THE SYNTHESIS OF ^{32}P AND ^{14}C-LABELLED O,S-DIMETHYL PHOSPHORAMIDOTHIOATE

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SUMMARY

O,S-Dimethyl phosphoroamidothioate has been synthesized with ^{32}P and with ^{14}C in the methoxy and the S-methyl group. Phosphorus trichloride was reacted with sulfur giving phosphorus thiochloride. The latter was reacted with methanol in the presence of acridine and calcium oxide yielding O-methyl phosphorodichloridothioate. Isomerization at 100°C gave S-methyl phosphorodichloridothioate. Finally, treatment with methanol and ammonia gave O,S-dimethyl phosphoroamidothioate. ^{32}P-labelled phosphorus trichloride and ^{14}C-methanol were appropriately introduced to yield the desired compound.

The intermediates and final product were characterized by NMR, mass spectra and TLC. Products were purified by solvent partition (S-methyl labelled) and anion exchange chromatography (^{32}P-labelled). Radiochemical purity was determined by TLC. Liquid Scintillation counting was used to determine specific activities. The ^{32}P-labelled compound had a specific activity of 163 μCi/mM while the ^{14}C-methoxy and S-methyl compounds had specific activities of 86 and 68 μCi/mM respectively.

INTRODUCTION

O,S-Dimethyl Phosphoroamidothioate is a relatively new insecticide of systemic activity sold under the brand names Monitor (Chevron Chemical Co. U. S. A.) and Tamaron (Bayer Chemical Co., Germany). In an effort to study

the mode of decomposition and fate of residues on soils and tomato plants it was necessary to synthesize the phosphoroamidothioate labelled with ^{14}C in the methoxy as well as in the S-methyl group. Since the phosphoroamidothioate is a relatively simple molecule its decomposition studies also required that it should be synthesized with ^{32}P. A thorough review of the literature shows no published procedure for the synthesis of the phosphoroamidothioate labelled with ^{14}C. Khasawinah[1] has synthesized the phosphoroamidothioate with ^{32}P using reactions different from those used in the present work and which produce a low yield and a low specific activity. Furthermore, it used a labelled starting material not readily available.

The approach chosen in this work was to start with a readily available ^{32}P-labelled material such as phosphorus trichloride. The synthetics steps chosen in this work which are shown in Figure 1 are advantageous in the fact that they give few undesired side reactions and they can be easily adapted to the synthesis of a radiolabelled material. The reaction of phosphorous trichloride with sulfur, in the presence of aluminium chloride, yields phosphorus thiochloride[2]. Peterson[3] reported yields of O-methyl phosphoro-

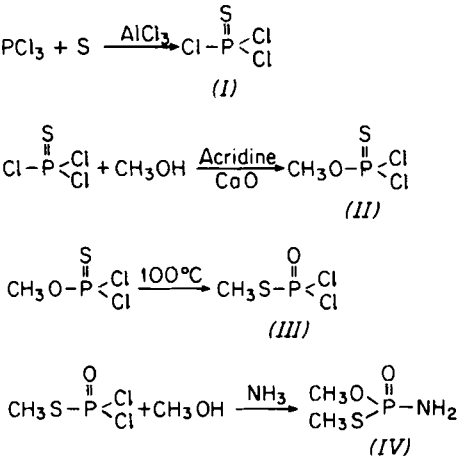


Figure 1 *Scheme for the Synthesis of O,S-Dimethyl Phosphoroamidothioate*

dichloridothioate (II) of 95% if acridine and CaO are used as catalysts in the reaction of phosphorus thiochloride with methanol. Hilgetag[4] *et al.* showed that heating (II) at 100°C will, after five hours, afford S-methyl phosphorodichloridothioate (III) in yields of about 79%. The final reaction step consists of a methoxy substitution as well as an amidation reaction yielding O,S-dimethyl phosphoroamidothioate (IV) as reported by Cölln[5] who obtained yields of 75%.

This work presents the synthesis of O,S-dimethyl phosphoroamidothioate labelled individually with ^{14}C in either carbon as well as with ^{32}P.

EXPERIMENTAL

Phosphorus Thiochloride-^{32}P (I)

Phosphorus trichloride (250 mg, 1.82 mM, 11.2 mCi) was diluted with non-labelled phosphorus trichloride (6.45 g, 47.0 mM). Then, sulfur (1.80 g, 56.2 mM) and anhydrous aluminum chloride (200 mg, 1.5 mM) were added and the reaction mixture was heated at 100°C in a sand bath and allowed to reflux for fifteen minutes. The product was distilled at 60°C (90 mm Hg) yielding 5.8 g (72%) of I.

