

Mechanistic Investigation in the Oxidation of O,O-Diethyl-S-Phenyl Phosphorothiolate-¹⁸O

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Abstract : O,O-Diethyl-S-phenyl phosphorothiolate-¹⁸O and other related compounds were prepared and oxidized with m-chloroperbenzoic acid (MCPBA). Each reaction was followed by ³¹P NMR and the products were analyzed by GC-MS. O,O-Diethyl-S-phenyl phosphorothiolate-¹⁸O was converted to diethyl methyl phosphate in methanol by MCPBA and it was confirmed to contain ¹⁸O,_j which proved that the originally proposed mechanism of Segall and Casida operates in the oxidative reaction (Received June 2, 1994; accepted July 5, 1994).

Introduction

The chemistry of phosphorothioates has been the subject of much attention, particularly with respect to defining the mechanism of action of insecticidally active compounds. Of the approximately 106 organophosphorus pesticides in current use, phosphorothioates account for more than two thirds¹⁾ and in biological systems these are converted to metabolites which can be more toxic or less toxic than the parent compounds. These processes are termed "bioactivation" and "detoxification", respectively. In many instances, these transformations are oxidatively induced, for example, the insecticide methamidophos exhibited high insecticidal activity but it is a poor anti-acetylcholinesterase *in vitro*.²⁾ This interesting bioactivation has been studied by many researchers.²⁻⁴⁾ and the anti-acetylcholinesterase activity of methamidophos increased substantially on oxidation with MCPBA.³⁾ Based on several evidences these workers proposed that the initially formed active intermediate was the sulfoxide, O,S-dimethyl phosphoramidothiolate-S-oxide.

In MCPBA oxidations of a series of S-alkyl phos-

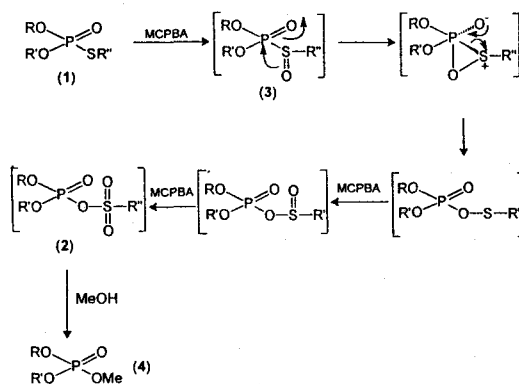
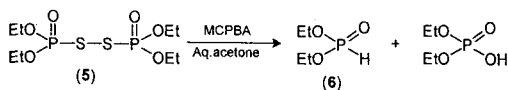


Fig. 1. Mechanism for the oxidation of thiolate.

phorothiolates (1), Segall and Casida^{5,6)} reported a new class of phosphinyloxy sulfonates (2) whose formation was rationalized by a novel rearrangement process of the phosphorothiolate-S-oxide (3) (Fig. 1) and further oxidation in various alcohols was studied.⁷⁾ The oxysulfonate (2) was reported to give methyl ester (4) in methanol.⁸⁾

In the oxidation of some acyclic phosphorothioates, however, oxidation of the disulfide (5) in aqueous acetone gave diethyl hydrogenphosphonate (6)⁹⁾ as one of the products.



In view of this observation another possible mechanism (Fig. 2) can be postulated in which the phosphorothiolate S-oxide (3) rearranges to phosphite (7). This phosphite (7) would then be oxidized further to the oxysulfonate (2) which will give methyl ester (4) in methanol as also shown in Fig. 1. The phosphite ester (8) could be formed by phosphorylating methanol and readily oxidized further to give the methyl ester (4). In aqueous media the phosphite (7) may also hydrolyse to produce hydrogenphosphonate (9) which would be relatively insensitive to further oxidation. Thus, the present study was conducted to distinguish the mechanism shown in Fig. 1 from an alternative in Fig. 2.

Materials and Methods

Synthesis of compounds

For the experiments, starting materials and other reference compounds were prepared based on the general procedures in Fig. 3.⁹⁾ All syntheses were carried out in an inert atmosphere (either nitrogen or argon) and solvents were dried before use.

