# LABELLED ORGANOPHOSPHORUS PESTICIDES IV. SYNTHESES OF OPTICALLY ACTIVE O-ARYL O-ETHYL PHENYLPHOSPHONOTHIOATES LABELLED WITH CARBON-14

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## SUMMARY

*Optically active O-p-cyanophenyl O-ethyl phenylphosphonothioate (cyanofenphos) (1) and O-ethyl O-p-nitrophenyl phenylphosphonothioate (EPN)(2), organophosphorus insecticides, were labelled with carbon-14 at the phenyl ring of the phenylphosphonothioic acid moiety for use in metabolic studies. The synthetic procedures were shown in Fig. 1. Treatment of benzene-$^{14}C_6$ with phosphorus trichloride in the presence of anhydrous aluminum chloride followed by sulfurization with sulfur gave phenyl-$^{14}C_6$-phosphonodichloridothioate (3). Reaction of 3 with ethanol gave O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioate (4). Hydrolysis of 4 gave (±)-O-ethyl phenyl-$^{14}C_6$-phosphonothioic acid (5). Optical resolution of 5 with brucine afforded (-)- and (+)-enantiomers (5a and 5b), which were chlorinated with oxalyl chloride to give (-)- and (+)-chloridothioates (4a and 4b), respectively. Condensation of 4a and 4b with p-cyanophenol yielded (+)- and (-)-cyanofenphos-(phenyl-$^{14}C_6$)(1a and 1b) in the overall yields of 4.0 and 4.5%, respectively. Similarly, condensation of 4a and 4b with p-nitrophenol afforded (+)- and (-)-EPN-(phenyl-$^{14}C_6$)(2a and 2b) in the overall yields of 4.2 and 5.5%, respectively.*

Key Words: Carbon-14, Organophosphorus Pesticide, Optically Active, Cyanofenphos, EPN

## INTRODUCTION

O-*p*-Cyanophenyl O-ethyl phenylphosphonothioate (cyanofenphos)(1) and O-ethyl O-*p*-nitrophenyl phenylphosphonothioate (EPN)(2) have insecticidal

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activities useful for the control of many insect pests[1-3]. Since these compounds contain an asymmetric phosphorus atom in the molecule, optical isomers are possible. It has been known that the enantiomers of organophosphorus compounds show differences in their biological activities and toxicities due in part to the differential metabolism of them[4-7]. To compare the metabolism of racemic, (+)- and (-)-cyanofenphos, the enantiomers labelled with carbon-14 at the cyano group were already synthesized[7]. In the investigations by using the labelled compounds, it became apparent that cyanofenphos was biodegradated in relatively early stage to give cyanophenol residues and phenylphosphonothioic acid residues. To clarify the metabolic fates of the acid residues, it was required to prepare the enantiomers labelled with carbon-14 at the acid moiety.

In this paper, we describe the synthetic methods for the preparation of optically active cyanofenphos-$^{14}C_6$ and EPN-$^{14}C_6$ labelled at the phenyl ring of the acid moiety.

## DISCUSSION

Figure 1 illustrates the reaction sequences for the syntheses of optically active cyanofenphos-(phenyl-$^{14}C_6$)(1a and 1b) and EPN-(phenyl-$^{14}C_6$)(2a and 2b). O-Ethyl phenyl-$^{14}C_6$-phosphonothioic acid (5), an important intermediate for these compounds, was synthesized in the following manner. Benzene-$^{14}C_6$ was reacted with phosphorus trichloride in the presence of anhydrous aluminum chloride[8] to yield a complex of dichlorophenyl-$^{14}C_6$-phosphine-aluminum chloride. The complex was immediately sulfurized with powdered sulfur, and subsequent hydrolysis of the resulting product with 5% hydrochloric acid gave phenyl-$^{14}C_6$-phosphonodichloridothioate (3) in 88% yield, based on benzene-$^{14}C_6$. Esterification of 3 with ethanol in the presence of triethylamine afforded O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioate (4) in 77% yield. In this reaction, a small amount of O,O-diethyl phenyl-$^{14}C_6$-phosphonothioate was produced as a by-product but removed by column chromatography on silica gel with hexane-benzene. Hydrolysis of 4 with 2N potassium hydroxide solution at room temperature for 70

