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Synthesis of High Specific Activity [ethyl-1, 2-^{3}H]-Labeled Chlorpyrifos Oxon and Diazoxon

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SUMMARY

[Ethyl-1,2-^{3}H]Chlorpyrifos oxon and [ethyl-1,2-^{3}H]diazoxon were synthesized at a specific activity of 79 and 58 Ci/mmol, respectively, by catalytic tritiation of the corresponding monovinyl analogs over Pd/C. The high specific activity results from isotope exchange of the terminal vinylic protons prior to saturation of the double bond. This radiosynthesis procedure is applicable to the toxicologically-important oxon metabolites of many commercial *O,O*-diethyl phosphorothioate pesticides.

Keywords: acetylcholinesterase, chlorpyrifos oxon, diazoxon, insecticide, tritium, isotope exchange.

INTRODUCTION

Chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] and diazinon [*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] are two of the most important and widely-used insecticides.[1] These

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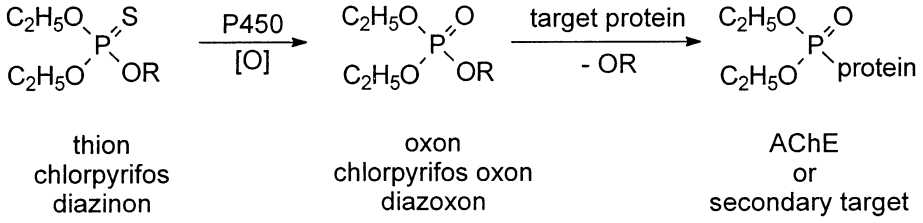
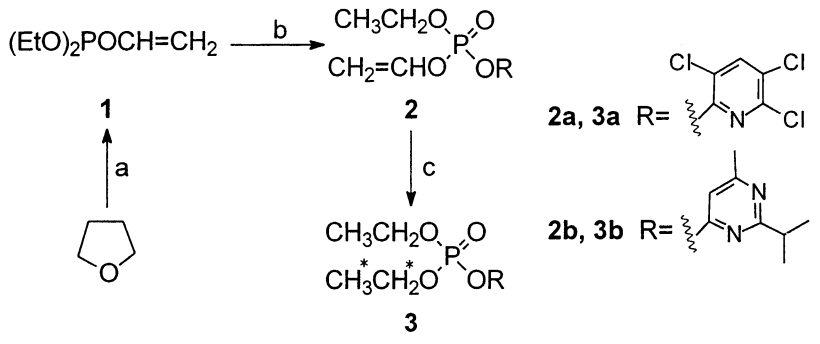
phosphorothionates (thions) are bioactivated by cytochrome P450-catalyzed oxidation to the corresponding phosphates (oxons), chlorpyrifos oxon and diazoxon. The oxons then inhibit acetylcholinesterase (AChE) activity by forming an inactive diethylphosphoryl derivative at the esteratic site, resulting in the primary toxicity to insects and mammals.[2,3]



Secondary targets are also of importance in evaluating the safety of organophosphorus insecticides. Chlorpyrifos oxon interacts with muscarinic acetylcholine receptors[4] and diazoxon inhibits kynurenine formamidase leading to teratogenic effects in chicken embryos.[5] These secondary biochemical lesions probably involve formation of diethylphosphorylated proteins and further understanding of their mechanisms and significance would be facilitated by the availability of ^{3}H-ethyl labeled compounds. We therefore synthesized [ethyl-1, 2-^{3}H]chlorpyrifos oxon (**3a**) and [ethyl-1, 2-^{3}H]diazoxon (**3b**) with high specific activities as reported here (Scheme 1).

