LABELLED ORGANOPHOSPHORUS PESTICIDES. I. SYNTHESIS OF CARBON-14 LABELLED O,O-DIMETHYL O-(3-METHYL-4-NITROPHENYL) PHOSPHOROTHIOATE (SUMITHION®).

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SUMMARY

O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate (I) (Sumithion®), an organophosphorus insecticide, was labelled with carbon-14 individually at the aryl methyl and the phenyl ring. The synthetic procedures are shown in Fig. 1 and 2. Coupling reaction of 3-methoxyphenylmagnesium bromide with methyl-^{14}C iodide followed by O-demethylation gave 3-methyl-^{14}C-phenol, which was nitrosated with sodium nitrite and subsequently oxidized with 30% nitric acid to give 3-methyl-^{14}C-4-nitrophenol in the overall yield of 48% from methanol-^{14}C. Condensation of 3-methyl-^{14}C-4-nitrophenol with O,O-dimethyl phosphorochloridothioate gave Sumithion-(aryl methyl-^{14}C) in 89% yield.

Grignard reaction of 1-bromo-3-methoxybenzene-$^{14}C_6$ with methyl iodide afforded 1-methoxy-3-methylbenzene-$^{14}C_6$ in good yield and the latter was converted to Sumithion-(phenyl-^{14}C) by the similar procedures used for the aryl methyl labelling. The overall yield of Sumithion-(phenyl-^{14}C) was 43% from 3-bromophenol-$^{14}C_6$.

Key Words: Carbon-14, 3-Methyl-4-nitrophenol, Organophosphorus Insecticide

INTRODUCTION

O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate[1], Sumithion® (I), is a famous insecticide being widely used for the control of both plant pests and insects of medical importance. In connection with metabolic studies[2-4] of this agent in mammals, radioactive compounds labelled in the O,O-dimethyl-phosphorothioic acid moiety with ^{14}C, ^{32}P and ^{35}S were already synthesized[5]. However, none of the previous investigations has clarified in detail the metabolic fate of the nitrocresol moiety. To make it clear, therefore, it was

required to synthesize radioactive Sumithion labelled in the nitrocresol moiety. This paper describes the synthesis of Sumithion-^{14}C labelled individually at both the aryl methyl and the phenyl ring.

DISCUSSION

The reaction sequence for the synthesis of Sumithion-(aryl methyl-^{14}C)(Ia) is shown in Figure 1.

In general, several procedures may be considered to incorporate carbon-14 into an aryl methyl group. For example, Downes *et. al.*[6] prepared 1-methoxy-2-methyl-^{14}C-benzene in 50% yield by carboxylation of 2-methoxyphenylmagnesium bromide with carbon-^{14}C dioxide followed by two-step reduction of the carboxyl-^{14}C group to methyl-^{14}C, while Hazue *et. al.*[7] synthesized 1-methoxy-3-methyl-4-methyl-^{14}C-benzene in 85% yield by coupling reaction of 1-lithium-4-methoxy-2-methylbenzene with methyl-^{14}C iodide.

Adaptation of these methods to the preparation of 1-methoxy-3-methyl-^{14}C-benzene gave lower yields and more erratic results in their reproducibility. In contrast, it was found that the Grignard reagent of 1-bromo-3-methoxybenzene underwent a coupling reaction with methyl-^{14}C iodide in excellent yield. Thus, treatment of a slight excess of 3-methoxyphenylmagnesium bromide with methyl-^{14}C iodide, which was prepared from methanol-^{14}C with 57% hydriodic acid, at 50-55° for 8 hr afforded 1-methoxy-3-methyl-^{14}C-benzene (II) in the overall yield of 78.1% from methanol-^{14}C.