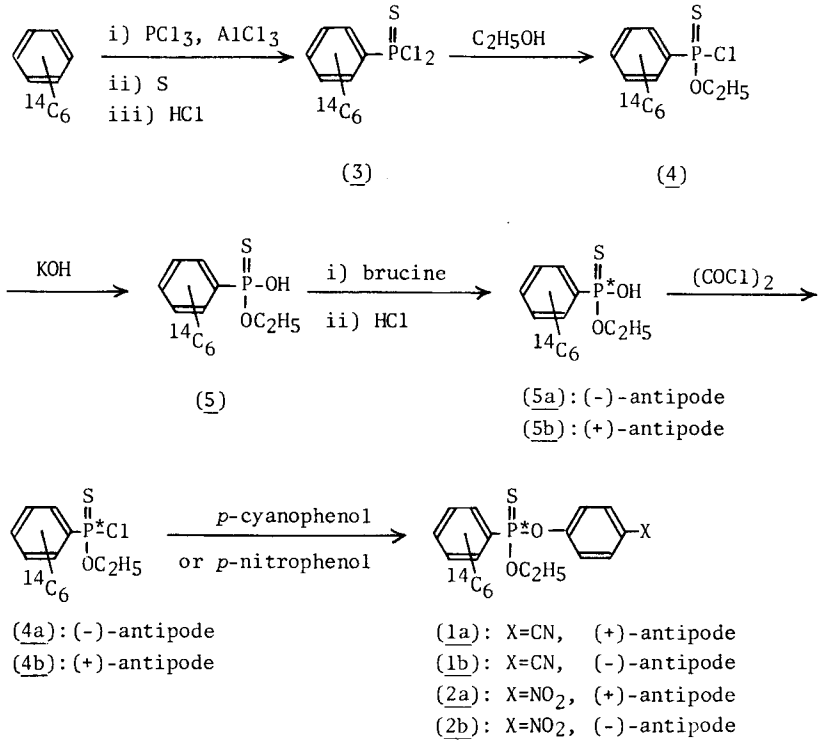


Fig. 1. The reaction sequences for the syntheses of optically active cyanofenphos-(phenyl-$^{14}C_6$) (1a,1b) and EPN-(phenyl-$^{14}C_6$) (2a,2b)

hr gave the racemic acid (5) in a quantitative yield.

Ohkawa *et al.* reported that unlabelled O-ethyl phenylphosphonothioic acid readily formed crystalline brucine salts, which were used to effect an optical resolution of the acid[7]. We successfully applied the method for this radioactive preparation. Thus, treatment of the racemic acid (5) with an equivalent of brucine in methanol gave the diastereomeric brucine salts. Recrystallization of the salts from acetone gave the salt of (-)-antipode as the head crop. The residue obtained from the mother liquid was then recrystallized from methanol to give the salt of (+)-antipode. Treatment of the salts of (-)- and (+)-antipodes with 1N hydrochloric acid gave the desired (-)- and (+)-acids (5a and 5b) in 17% and 21% yields, respectively. The $[\alpha]_D^{20}$ values in chloroform were -12.6° (c= 4.76) for 5a and +11.1° (c=5.68) for 5b. Some interesting observations have

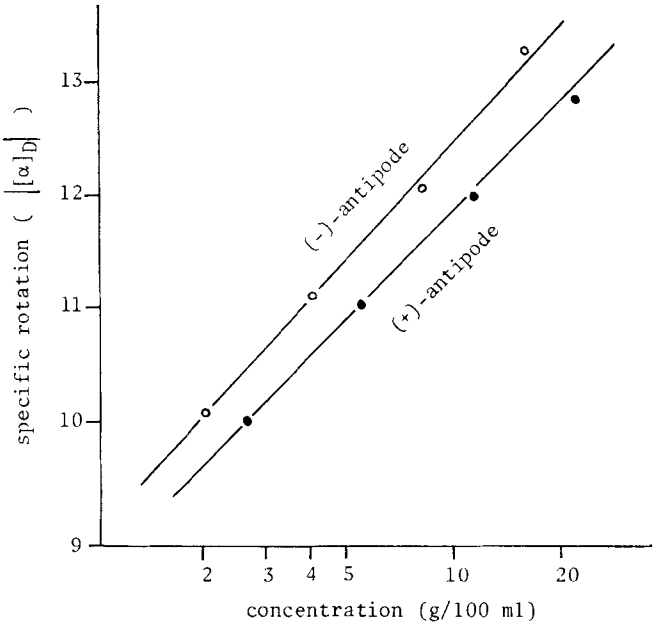


Fig. 2. Effect of concentration on the specific rotation of O-ethyl phenylphosphonothioic acid in chloroform

been made with respect to the specific rotation of the acids (5a and 5b), the results of which are shown in Fig. 2. As can be seen from Fig. 2, the specific rotations of the acids are affected by their own concentrations in chloroform. For example, by increasing the concentrations of the acids, one obtains larger absolute values for the specific rotations. Aaron *et al.* reported similar observations on the specific rotation of O-ethyl ethylphosphonothioic acid, and suggested that it should be possible to correlate the acid dissociation constant and the observed rotation[9].

