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SYNTHESIS OF [^{35}S]PHOSPHOROTHIONATE INSECTICIDES : THE EXAMPLE OF [^{35}S]FENTHION

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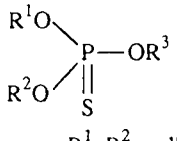
SUMMARY

A general method for the synthesis of [^{35}S]phosphorothionates is proposed, based on the example of [^{35}S]fenthion. This consists of reacting molecular ^{35}S with the corresponding phosphites which are easily prepared from commercial precursors.

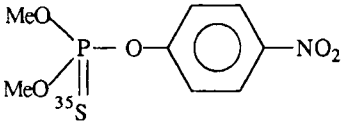
Key words: Organophosphorus compounds, insecticides, ^{35}S labelling.

INTRODUCTION

The phosphorothionates **1** are an important family among the organophosphorus insecticides.



1 R^1, R^2 = alkyl; R^3 = aryl



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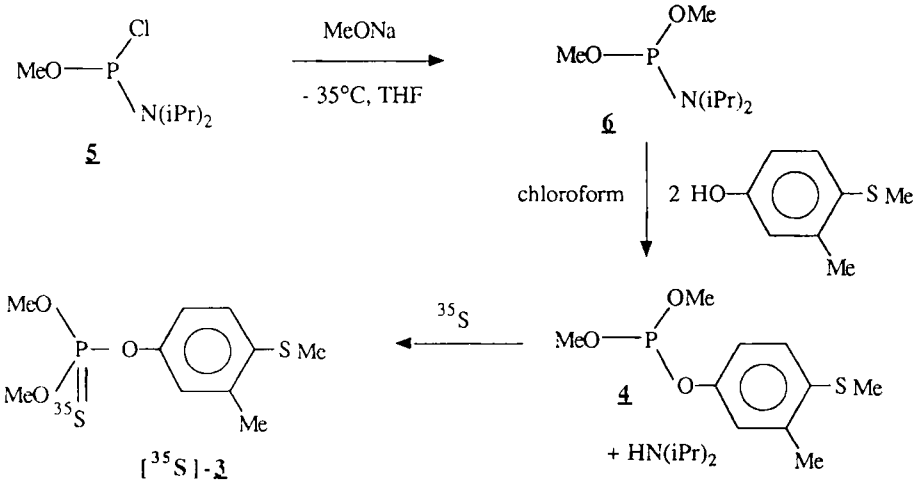
These compounds are converted, both in mammals and insects, by the cytochrome P450-containing monooxygenase enzyme systems, to the corresponding phosphate triesters which are potent inhibitors of acetylcholinesterase (1). It has been shown from experiments using [^{35}S]parathion **2**, that sulphur was liberated and bound to a cysteine residue of the active site to give a hydrodisulphide group, inducing the deactivation of the monooxygenase enzyme (1). Thus, parathion appears to be a suicide substrate for this type of enzyme. Despite their interest as reagents for studying the biological behaviour of the phosphorothionates, their [^{35}S]-labelled analogues are not commercially available. We required [^{35}S]-fenthion [^{35}S]-**3** to study its interactions with tomato plants. We report herein a simple synthesis of this labelled compound, which may be generalized to the syntheses of other phosphorothionates.

RESULTS AND DISCUSSION

The most direct route to obtain [^{35}S]phosphorothionates would be to introduce sulphur into the corresponding phosphites. Although many sulphur transfer reagents have been designed for this purpose (2), none could be easily obtained as a labelled compound. Cold molecular sulphur S_8 can react with phosphites to give phosphorothionates directly (3). Molecular ^{35}S being a commercial radiochemical, we chose this reaction for the synthesis of radiolabelled fenthion [^{35}S]-**3** (scheme):



Scheme

Thus, for preparing [^{35}S]-**3**, we needed the phosphite **4**, which was obtained by simple methods. We used as a starting material the chlorophosphoramidite **5**, a reagent designed for oligonucleotide synthesis (4). This compound **5**, in the presence of sodium methanolate at low temperature, gave the dimethylphosphoramidite **6** in 89% yield. **6** Reacted with 2 equivalents of 4-methylthiometacresol in chloroform at room temperature to yield quantitatively the phosphite **4**. The excess phenol in this reaction was required to protonate the diisopropylamino group, thus allowing the substitution.This role in oligonucleotide synthesis is usually played by the weak acid tetrazole (4), which was not useful in our reaction. Attempts to purify the phosphite **4** by chromatography before reaction with sulphur were unsuccessful, resulting in decomposition either on a reversed phase column, or on silica gel or basic alumina. Furthermore, isolation by chromatography on neutral alumina was only partially successful, giving the phosphite **4** as a mixture with diisopropylamine. Thus, in subsequent experiments sulphur was reacted with the phosphite **4** without prior purification.

Addition of molecular sulphur S_8 to the reaction medium allowed the obtention of fenthion **3** in 73% yield for the purified product. The same reaction with ^{35}S and an aliquot of this reaction mixture, containing a large excess of phosphite **4**, gave [^{35}S]-**3** in 38% radiochemical yield. The product was purified by reversed phase chromatography, first on a preparative and then on an analytical column. Sulphur addition to phosphites usually requires the presence of bases such as pyridine or 2,6-lutidine in order to avoid side reactions (3). In this reaction the role of the base was played by diisopropylamine liberated in the preparation of the phosphite **4**.