General methods

Melting points (m.p.) were determined on Kofler

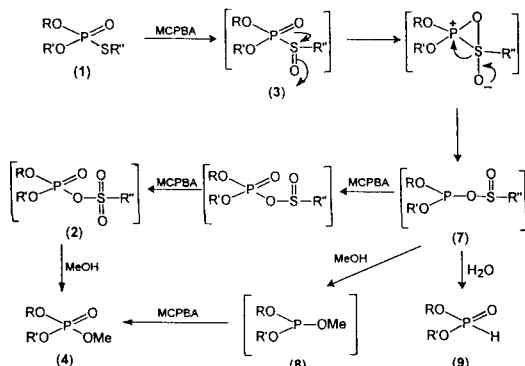


Fig. 2. Proposed alternative mechanism of the oxidation of thiolate.

Hot Stage Microscope melting point apparatus and are uncorrected. Analytical t.l.c. utilized layers of silica gel GF 254 type 60 (Merk) of 0.25 mm thickness and the plates were visualised either under U.V. light, with iodine vapour, or for the compounds containing the P-S linkage by spraying with an acidified (HCl) aqueous PdCl₂ solution (0.5%).

IR spectra were recorded with either an Hitachi EPI-G grating spectrometer or a Perkin Elmer 580B spectrometer. ¹H NMR and ¹³C NMR spectra were recorded either on a Bruker AM 500 (500 MHz for ¹H or 125.77 MHz for ¹³C), or CXP-300 (300 MHz) with CDCl₃ as internal standard unless otherwise specified. ³¹P NMR spectra were recorded CXP-300 (121.47 MHz) spectrometer generally with broad band proton decoupling, and with 85% H₃PO₄ in D₂O as external standard unless otherwise specified. Mass spectra were taken on a AEI-MS12 spectrometer (operating at 70 eV, electron impact mode) attached to a VG-display digispec data acquisition

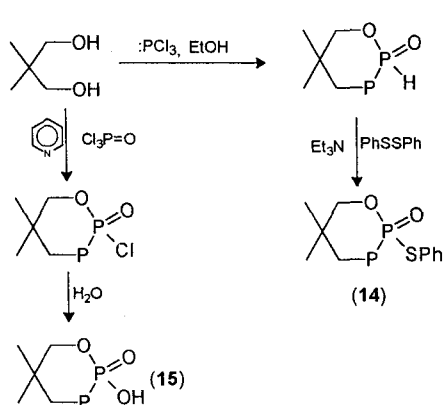
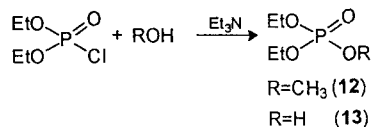
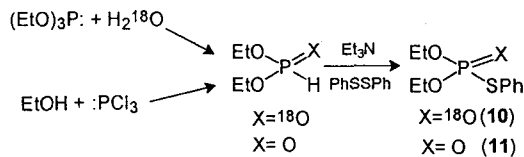


Fig. 3. Synthesis of compounds.

system computer.

O,O-Diethyl-S-phenyl phosphorothiolate- ^{18}O (**10**).

Analysis ($\text{C}_{10}\text{H}_{15}\text{O}_2\text{PS}^{18}\text{O}$), Found (%): C, 48.33; H, 6.25, Calculated (%): C, 48.38; H, 6.09, MS, m/z (%): 248 (M^+ , 96), 220 (31), 192 (25), 111 (68), 110 (100), 109 (56), ^{31}P NMR (EtOAc): δ 22.5, ^1H NMR (CDCl_3): δ 7.25, 7.34 (m, 5 H, aromatic), 4.19 (m, 4 H, $\text{CH}_3\text{CH}_2\text{OP}$), 1.30 (d, t, $J=0.98$ Hz, 7.07 Hz, 6 H, $\text{CH}_3\text{CH}_2\text{OP}$), ^{13}C NMR (CDCl_3): δ 134.4, 134.4, 129.20, 128.9, 126.5, 126.4 (m, SPh), 63.9 (d, $J=6.0$ Hz, $\text{CH}_3\text{CH}_2\text{OP}$), 15.9 (d, $J=7.0$ Hz, $\text{CH}_3\text{CH}_2\text{OP}$), IR (Neat): 3560 (w, br), 3490 (w, br), 2985 (s), 2940 (m), 2910 (m), 1582 (m), 1477 (s), 1442 (s), 1395 (s), 1255 (m), 1225 (s), 1162 (s), 1100 (m), 1020 (s, br), 970 (s, br), 790 (s, br), 750 (s, br), 702 (s), 693 (s).