The reaction of II with boron tribromide at room temperature for several hours gave 3-methyl-^{14}C-phenol (III) in good yield. O-Demethylation with this reagent was more attractive than with conventional strong acids, since the reaction could be carried out under moderate conditions and the decomposition of the product could be considerably avoided. 3-Methyl-^{14}C-phenol thus obtained was radiochemically pure. However, it contained approximately 30% of phenol as an inactive impurity which apparantly originated from the excess of 3-methoxyphenylmagnesium bromide used for the coupling reaction. Since it was observed that the contamination with phenol caused undesirable results in the following

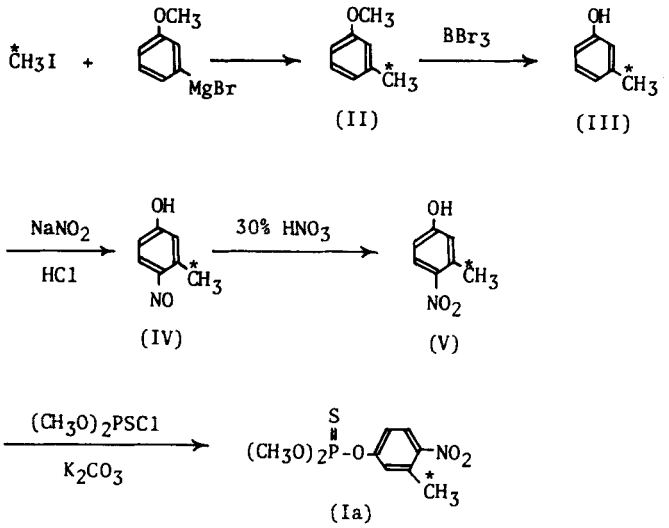

Figure 1. The reaction sequence for the synthesis of Sumithion-(aryl methyl-^{14}C)

reaction, the cresol-^{14}C was purified by partition chromatography on a column of silica gel containing 38% of water with hexane as a mobile phase. After chromatography, the purity was nearly 100% both radiochemically and chemically and the yield was 76.0% from II.

3-Methyl-^{14}C-phenol was treated with sodium nitrite[1] in the presence of concentrated hydrochloric acid to yield 3-methyl-^{14}C-4-nitrosophenol (IV), which was immediately oxidized with 30% nitric acid at 40° to give 3-methyl-^{14}C-4-nitrophenol (V) in 80.6% from III. Its radiochemical purity was 98% as determined by radio-thinlayerchromatography and no isomeric nitrocresols, but 3-methyl-^{14}C-4-nitrosophenol was contained as an impurity.

Conversion of V to Sumithion-(aryl methyl-^{14}C)(Ia) was achieved by allowing V to react with O,O-dimethyl phosphorochloridothioate[1] in the presence of potassium carbonate at 80° for 3 hr and followed by column-chromatography on silica gel. The product (Ia) had a specific activity of 3.58 mCi/mmole and was identical in all respects with the unlabelled authentic sample. The overall yield of Ia was 42.4% from methanol-^{14}C.

Figure 2 illustrates the procedure for the synthesis of Sumithion-(phenyl-^{14}C)(Ib). 3-Bromophenol-$^{14}C_6$ (VI), which was synthesized by Moriya[8], was used as the starting material for the synthesis of Ib. O-Methylation of VI with dimethyl sulfate in 10% sodium hydroxide solution gave 1-bromo-3-methoxybenzene-$^{14}C_6$ (VII) in 95% yield.

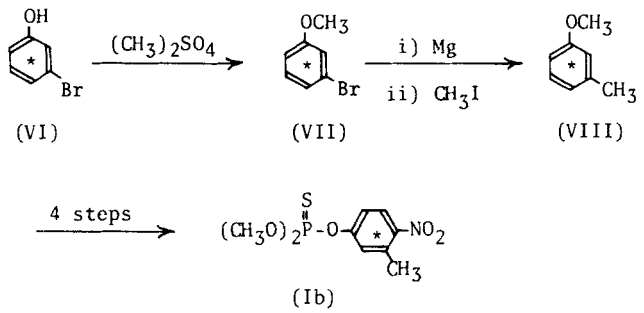

Figure 2. The reaction sequence for the synthesis of Sumithion-(phenyl-^{14}C)

Grignard reaction of VII with magnesium and an excess of methyl iodide gave a mixture of 1-methoxy-3-methylbenzene-$^{14}C_6$ (VIII), methoxybenzene-$^{14}C_6$ and unknown radioactive by-products; each ratio was 80, 10 and 10% respectively as determined by radio-gaschromatography. The radiochemical yield of the mixture was 96.6% from VII. Without purification the mixture was employed for the next reaction.