Attempts to obtain optically active O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioates (4a and 4b) by chlorination of the acids (5a and 5b) with commonly used phosphorus pentachloride[7,10] afforded unsatisfactory results. For example, when 5a was treated with less than 1.5 equivalents of phosphorus pentachloride which was freshly sublimed before use, the desired product (4a) was obtained only below 30% yield although its specific rotation was maintained as high as

that of the unlabelled authentic sample. On the other hand, when a large amount of the chlorinating agent was used, the yield of 4a increased but its specific rotation considerably decreased due to the racemization taking place during the reaction. After intensive investigations, 4a and 4b were found to be readily accessible in moderate yields and higher specific rotations by treatment of the acids (5a and 5b) in halogenohydrocarbon medium with oxalyl chloride. Chlorination of 5a and 5b in chloroform with 3 equivalents of freshly distilled oxalyl chloride at room temperature for 4 hr gave 4a [$[\alpha]_D^{20}$ -80.6° (c= 4.71)] and 4b [$[\alpha]_D^{20}$ +77.4° (c=5.27)] in 86% and 87% yields, respectively. The $[\alpha]_D$ values were higher than those described in the literature[7].

Condensation of 4a and 4b with *p*-cyanophenol in the presence of anhydrous potassium carbonate[7] gave optically active cyanofenphos-(phenyl-$^{14}C_6$), 1a [$[\alpha]_D^{20}$ +39.2° (c=2.71)] and 1b [$[\alpha]_D^{20}$ -38.9° (c=3.21)], in the overall yields of 4.0% and 4.5% from benzene-$^{14}C_6$, respectively. Recrystallization of 1a and 1b from hexane resulted in the improvement of their specific rotations; $[\alpha]_D^{20}$ +41.9° (c=2.40) and -43.0° (c=2.84) for 1a and 1b, respectively. Similarly, recrystallization of the unlabelled 1b from hexane also improved its $[\alpha]_D$ value of -32.2° (c=4.30) to -40.5° (c=2.52). The residue ($[\alpha]_D^{20}$ -8.2°) recovered from the mother liquid was confirmed to be chemically pure by TLC and GC analyses, and to be identical with the authentic sample (1) in the analyses by IR and NMR spectra. The facts indicate that the improvement in the optical purity of 1a or 1b by the recrystallization was resulted by a kind of spontaneous resolution[11] occurring in a solution where existed a large excess of optically active compound as compared to the corresponding racemic form.

Condensation of 4a and 4b with *p*-nitrophenol in the similar manner as described above gave optically active EPN-(phenyl-$^{14}C_6$), 2a [$[\alpha]_D^{20}$ +36.6° (c= 2.81)] and 2b [$[\alpha]_D^{20}$ -36.2° (c=3.54)] in 4.2% and 5.5% yields based on benzene-$^{14}C_6$, respectively. In the cases of these compounds, recrystallization from any solvents was unsuccessful because of their low melting points.

## EXPERIMENTAL

Radiogaschromatography (RGC) was carried out on Yanako G80 Chromatograph (Yanagimoto MFG Co., Ltd., Japan) equipped with a gas-flow type of GM-counter. A glass column (2 m, 3 mm I.D.) packed with 2% SE-30 on Chromosorb W was used for phenylphosphonodichloridothioate (3) and O-ethyl phenylphosphonochloridothioate (4). Column temperature was 120°; carrier gas He (40 ml/min); detector TCD; counting gas propane (50 ml/min); oxidation temperature 600°. Retention times: 6.2 min for 3, 9.7 min for 4. A glass column (2 m, 3 mm I.D.) packed with 5% XE-60 on Chromosorb W was used for cyanofenphos (1) and EPN (2). Operating condition: column temperature 250°, carrier gas He (40 ml/min), detector FID ($H_2$ 45 ml/min). Retention times: 8.8 min for 1, 11.0 min for 2. Specific rotations were measured at 20° in chloroform in a cell (1 cm) on Perkin-Elmer 141 Spectropolarimeter.