O-Methyl-Phosphorodichloridothioate-^{32}P (II)

Phosphorus thiochloride-^{32}P (5.8 g, 34.2 mM) and non-labelled thiochloride (600 mg, 3.5 mM) were dissolved in 30 ml of benzene. Then, acridine (0.400 g, 2.2 mM) and calcium oxide (4.5 g, 80.2 mM) were added while the entire mixture was kept at 15°C. Methanol (1.74 g, 54.4 mM) was added during a period of one hour while the mixture was thoroughly stirred. After an additional stirring period of 3 hours, the solids were filtered out of the reaction mixture and washed repeatedly with benzene which removed traces of entrained II. Acridine was removed by washing the benzene phase twice with 2% HCl cooled to 2°C. The benzene was evaporated and the compound was subjected to the isomerization

reaction.

O-^{14}C-Methyl-Phosphorodichloridothioate (II)

By using ^{14}C-methanol in step 2 the labelled methoxy group is introduced in the molecule and thus after isomerization the ^{14}C-activity will be in the S-methyl group. ^{14}C-methanol (specific activity 59 mCi/mM, 1.6 mg, 0.051 mM) diluted with non-labelled methanol (1.74 g, 54.4 mM) was added to phosphorus thiochloride (6.4 g, 37.8 mM) using the same procedure and amounts of other reagents as described previously for the ^{32}P-labelled material. Compound II was also synthesized with non-labelled material in order to characterize the intermediate and also to produce sufficient S-methyl phosphorodichloridothioate (III) as starting material for the synthesis of (IV) labelled in the methoxy position. By using 0.5 mol of phosphorus thiochloride, 1 mol of CaO, 1.0 mM of acridine, 0.75 mol of methanol and by using the procedure previously described, 67.5 g (82%) of II were obtained after distilling the reaction product at 60°C and 55 mm Hg. However, in the synthesis of labelled II, the distillation was obviated so as to minimize losses. A NMR spectrum taken with a Varian XL-100 MHZ high resolution spectrometer yielded a doublet centered at δ 4.00 ppm (d, 3H, CH_3O-P, J = 19 Hz, solvent $CDCl_3$).

S-Methyl-Phosphorodichloridothioate (III)

^{32}P-Labelled Compound

The reaction product containing II was placed in a round bottom flask and heated in a sand bath at 100°C for a period of five hours. The heating was stopped and the reaction product distilled (75.5°C, 10 mm Hg). The NMR spectrum of this compound showed a doublet centered at δ 2.65 ppm (d, 3H, CH_3S-P, J = 21 Hz, solvent $CDCl_3$).

^{14}C-Labelled Compound

The same procedure as described above in the phosphorus synthesis was ap-

plied here and 2.0 g of compound III were obtained.

Non-Labelled Compound

Compound II (60 g, 0.36 mol) was heated as described for the ^{32}P-labelled material. Aliquots of the reaction mixture were removed and NMR spectra were obtained. Distillation yielded 46.8 g (78.0%) of compound III.

O-Methyl-S-Methyl Phosphoroamidothioate (IV)

^{14}C-Methoxy-Labelled Compound

To a three-necked flask, III (2.76 g, 16.7 mM) was mixed with 150 ml of methylene chloride. The solution was continuosly stirred and kept in an ice bath at -8°C. Subsequently ^{14}C-methanol (specific activity 59 mCi/mM, 1.6 mg, 0.051 mM) diluted with non-labelled methanol (0.793 g, 24.7 mM) was added. After five minutes the solution was saturated with dry ammonia gas and the reaction mixture was filtered to eliminate NH_4Cl. The methylene chloride was removed in a rotary vacuum evaporator and the remaining slightly colored and hygroscopic oil was lyophilized to remove water giving IV as a white solid (1.35 g, 57.2% based on III).

S-^{14}C-Methyl Compound

Compound III labelled with ^{14}C (1.95 g, 11.8 mM), was dissolved in 100 ml of CH_2Cl_2 and methanol (0.510 g, 15.9 mM) was added. After completing the procedure described above, 1.30 g (77% yield) of IV was obtained.

^{32}P-Labelled Compound

^{32}P-labelled III (3.0 g, 18.2 mM) and methanol (0.793 g, 24.7 mM) were reacted in similar manner as described previously. IV was obtained (2.05 g, 77%).

