a higher field even than that of 6 suggests an ionic character of the species, possibly consistent with the phosphoxathiiranium structure for B. The δ −8.71 peak might be attributable to either C or D. Comparing the chemical shifts of 2 (δ 18.92) and 5 (δ 17.82) shows that separation of the phenyl substituent by an additional oxygen (P–SPh versus P–SOPh) does not cause a significant upfield shift. An even weaker influence is expected for incorporation of a less electronegative sulfur atom. Since the chemical shifts are almost identical for the δ −8.71 intermediate and 4 (δ −8.74), it seems reasonable to regard the intermediate as sulfenate C. Thus, the thermolabile intermediates on oxidation of 2 are considered to be B and C. Sulfinate D is not observed, probably undergoing rapid oxidation to 6. The phosphorylating agent(s) formed on oxidation of 2 is much more reactive toward alcohols than 6, which is both a phosphorylating and sulfonylating agent and is likely to be one or more of B–D, but the available data are not sufficient to distinguish between these alternatives. In light of these findings, ^{13}C NMR data for MCPBA oxidation of methamidophos [$MeO(NH_2)P(O)SMe$] performed at room temperature with signals attributed to the phosphorothiolate *S*-oxide (Thompson et al., 1984) should be reconsidered. Although synthesis of an analogous phosphonothiolate *S*-oxide [EtO(Et)P(S)S(O)Ph] is reported (Bellet and Casida, 1974), we did not succeed in our attempts to synthesize B–D or their analogues by reactions of diethyl and triethyl phosphites with propylsulfinyl chloride and diethyl phosphate with propyl, *tert*-butyl, or benzenesulfenyl chloride. Although the oxaziridine readily oxidizes 1 and 3, it is too weak an oxidant to react with phosphorothiolate 2.

Phosphorodithioate 3, on treatment with MCPBA (1:3) at 25 °C, is converted to equimolar 2 and 7, presumably via the phosphoxanthiirane. The δ +57.52 intermediate evident on oxidation of 3 at −35 °C is referred to as phosphoxathiirane E on analogy with the oxidation of 1 to A. E apparently rearranges exclusively to 7 at <−10 °C or gives both 2 and 7 at higher temperatures. This observation favors the hypothesis of rearrangement over that of cleavage and recombination of fragments (Miyamoto and Yamamoto, 1977) in the conversion of 3 to 7. On oxidation of 3 with a large excess of MCPBA (1:>5), there is the expected initial formation of 2 and 7 in about a 1:1 ratio but further oxidation does not give 6, as obtained on direct MCPBA oxidation of 2; probably the initially formed intermediate(s) derived from 2 reacts with 7 or other thiophosphorus intermediates giving 15. Disulfide 7 is further oxidized with MCPBA to yield compounds proposed to be thiosulfinate 8 and ultimately thiosulfonate 9. Thiosulfinate 8, in contrast to its oxygen analogue D, can be easily detected even at room temperature. Oxidation of 3 with the oxaziridine gives only 2 at 25 °C. The rate for phosphorylation of methanol decreases in the order of oxidized 2 > oxidized 3 or 1 > 8 > 9 > 4, suggesting that the phosphorylating intermediate(s) derived from 3 are of similar or slightly lower reactivity than that derived from 1 and much lower than those from 2. Methanol is phosphorylated on oxidation of 3 with equimolar MCBPA, consistent with the initial oxidation product(s) being responsible. The phosphoxathiirane or a rearrangement product thereof derived from the phosphorodithioate is a candidate for the initially formed phosphorylating agent and the phosphorothiolate *S*-oxide or phosphorylsulfenate for an additional phosphorylating agent formed via the phosphorothiolate.

The bioactivated forms of phosphorothionates and phosphorodithioates responsible for phosphorylation of AChE or other esterases or proteins are generally considered to be the corresponding phosphates and phosphorothiolates (Eto, 1974) although the phosphorothiolates may be further bioactivated by S-oxygenation (Wing et al., 1983). The present study leads to an additional or alternative possibility that the bioactivated phosphorylating agents are in fact the phosphoxathiiranes themselves or rearrangement products thereof derived from the phosphorothionates and phosphorodithioates rather than their

desulfurated derivatives, the oxons. Because of their high reactivity, the phosphoxathiiranes or their rearrangement products might be expected to react in the most part within the organs or cells in which they are formed. Thus, the potential relevance, if any, of the MCPBA/methanol model in nonaqueous systems to the cytochrome P_{450}/AChE systems in vivo remains to be established.

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Supplementary Material Available: Complete ^1H and ^{13}C NMR and CI-MS data for the standard compounds (3 pages). Ordering information is given on any current masthead page.

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