5,5-Dimethyl-2-oxo-thiophenyl-1,3,2-ioxaphosphorinane (**14**).

m.p.: $121\sim 122^\circ\text{C}$, Analysis ($\text{C}_{11}\text{H}_{15}\text{O}_3\text{PS}$), Found (%): C, 51.10; H, 6.05, Calculated (%): C, 51.16; H, 5.81, MS, m/z (%): 258 (M^+ , 52), 246 (38), 218 (40), 190 (39), 110 (87), 109 (100), ^{31}P NMR (CH_2Cl_2): δ 14.7, ^1H NMR (CDCl_3): δ 7.64, 7.36 (m, 5 H,

aromatic), 4.20 (d, d, $J=4.1$ Hz, 10.8 Hz, 2 H_{ax}, CH_2OP), 3.93 (d, d, t, $J=11.22$ Hz, 23.73 Hz, 1.5 Hz, 2 H_{eq}, CH_2OP), 1.28 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), ^{13}C NMR (CDCl_3): δ 134.7 (d, $J=5.0$ Hz), 129.5, 129.2, 124.8 (d, $J=6.0$ Hz)(m; SPh), 78.2 (d, $J=7.3$ Hz, CH_2OP), 32.5 (d, $J=6.5$ Hz, C_4), 22.0 (s, CH_3), 20.4 (s, CH_3), IR (KBr): 3432 (w), 2970 (w), 1478 (s), 1442 (w), 1273 (s), 1052 (s), 997 (s), 975 (s), 846 (s), 786 (s), 755 (s), 564 (s), 493 (s), 482 (m)

Oxidation

Oxidation of O,O-diethyl-S-phenyl phosphorothiolate- ^{18}O (**10**) by MCPBA in CH_3OH

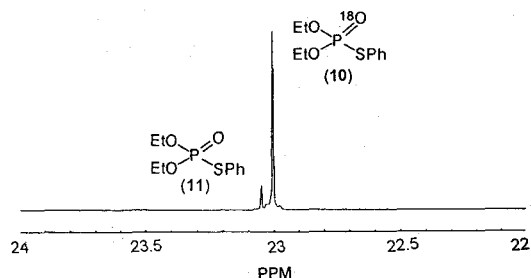


Fig. 4. ^{31}P NMR spectrum of O,O-diethyl-S-phenyl phosphorothiolate- ^{18}O (**10**).

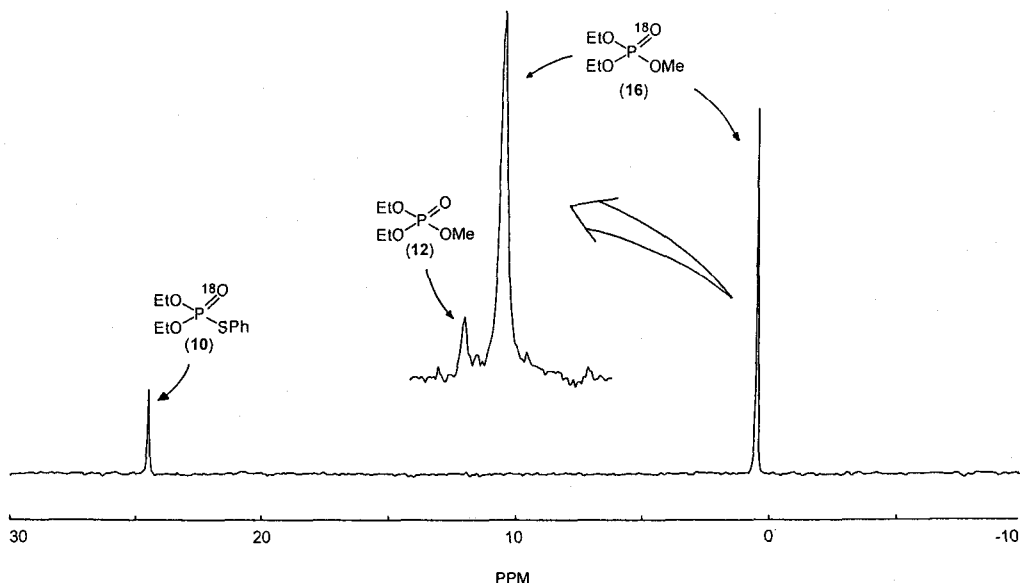


Fig. 5. ^{31}P NMR spectrum of reaction mixture from oxidation of ^{18}O -thiolate (**10**) in CH_3OH with MCPBA.