Conversion of VIII to Sumithion-(phenyl-^{14}C)(Ib) was carried out by adapting the procedures used for the aryl methyl labelling as described above; which comprised O-demethylation of VIII with boron tribromide, nitrosation with sodium nitrite, oxidation with 30% nitric acid and then condensation of the resultant 3-methyl-4-nitrophenol-$^{14}C_6$ with O,O-dimethyl phosphorochloridothioate. The results are described in detail in the experimental. The overall yield of Ib from 3-bromophenol-$^{14}C_6$ was 43.4% and the purity over 99%. The product (Ib) had a specific activity of 5.54 mCi/mmole and was identical in every respect with the unlabelled authentic sample.

EXPERIMENTAL

The purities of the volatile products were evaluated by radio-gaschromatography, which was conducted on 1 m long 3 mm diameter glass tube packed with 20% DC-550 on Chromosorb W (60-80 mesh). Column temperature was 90°; carrier gas was helium (30 ml/min); the detector was TCD; the radioactivity was counted by a gas-flow type of GM-counter (Nihon-Musen Co., Ltd., Japan). The retention times of the authentic materials were: 3-bromophenol (16.5 min), 1-bromo-3-methoxybenzene (12.0 min), methoxybenzene (1.8 min), 1-methoxy-3-methylbenzene (3.3 min), phenol (2.5 min) and 3-methylphenol (4.5 min).

Methyl-^{14}C Iodide -- Methyl-^{14}C iodide was prepared from methanol-^{14}C (68.8 mCi, 8.42 mmol) by the well-known method[9] which comprised iodination with refluxing 57% hydriodic acid. The product was purified by distillation and employed for the following reaction without measuring the radioactivity.

1-Methoxy-3-methyl-^{14}C-benzene -- A solution of 1-bromo-3-methoxybenzene (2.24 g, 12.0 mmol) in anhydrous tetrahydrofuran (5 ml) was added dropwise to a suspension of magnesium turnings (0.32 g, 13 mmol) in anhydrous tetrahydrofuran (5 ml) under gentle refluxing during 0.5 hr. The reaction mixture was then heated to reflux for 1 hr. After cooling, the reaction flask was connected to a vacuum manifold and the mixture was frozen with liquid nitrogen. To the frozen mixture was added under reduced pressure by distillation the methyl-^{14}C iodide obtained above. After addition of anhydrous ether (10 ml), the mixture was heated at 50-55° under atomospheric pressure for 8 hr. The mixture was acidified with 10% hydrochloric acid and extracted with ether. The extract was washed with 5% sodium thiosulfate solution and then water, dried over sodium sulfate and evaporated under atomospheric pressure to give 1-methoxy-3-methyl-^{14}C-benzene (53.7 mCi, 78.1% from methanol-^{14}C). The radio-gaschromatography of the product showed that its purity was radiochemically 99% and chemically 75%; methoxybenzene was contained as a cold impurity. The product was used for the next reaction without any purification.