RESULTS AND DISCUSSION

Vinyl analogs of chlorpyrifos oxon and diazoxon (**2a** and **2b**) were selected as the precursors for radiolabeling **3a** and **3b**. They were prepared from the reaction of diethyl vinyl phosphite with iodine, followed by coupling with 3, 5, 6-trichloro-2-pyridinol and 2-isopropyl-6-methyl-4-pyrimidinol, respectively. Diethyl vinyl phosphite (**1**) was made from the reaction of diethyl phosphorochloridite with the enolate of acetaldehyde,[6] which was generated by the treatment of anhydrous THF



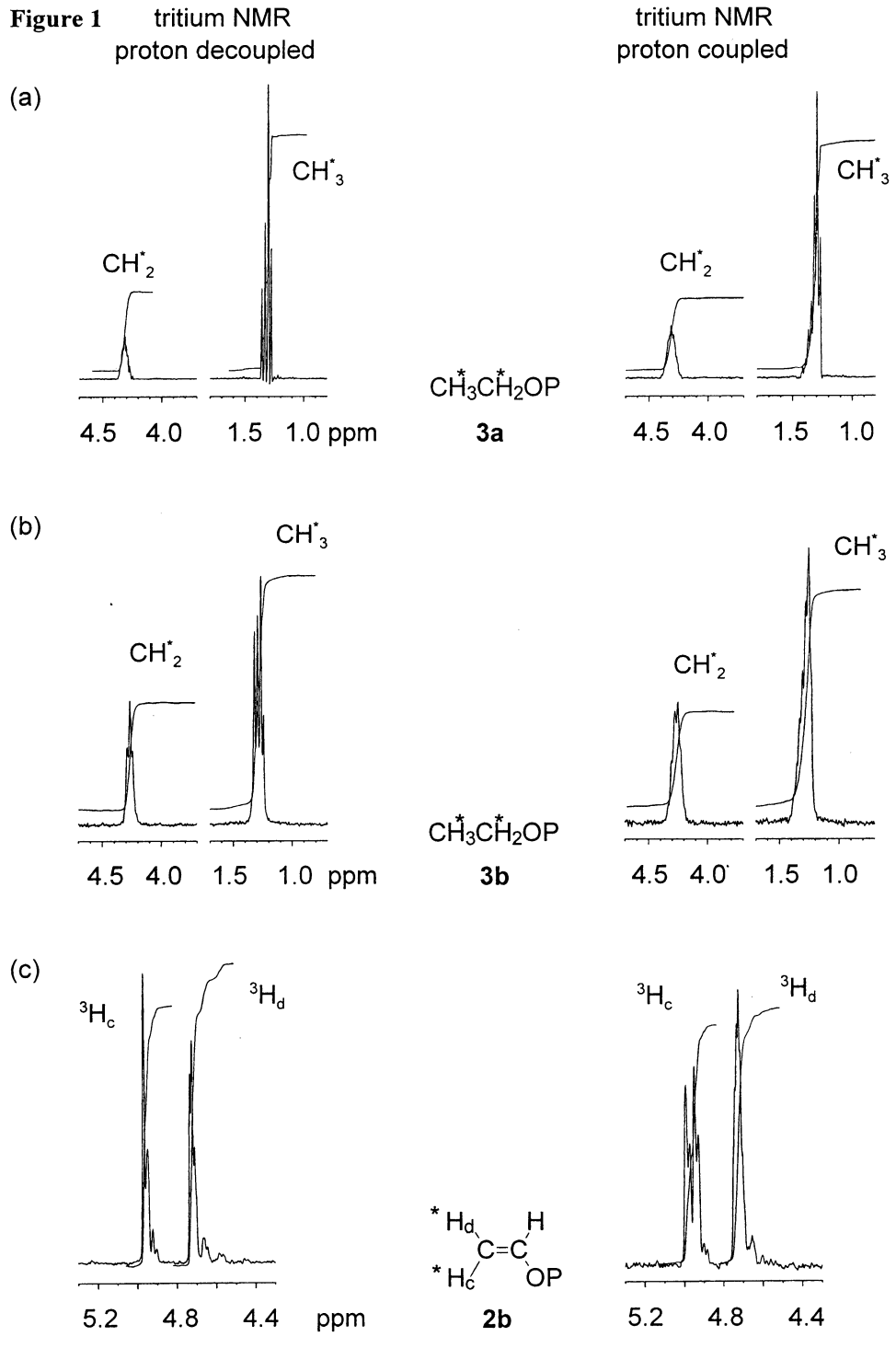
a: 1) BuLi, 16 h; 2) $(EtO)_2PCl$, -78°C. b: 1) I_2/CH_2Cl_2; 2) ROH/NEt_3. c: $^3H_2$, 10% Pd/C.