Phenyl-$^{14}C_6$-phosphonodichloridothioate (3) -- A mixture of benzene-$^{14}C_6$ (100 mCi, 1.56 g, 20 mmol), phosphorus trichloride (5.36 ml, 61 mmol) and anhydrous aluminum chloride (5.33 g, 40 mmol) was heated under stirring at 80° for 3 hr. After cooling, powdered sulfur (0.68 g, 21 mmol) was added to the mixture. The mixture was heated under stirring at 85° for 20 min, cooled, and then added dropwise to 5% hydrochloric acid (10 ml) at 0°. After stirring for 30 min, the mixture was extracted with benzene, and the extract washed with water and dried over sodium sulfate. Concentration of the extract under reduced pressure gave phenyl-$^{14}C_6$-phosphonodichloridothioate (3)(87.9 mCi); the radiochemical purity being 92% on RGC and radiothinlayerchromatogram (RTLC)(silica gel, hexane/benzene=2/1 v/v, $R_f$-value 0.50). The product was used for the next reaction without any purification.

O-Ethyl Phenyl-$^{14}C_6$-phosphonochloridothioate (4) -- To a stirred solution of phenyl-$^{14}C_6$-phosphonodichloridothioate (3)(87.9 mCi, 17 mmol) in benzene (10 ml) was added dropwise a mixture of ethanol (0.89 g, 19 mmol) and triethylamine (2.1 g, 20 mmol) below 30°. The mixture was stirred at room temperature for

3 hr, acidified with 5% hydrochloric acid and extracted with benzene. The extract was washed with water, dried over sodium sulfate and evaporated under reduced pressure to leave an oily residue. Column chromatography of the residue on silica gel, eluted with hexane-benzene (2:1 v/v), gave O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioate (4)(67.5 mCi, 3.08 g); the radiochemical purity being 97% on RGC and RTLC (silica gel, hexane/benzene=2/1 v/v, $R_f$-value 0.37); NMR spectrum (δ, $CDCl_3$): 1.40 (3H, t, J=6 Hz, $-CH_2\underline{CH_3}$), 3.81-4.73 (2H, m, $O-\underline{CH_2}CH_3$), 7.19-8.24 (5H, m, aromatic H); IR spectrum ($CHCl_3$) being identical with that of the unlabelled authentic sample[7].

O-Ethyl Phenyl-$^{14}C_6$-phosphonothioic Acid (5) -- A mixture of O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioate (4)(67.5 mCi, 3.08 g, 13 mmol) and 2N potassium hydroxide solution (20 ml) was vigorously stirred at room temperature for 70 hr. The mixture was washed with benzene, then acidified with concentrated hydrochloric acid, and extracted with ethyl acetate. The extract was washed with water, dried over sodium sulfate and evaporated under reduced pressure to give O-ethyl phenyl-$^{14}C_6$-phosphonothioic acid (5)(64.4 mCi, 2.60 g); the radiochemical purity being 99% on RTLC (silica gel, toluene/ethyl formate/formic acid=5/7/1 v/v/v, $R_f$-value 0.34); NMR spectrum (δ, $CDCl_3$): 1.32 (3H, t, J=6 Hz, $-CH_2\underline{CH_3}$), 3.88-4.42 (2H, m, $O-\underline{CH_2}CH_3$), 7.22-8.15 (5H, m, aromatic H), 7.78 (1H, s, $\underline{OH}$); IR spectrum ($CHCl_3$) being identical with that of the unlabelled authentic sample[7].

(-)- and (+)-O-Ethyl Phenyl-$^{14}C_6$-phosphonothioic Acids (5a and 5b) -- To a solution of racemic O-ethyl phenyl-$^{14}C_6$-phosphonothioic acid (5)(64.4 mCi, 2.60 g, 13 mmol) in methanol (100 ml) was added brucine dihydrate (5.76 g, 13 mmol) at room temperature, and the mixture stirred at the same temperature for 10 min. After evaporating the mixture to dryness, a residue obtained was recrystallized four times from acetone (25-30 ml each) to give a brucine salt of (-)-antipode (1.56 g). The salt was suspended in ethyl acetate (10 ml), treated with 1N hydrochloric acid (10 ml) and then extracted with ethyl acetate. The extract was washed with water, dried over sodium sulfate and evaporated to give (-)-O-

ethyl phenyl-$^{14}C_6$-phosphonothioic acid (5a)(10.9 mCi, 0.44 g, 16.9% yield); $[\alpha]_D^{20}$ -12.6° (c=4.76). A residue recovered from the mother liquid was recrystallized from methanol (25-30 ml each) four times to give a brucine salt of (+)-antipode (2.27 g). The salt was worked up in the same manner as described above to give (+)-O-ethyl phenyl-$^{14}C_6$-phosphonothioic acid (5b)(13.2 mCi, 0.53 g, 20.5% yield); $[\alpha]_D^{20}$ +11.1° (c=5.68). Both of the products had the radiochemical purities of 99% on RTLC.