This simple scheme would thus appear to be suitable for the preparation of any of the phosphorothionates **1** and their ^{35}S-labelled analogues, from the corresponding phenols and chlorophosphoramidites.

EXPERIMENTAL

Radioactivity was determined with a Beckman Model 1800 liquid scintillation counter using the Beckman Ready Value® cocktail as scintillation medium. ^{1}H NMR spectra were recorded on a Varian EM 360 spectrometer using tetramethylsilane as an internal standard. HPLC purifications were performed on a Waters apparatus on Merck columns : column A was a preparative glass column (240x10 mm) loaded with Licroprep RP18 (40-63 µm) and column B was a steel analytical column

(125x4 mm) loaded with Lichrospher 100 RP 18 (5 µm). Gradient elutions were run in methanol-water mixtures (flow rate 1 mL.min^{-1}). Molecular ^{35}S was purchased from Amersham (toluene solution - specific activity 1.233 Ci.mA^{-1}).

Dimethyl-N,N-diisopropylphosphoramidite **6**.

N,N-diisopropylmethylphosphonamidic chloride **5** (2.54 g, 12.8 mmol) in 30 mL of THF was added dropwise, under nitrogen atmosphere, to a stirred solution of sodium methanolate (0.85 g, 15.7 mmol) in 40 mL of THF, cooled to - 35°C. The cooling bath was removed, and the reaction medium was allowed to warm to room temperature. The solvent was then evaporated under reduced pressure, and the residue taken up with 50 mL of diethyl ether. The solution was washed with water (30 mL), saturated sodium hydrogenocarbonate (30 mL) and again with water (30 mL). After drying over Na_2SO_4, the solvent was evaporated to give **6** as a pale yellow oil (2.19 g, 88%); ^1H NMR ($CDCl_3$) δ 1.18 (d, J = 6.8 Hz, 12H, 2 $(CH_3)_2CH$), 3.58 (m, J = 6.8 Hz, 2H, 2 CH), 3.4 (d, H = 13.4 Hz, 6H, 2 CH_3O). Compound **6** decomposed slowly when left under air atmosphere, and was used without further purification.

Dimethyl-(3-methyl-4-methylthiophenyl)phosphite **4**.

To 1.8 g (9.3 mmol) of the phosphoramidite **6** in 30 mL of chloroform, was added 4-methylthiometacresol (2.88 g, 18.7 mmol), and the mixture was stirred at room temperature overnight. NMR monitoring showed that the reaction was complete in this period. The solvent was evaporated under reduced pressure and the residue was purified by chromatography on neutral alumina with hexane-dichloromethane (50:50) as eluent. A fraction was isolated which contained 0.595 g (26%) of **4** and 66 mg of diisopropylamine (NMR determination). Attempts to purify **4** on silica gel or basic alumina resulted in complete decomposition. **4**: ^1H NMR δ 2.25 and 2.30 (2s, 2x3H, CH_3S and CH_3phenyl), 3.45 (d, J = 10 Hz, 6H, 2 CH_3O), 6.6-6.9 (m, 3H, Hphenyl).

Dimethyl-(3-methyl-4-methylthiophenyl)thiophosphate **3**.

To the reaction mixture containing the *in situ* product **4**, was added molecular sulphur (595 mg, 18.6 mmol), and the mixture was stirred at room temperature for 3 h. Evaporation of the solvent

under reduced pressure and chromatography of the residue on silica gel [eluent hexane-dichloromethane (50:50)] gave 1.88 g (73%) of **3**, as a colorless oil. ^{1}H NMR δ 2.27 and 2.37 (2s, 2x3H, CH_3S and CH_3phenyl), 3.78 (d, J = 14 Hz, 6H, 2 CH_3O), 6.8-7.3 (m, 3H, Hphenyl). This spectrum was identical to that obtained from an authentic sample of fenthion.

[^{35}S]-Dimethyl-(3-methyl-4-methylthiophenyl)thiophosphate [^{35}S]-**3**.

To 3.5 ml of the reaction mixture of compound **4** [expected to contain about 300 mg (1.09 mmol) of **4**] was added 0.15 mL of a solution of ^{35}S in toluene (2.52 mCi), followed by stirring at room temperature for 2 days. The solvents were removed under reduced pressure and the residue taken up with a mixture of 0.65 mL of methanol and 0.85 mL of water. The resulting suspension was filtered through a Millex® filter before injection onto column A equilibrated with methanol-water (45:55). The column was eluted using a linear gradient (45% to 100% methanol over 60 min), then isocratic. The fractions containing the main peak of radioactivity were combined and the solvents evaporated. The residue was taken up with 2 mL of 45% aqueous methanol and the solution was injected onto column B, using a Rheodyne injector equipped with a 2 mL sample loop. The chromatography was effected using a linear gradient (45% to 100% methanol over 10 min), then isocratic elution. The peak corresponding to fenthion was collected. This contained 0.90 mCi (38% radiochemical yield after ^{35}S decay correction). The chemical purity of [^{35}S]-**3** was checked by HPLC and was found to be greater than 99%.

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