Scheme 1 Synthesis of [ethyl-1,2-^{3}H]-labeled chlorpyrifos oxon and -diazoxon

with butyllithium. Hydrogenation of **2a** and **2b** with homogenous Wilkinson's catalyst was not successful. However, under normal pressure at room temperature, **2a** and **2b** were hydrogenated completely to chlorpyrifos oxon and diazoxon in two hours using 20% by weight of Pd/C (10%) as catalyst. Under these conditions, no dechlorinated products were found in the hydrogenation of **2a**, as evidenced by the ^{1}H NMR spectrum of the reaction mixture. Tritiation of **2a** and **2b** was therefore performed with $^3H_2$ over Pd/C using conditions optimized by hydrogenation. The reaction of **2b** was carried out for 4 h whereas that of **2a** was stopped at 3 h to minimize or prevent possible dechlorination. The tritiated products (purified by normal-phase HPLC) showed a nearly complete conversion of **2a** to **3a** but, to our surprise, **2b** was only converted to **3b** in low yield, with a large amount of **2b** recovered. The specific activities of [ethyl-1, 2-^{3}H]chlorpyrifos oxon and [ethyl-1, 2-^{3}H]diazoxon were 79 and 58 Ci/mmol, respectively. The labeling positions of **3a** and **3b** were confirmed by tritium NMR (Figure 1).

The very high specific activity of **3b** and particularly **3a** results from tritium-proton exchange at the vinylic position before the saturation reaction observed here with monovinyl phosphates and with several non-phosphorus compounds earlier.[7,8] The tritium NMR spectra of **3a** showed that all tritium was incorporated in the ethyl

Figure 1



group and no tritium was incorporated by tritio-dechlorination. Multiple tritium signals were observed at both the methyl and methylene position with a complex pattern comprised of overlaid spectra from a host of isotopomers.[9] Such exchange reaction over Pd/C was further confirmed in the tritiation of **2b** in which a large amount of starting material was recovered and shown to have significant tritium signals in and only in its terminal methylene moiety (δ ~4.7 and 5.0 ppm) but not in the methine position (δ ~6.8 ppm). This result also established that the tritium-proton exchange reaction in the vinyl group occurs prior to the saturation reaction and regio-specifically occurs at the terminal methylene position.

EXPERIMENTAL

All reactions were performed under nitrogen atmosphere, unless otherwise indicated. Solvents were dried by standard methods.[10] 2-Isopropyl-6-methyl-4-pyrimidinol was purchased from Aldrich Chemical Co. 3,5,6-Trichloro-2-pyridinol was made from 6-chloro-2-pyridinol and diethyl vinyl phosphite (**1**) was prepared from diethyl phosphorochloridite and THF by reported methods.[6,11]

^{1}H, ^{13}C and ^{31}P NMR spectra of unlabeled chemicals were determined in $CDCl_3$ at 300, 75 and 121 MHz, respectively, with a Bruker AM-300 spectrometer. NMR spectroscopy on [^{3}H]-labeled compounds was carried out in CD_3OD using a Bruker DMX 300 spectrometer (^{3}H at 320 MHz, ^{1}H at 300 MHz) and a $^{3}H/^{1}H$ 5-mm dual probe. HPLC purification was performed on a Zorbax normal-phase column (4.6 mm ID x 250 mm) at a flow rate of 1.5 ml/min, with THF-hexane (1:4 for **3a** and 3:7 for **3b**) as elution solvents. The purification was monitored by both UV (260 nm for **3a** and 255 nm for **3b**) and radioactivity detectors.