3-Methyl-^{14}C-phenol -- To a stirred solution of 1-methoxy-3-methyl-^{14}C-benzene (53.7 mCi, 6.57 mmol) in anhydrous dichloromethane (30 ml) was added boron tribromide (5.0 g, 20 mmol) at -5°. The mixture was stirred at 0° for 0.5 hr and then at room temperature for 1 hr. To the mixture was cautiously added at 0° 10% sodium bicarbonate solution (50 ml) and the resultant slurry was extracted with ether. The ethereal solution was extracted with 10% sodium hydroxide solution. The basic extract was acidified with concentrated hydrochloric acid, extracted with ether and the the ethereal extract dried over sodium sulfate. Removal of the solvent under atomospheric pressure gave an oily residue (52.3 mCi), which was subjected to partition chromatography on a column of silica gel containing 38% of water and eluted with hexane and 10% ether-hexane successively. The hexane eluate was evaporated under atomospheric pressure to give 3-methyl-^{14}C-phenol (40.8 mCi, 540 mg, 8.16 mCi/mmol, 76%); the radio-gaschromatography showing its purity of nearly 100% both radiochemically and chemically.

3-Methyl-^{14}C-4-nitrophenol -- To a mixture of 3-methyl-^{14}C-phenol (40.8 mCi, 540 mg, 5.0 mmol), diluted with inactive 3-methylphenol (690 mg, 6.4 mmol), and concentrated hydrochloric acid (4.2 ml) in isopropyl alcohol (1.5 ml) was added dropwise with stirring a solution of sodium nitrite (1.10 g, 16 mmol) in water (1.6 ml) at -10° to -5° over a period of 15 min. After stirring at the same temperature for 2 hr, the crude product which was precipitated was collected by filtration and the pale yellow crystals were washed with cold water to afford 3-methyl-^{14}C-4-nitrosophenol. The product showed an identical R_f-value (0.45) with that of the authentic sample on the silica gel TLC developed in nitromethane and had 98% radiochemical purity. The crystals were then added to 30% nitric acid (2.6 ml) at room temperature and the mixture was heated with stirring at 40° for 2 hr. After cooling in an ice-bath, the precipitate was filtered, washed with water and dried *in vacuo* to give 3-methyl-^{14}C-4-nitrophenol (32.9 mCi, 1.40 g, 3.58 mCi/mmol, 80.6%) as pale yellow needles; having mp 128-129° and a R_f-value of 0.55 on the silica gel TLC with nitromethane. The product was identical in every respect with the unlabelled authentic sample.

O,O-Dimethyl O-(3-Methyl-^{14}C-4-nitrophenyl) Phosphorothioate (Ia) -- To a mixture of O,O-dimethyl phosphorochloridothioate (1.73 g, 10.8 mmol) and potassium carbonate (1.48 g, 10.8 mmol) in methyl isobutyl ketone (25 ml) was added dropwise a solution of 3-methyl-^{14}C-4-nitrophenol (32.9 mCi, 1.40 g, 9.18 mmol) in the same solvent (25 ml) at room temperature during 0.5 hr and the mixture was heated at 80° for 3 hr. After cooling, the mixture was poured into ice-water and extracted with benzene. The extract was washed with water, dried over sodium sulfate and evaporated under reduced pressure to leave an oily residue (32.1 mCi). The residue was chromatographed on silica gel and eluted with benzene. Evaporation of the solvent gave O,O-dimethyl O-(3-methyl-^{14}C-4-nitrophenyl) phosphorothioate (Ia) (29.2 mCi, 2.26 g, 3.58 mCi/mmol, 88.8%) which was identical in every respect with the unlabelled authentic sample.

1-Bromo-3-methoxybenzene-$^{14}C_6$ -- To a solution of 3-bromophenol-$^{14}C_6$ (41.0 mCi, 900 mg, 6.30 mmol, 6.51 mCi/mmol) in 10% sodium hydroxide solution (4 ml) was added dropwise dimethyl sulfate (1.13 g, 9.0 mmol) at room temperature during 10 min. The mixture was stirred at the same temperature for 15 min and then heated at 100° for 1.5 hr. After cooling, the mixture was extracted with ether. The extract was washed with water, dried over sodium sulfate and evaporated under atomospheric pressure to give an oily residue. In order to remove the moisture in the residue, the residue was taken up in benzene (10 ml) and the solvent was evaporated under atomospheric pressure to afford 1-bromo-3-methoxybenzene-$^{14}C_6$ (38.9 mCi, 94.9%). The radio-gaschromatography of the product showed its radiochemical and chemical purities to be nearly 100%.