MCPBA (34.3 mg) was added to a solution of thiolate-¹⁸O (**10**, 12.3 mg) in CH₃OH (0.5 ml) and the reaction was monitored by ³¹P NMR for 10 hours. And then the reaction mixture was analysed by GC-MS. As a control experiment unlabelled thiolate (**11**, 11.7 mg) was oxidized with MCPBA (35.3 mg) in CH₃OH with monitoring on ³¹P NMR and after 10 hours the reaction mixture was also analysed by GC-MS for direct comparison with the labelled experiment. Another control experiment carried out with a solution of thiolate (**11**, 23.6 mg) in CH₃OH (0.5 ml) for 56 hours.

Oxidation of *O,O*-diethyl-S-phenyl phosphorothiolate (**11**) by MCPBA in aqueous acetone

MCPBA (43.7 mg) was added to a solution of thiolate (**11**, 14.4 mg) in aqueous acetone [0.5 ml, 1:1 (v/v)] and the reaction was monitored by ³¹P NMR for 4 hours and 10 mins. For a control experiment thiolate (**11**) (23.6 mg) in aqueous acetone was monitored for 15 hours.

Oxidation of 5,5-dimethyl-2-S-phenyl-2-oxo-1,3,2-dioxophosphorinane (**14**) by MCPBA in aqueous

acetone

Oxidation of the cyclic thiolate (**14**) (8.0 mg) with MCPBA in aqueous acetone was followed by ³¹P NMR for 11 hours. In a control experiment, cyclic thiolate (**14**) (6.0 mg) and MCBA (11.6 mg) was dissolved in aqueous acetone and the reaction was monitored for 11 hours.

Results and Discussion

All compounds were prepared in reasonable yield and ³¹P NMR chemical shifts are shown in Table 1 for further reference. On ³¹P NMR ¹⁸O-thiolate (**10**) resonated slightly upper field than unlabelled thiolate (**11**) and they were separated by 5.6 Hz (Fig. 4). The degree of incorporation of ¹⁸O was calculated as 88% based on ³¹P NMR and mass spectra.

Oxidation of ¹⁸O-thiolate (**10**) by MCPBA in CH₃OH gave ¹⁸O-diethyl methyl phosphate (**16**) as the only product (73%) and it was found that **16** contained about 12% of **12** on ³¹P NMR (Fig. 5) and GC-MS analyses (Fig. 6).

In mass spectra, although the molecular ion (M⁺ = 170) was not seen, a series of fragment ions were

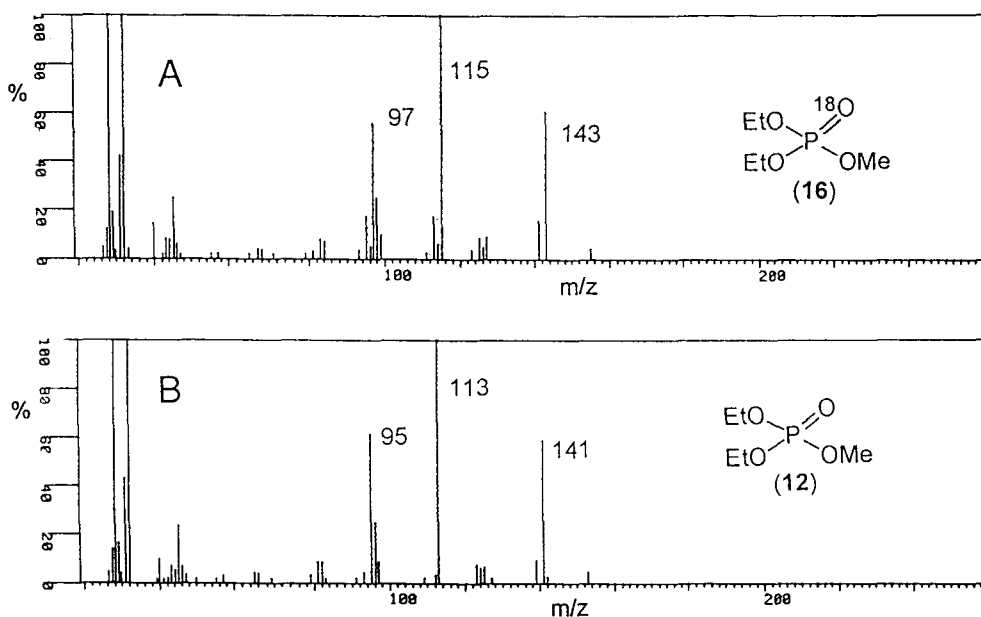
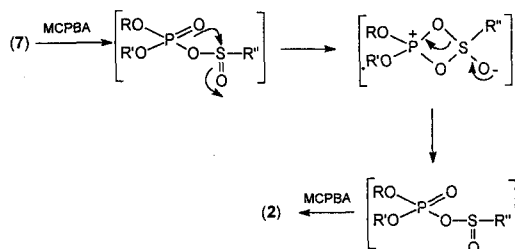