Tritium gas for the tritiation contained 97.9% $^{3}H_2$ and 1.76% $^{2}H^{3}H$. Specific activities were determined by comparison of the UV absorption with that of a standard analytical sample and liquid scintillation counting of the isolated HPLC peak. Total activities were determined by liquid scintillation counting.

Vinyl Chlorpyrifos Oxon (2a) was prepared by a general procedure for vinyl and divinyl phosphates.[6] Into a solution of diethyl vinyl phosphate (**1**) (175 mg, 1.07 mmol) in anhydrous methylene chloride (2.0 ml) was added solid iodine (200 mg, 0.79 mmol) at room temperature. The solution was stirred for 5 min and then treated with 3,5,6-trichloro-2-pyridinol (150 mg, 0.76 mmol) followed by anhydrous triethylamine (300 mg, 3.0 mmol). The solution was stirred for another 10 min at room temperature, after which ether (50 ml) was added and the solution was filtered. The filtrate was concentrated and subjected to silica gel flash chromatography with EtOAc and hexane (1:2) to provide compound **2** (185 mg, 73% yield; characterized by ^{31}P, ^{1}H, ^{13}C NMR and FAB-HRMS). [6]

Vinyl Diazoxon (2b) was prepared as above, except 3,5,6-trichloro-2-pyridinol was replaced with 2-isopropyl-6-methyl-4-pyrimidinol. Pure **2b** was obtained by chromatography on silica gel with EtOAc and hexane (2:1). The chemical yield was 98% and the final compound was characterized by ^{31}P, ^{1}H, ^{13}C and FAB-HRMS.[6]

[ethyl-1,2-^{3}H]Chlorpyrifos Oxon (3a). Vinyl chlorpyrifos oxon (**2a**) (10.0 mg, 0.030 mmol) was dissolved in EtOAc (1.0 ml). The solution was frozen in liquid N_2 and then thawed under vacuum to degas the solution, which was repeated twice before the addition of 10% Pd/C (2.0 mg). Then tritium gas was introduced and the reaction was allowed to proceed at ~720 mm Hg for 3 h, after which tritium gas was recovered onto a uranium bed. The solvent was removed under vacuum. Methanol (2 x 1.0 ml) was added to exchange any possible labile tritium and pumped away under vacuum. The residue was then dissolved in EtOAc (2.0 ml) and filtered, washing the residue several times with EtOAc. The filtrate was purified by HPLC with hexane and THF (4:1), giving pure [ethyl-1,2-^{3}H]chlorpyrifos oxon (**3a**) (t_R=5.8 min) with a total activity of 1.5 Ci, a specific activity of 79 Ci/mmol, and a radiochemical yield of 63%. The radiochemical purity determined by TLC cochromatography (silica gel, hexane-EtOAc 2:1, R_f 0.6) and autoradiography was > 95% without further purification.

[ethyl-1,2-^{3}H]Diazoxon (3b). Under the same conditions as the previous tritiation, a solution of vinyl diazoxon (**2b**) (10.0 mg, 0.035 mmol) in EtOAc (1.0 ml) was tritiated for 4 h. Tritium gas was then recovered and labile tritium was removed. The catalyst was filtered off and the filtrate was purified by HPLC with THF and hexane (3:7). The principal material recovered was unsaturated starting compound **2b** (t_R=5.1 min) but there was also pure [ethyl-1,2-^{3}H]diazoxon (**3b**) (t_R=7.2 min) obtained with a total activity of 0.15 Ci, a specific activity of 58 Ci/mmol and a radiochemical yield of 7.5%. The radiochemical purity of **3b** determined as above (silica gel, hexane-EtOAc 1:2, R_f 0.5) was >95%.

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