1-Methoxy-3-methylbenzene-$^{14}C_6$ -- To a suspension of magnesium turnings (175 mg, 7.20 mmol) in anhydrous tetrahydrofuran (2 ml) was added under gentle refluxing a solution of 1-bromo-3-methoxybenzene-$^{14}C_6$ (38.9 mCi, 6.00 mmol, 6.51 mCi/mmol) in anhydrous tetrahydrofuran (5 ml) during 0.5 hr. The mixture was continued to reflux for 1 hr. After cooling, to the mixture at room temperature was added methyl iodide (2.8 g, 20 mmol). The mixture was stirred at the same temperature

for 15 min and then at 50° for 2.5 hr. After addition of 10% sulfuric acid (10 ml) under cooling, the resulting slurry was extracted with ether. The extract was washed with 5% sodium thiosulfate solution and water, dried and evaporated to afford an oily residue, which was taken up in hexane (20 ml) and the solution was concentrated to give 1-methoxy-3-methylbenzene-$^{14}C_6$ (37.6 mCi, 96.6%). The radiochemical purity of the product was 80%; methoxybenzene-$^{14}C_6$ (10%) and unknown radioactive by-products (10%) were contained.

3-Methylphenol-$^{14}C_6$ -- To a solution of 1-methoxy-3-methylbenzene-$^{14}C_6$ (37.6 mCi, 5.78 mmol) in anhydrous dichloromethane (15 ml) was added at -10° under stirring boron tribromide (3.77 g, 15 mmol). The mixture was stirred continuously at -5° for 15 min, at 0° for 0.5 hr, and at room temperature for 0.5 hr. To the mixture was added ice-water and the slurry extracted with ether. The extract was worked up in the way described for 3-methyl-^{14}C-phenol and purified by partition chromatography to give 3-methylphenol-$^{14}C_6$ (25.1 mCi, 6.51 mCi/mmol, 67.3%); its purity being nearly 100% both radiochemically and chemically.

3-Methyl-4-nitrophenol-$^{14}C_6$ -- To a mixture of 3-methylphenol-$^{14}C_6$ (25.1 mCi, 416 mg, 3.85 mmol), inactive 3-methylphenol (80 mg, 0.75 mmol) and concentrated hydrochloric acid (1.7 ml) in isopropyl alcohol (0.5 ml) was added dropwise a solution of sodium nitrite (480 mg, 7.0 mmol) in water (0.7 ml) at -10° during 0.5 hr. Working up in the manner described for the methyl labelling gave 3-methyl-4-nitrosophenol-$^{14}C_6$ (98% purity), which was added portionwise at room temperature to 30% nitric acid (2.5 ml) and the mixture stirred at 37° for 5 hr. After cooling in an ice-bath, the crystalline precipitate was filtered and dried under vacuum to give 3-methyl-4-nitrophenol-$^{14}C_6$ (20.8 mCi, 5.54 mCi/mmol, 83.2%); the purity as determined by radio-TLC was 97%.

O,O-Dimethyl O-(3-Methyl-4-nitrophenyl-$^{14}C_6$) Phosphorothioate (Ib) -- A mixture of 3-methyl-4-nitrophenol-$^{14}C_6$ (20.8 mCi, 3.75 mmol), O,O-dimethyl phosphorochloridothioate (660 mg, 4.58 mmol), potassium carbonate (630 mg, 4.58 mmol) and methyl isobutyl ketone (30 ml) was stirred at 70° for 2 hr. After cooling,

the mixture was worked up in the same manner as described for the methyl labelling to yield O,O-dimethyl O-(3-methyl-4-nitrophenyl-$^{14}C_6$) phosphorothioate (Ib) in 85% yield (17.8 mCi, 889 mg, 5.54 mCi/mmol); the purity was 99% both radiochemically and chemically.

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