The NMR spectra of all three labelled compounds were taken and found to be identical to that obtained with non-labelled phosphoroamidothioate synthesized

by the Bayer Chemical Co. The NMR spectrum yields two doublets for each methyl group due to the proton-phosphorus interaction. The O-methyl protons show a doublet centered at δ 3.69 ppm (d, 3H, CH_3O-P, J = 12 Hz, solvent $CDCl_3$) while the S-methyl protons yield a doublet centered at δ 2.32 ppm (d, 3H, CH_3S-P, J = 14 Hz, solvent $CDCl_3$). These values are similar to those previously reported by Fahmy[6] *et al.* which showed a doublet centered at δ 2.2 ppm (d, 3H, CH_3S-P, J = 11 Hz) and at δ 3.7 ppm (d, 3H, CH_3O-P, J = 12 Hz). The mass spectrum of IV labelled in the methoxy and S-methyl group was found to be identical to the one obtained from a standard non-labelled sample (Bayer Chemical Co., Germany). The spectra showed a molecular ion at m/e 141 as well as ions at m/e 126 [$O_2P(SCH_3)$ (NH_2)], 111 [M-(CH_2=O)], 110 [M-(CH_3O)], 95 [M-(S=CH_2)], 94 [M-(SCH_3)], 80 [M-(S=CH_2+CH_3)], 79 [M-(SCH_3+CH_3)], 64 [M-(SCH_3+CH_2O)].

Radiochemical Purity and Specific Activity

The purity of the synthetized phosphoroamidothioate was studied by TLC as shown in Table 1. The plates were scanned with a Berthold Thin-Layer Scanner provided with a 2Π proportional counter. The ^{32}P-labelled compound was passed through a Dowex 1 x 8 anion exchange column in the hydroxide form. The radiochemical purity increased from 84.3% to 98.0%. The Rf values shown in Table 1 for the phosphoroamidothioate are identical to the ones obtained for the non-labelled standard material chromatographed under identical conditions but made visible by heating the plate after spraying with a 1% solution in hexane of 2,6-Dibromoquinone-4-chloroamide. Liquid scintillation counting yielded a specific activity of 163 μCi/mM for the purified compound at the end of the synthesis.

The S-^{14}C-methyl phosphoroamidothioate synthesized was disolved in 1 ml of H_2O and shaken with 2 ml of benzene. The benzene layer was removed and the aqueous layer was evaporated *in vacuo* and subsequently dried by lyophilization.

The radiochemical purity increased from 91.3% to 99.0%. The purified product gave by liquid scintillation counting a specific activity of 67.2 μCi/mM.

The ^{14}C-methoxy labelled phosphoroamidothioate had after synthesis a radiochemical purity of 96.2%. Further purification was found unnecessary. The specific activity obtained by liquid scintillation counting was 82.6 μCi/mM.

TABLE 1

TLC Studies of the Labelled O,S-Dimethyl Phosphoroamidothioate

Labelling	Rf Phosphoro-amidothioate	Rf Impurities	Purification Method	Radiochemical Purity
^{32}P-	0.76 [a]	0.58	Anion Exchange	98.0
$^{14}CH_3S$-	0.14 [b]	0.35	Benzene Partition	99.0
$^{14}CH_3O$	0.60 [c]	0.00	d	96.2

a Cellulose/95:5 Ethanol-Ammonia

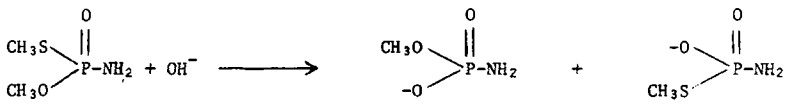
b Silica gel/Toluene

c Neutral Aluminum Oxide/2-Methoxyethanol

d Purification found unnecessary

Specificity of Labelling of the ^{14}C-Labelled Compounds

Fahmy[6] has shown that the alkaline hydrolysis in water and in 50% aqueous acetone yields the following products:



IV V VI

The reaction produces two volatile products: CH_3OH and $(CH_3)_2S$. In aqueous

alkaline hydrolysis, 82% of VI and 18% of V are produced while if the hydrolisis is carried out in 50% acetone the yields are 17% of VI and 83% of V. The two products can be separated by TLC[1]. Using Silicagel and 95% ethanol as the solvent, an Rf of 0.12 and an Rf of 0.37 are obtained for V and VI respectively by using 2,6 -Dibromoquinone-4-cloroamide in order to visualize the spots. When chromatographing IV, hydrolyzed in aqueous conditions and using as starting compound IV labelled in the methoxy position, the spot at Rf = 0.37 had no activity while the spot having Rf of 0.12 was active. Hydrolyzing IV, labelled in the methoxy position but utilizing as solvent 50% acetone, yields a spot having an Rf 0.12 which has activity but the spot having an Rf of 0.37 again shows no activity. However, if both types of hydrolysis are carried out with IV labelled in the S-methyl group, the spot having an Rf of 0.12 does not present activity, while the spot at Rf = 0.37 always presented activity. This conclusively shows the specificity of the labelling in the case of IV labelled with ^{14}C.

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