Fig. 6. Mass spectra of diethyl methyl phosphate-¹⁸O (**16**) (Spectrum A) and diethyl methyl phosphate (**12**) (Spectrum B).

Table 1. ^{31}P NMR chemical shifts (δ) of the synthesized compounds. (85% H_3PO_4 in D_2O as external standard)

Compound	(10)	(11)	(12)	(13)	(14)	(15)
Solvent	EtOAc	CHCl_3	CH_2Cl_2	CH_2Cl_2	CH_2Cl_2	CH_2Cl_2
δ	22.5	23.6	0.9	0.7	14.7	-3.9

Fig. 7. Possible mechanistic pathway from **7** to **2** for Fig. 2

observed at m/z 143, 115, 97 indicating the presence of ^{18}O (Fig. 6-A) when compared with diethyl methyl phosphate (**12**) which gave fragment ions at m/z 141, 113, 95 (Fig. 6-B) from a control experiment using unlabelled thiolate (**11**).

Either possible hydrolysis product **13** or methanolysis product **12** was not observed from other control reactions. This result suggests that compounds **16** and **12** were oxidatively produced and the originally proposed pathway in Fig. 1 is followed because unlabelled diethyl methyl phosphate (**12**) would be obtained if the reaction followed the pathway of Fig. 2.

Further experiments in aqueous acetone were carried out to investigate the formation of hydrogenphosphonate (**9**) because hydrolysis of key intermediate phosphite (**7**) would lead to the compound (**9**). However, oxidation of thiolate (**11**) in aq. acetone gave acid **13** as the only product (100%), and cyclic thiolate (**14**) also gave cyclic acid (**15**) only (86%) in similar reaction condition. Since formation of acids **13** and **15** was not observed in respective control reaction it was concluded that those acids were induced by oxidative processes. Another plausible pathway from **7** to **2** (in Fig. 2) which could result in **16** was considered (Fig. 7) but, it seems not to work because no hydrogenphosphonate such

as **9** (probably from **7**) was found in the oxidation of **11** or **14** in aqueous media.

These observations could be rationalized in accord with the earlier work by Bielawski and Casida⁸ and Yang et al.,¹⁰ therefore, the acids **13** and **14** should be formed from the hydrolysis of the reactive sulfoxide such as **3** before the further oxidation of sulfur.

Acknowledgement

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O,O-Diethyl-S-Phenyl Phosphorothiolate-¹⁸O의 산화반응기작

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Abstract-O,O-Diethyl-S-phenyl phosphorothiolate-¹⁸O와 관련화합물들을 합성하고 MCPBA로 산화시켰다. 각 반응을 ³¹P NMR로 추적하였고 반응산물을 GC-MS로 분석하였다. O,O-Diethyl-S-phenyl phosphorothiolate-¹⁸O은 메탄올에서 MCPBA에 의해 diethyl methyl phosphate로 전환되며 ¹⁸O를 포함하고 있어 이 산화반응은 Segall과 Casida가 제안하였던 반응기작을 따르고 있음이 증명되었다.