(-)- and (+)-O-Ethyl Phenyl-$^{14}C_6$-phosphonochloridothioates (4a and 4b) -- To a stirred solution of (-)-O-ethyl phenyl-$^{14}C_6$-phosphonothioic acid (5a)(10.9 mCi, 0.44 g, 2.2 mmol) in anhydrous chloroform (20 ml) was added freshly distilled oxalyl chloride (0.84 g, 6.6 mmol) in one portion at -15°. The mixture was stirred at 0° for 30 min and then at room temperature for 3 hr. The mixture was extracted with chloroform, and the extract washed with water and dried over sodium sulfate. Removal of the solvent under reduced pressure left an oily residue, which was chromatographed on silica gel with benzene. Evaporation of the main fraction gave (-)-O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioate (4a) (9.42 mCi, 0.42 g); $[\alpha]_D^{20}$ -80.6° (c=4.71); the radiochemical purity being 98% on RGC and RTLC; the structure being confirmed by NMR and IR spectra. (+)-O-Ethyl phenyl-$^{14}C_6$-phosphonothioic acid (5b) (13.2 mCi, 0.53 g, 2.6 mmol) was similarly chlorinated with oxalyl chloride (1.0 g, 7.9 mmol) to give (+)-O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioate (4b)(11.5 mCi, 0.51 g); $[\alpha]_D^{20}$ +77.4° (c=5.27); the radiochemical purity being 98% on RGC and RTLC.

(+)- and (-)-O-*p*-Cyanophenyl O-Ethyl Phenyl-$^{14}C_6$-phosphonothioates (1a and 1b) -- A mixture of (-)-O-ethyl phenyl-$^{14}C_6$-phosphonochloridotioate (4.71 mCi, 0.21 g, 0.94 mmol), *p*-cyanophenol (0.64 g, 5.4 mmol) and anhydrous potassium carbonate (0.74 g, 5.4 mmol) in anhydrous toluene (8 ml) was refluxed under stirring for 3 hr. After cooling, the precipitates were filtered off, and the filtrate evaporated under reduced pressure to give an oily residue. The residue was chromatographed on silica gel and eluted with hexane-benzene (1:2 v/v).

Evaporation of the main fraction gave a crystalline residue [4.46 mCi, $[\alpha]_D^{20}$ +39.2° (c=2.71)], which was recrystallized from hexane to give (+)-O-*p*-cyanophenyl O-ethyl phenyl-$^{14}C_6$-phosphonothioate (1a)(3.97 mCi, 0.24 g, 5.01 mCi/mmol); $[\alpha]_D^{20}$ +41.9° (c=2.40). Similarly, from (+)-O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioate (4b)(5.77 mCi, 0.25 g, 1.2 mmol) there was obtained a crystalline product [4.98 mCi, $[\alpha]_D^{20}$ -38.9° (c=3.21)], which on recrystallization from hexane gave (-)-O-*p*-cyanophenyl O-ethyl phenyl-$^{14}C_6$-phosphonothioate (1b) (4.48 mCi, 0.28 g, 4.98 mCi/mmol); $[\alpha]_D^{20}$ -43.0°. The purities of both products were radiochemically and chemically 99% on RGC and RTLC [(i) hexane/benzene=1/2 v/v ($R_f$-value 0.23), (ii) toluene/acetic acid=7/1 v/v ($R_f$-value 0.55), (iii) ethyl acetate/ethanol/water=10/2/1 v/v/v ($R_f$-value 0.78)]. Both products had mp 105-106° and showed similar spectra; IR spectrum ($cm^{-1}$, $CHCl_3$): 2250 (CN); NMR spectrum (δ, $CDCl_3$): 1.33 (3H, t, J=6 Hz, $-CH_2\underline{CH_3}$), 4.01-4.60 (2H, m, $-\underline{CH_2}-$), 7.05-8.26 (9H, m, aromatic H). The products were identical in every respect with the unlabelled authentic sample[7], respectively.

(+)- and (-)-O-Ethyl O-*p*-Nitrophenyl Phenyl-$^{14}C_6$-phosphonothioate (2a and 2b) -- A mixture of (-)-O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioate (4a)(4.71 mCi, 0.21 g, 0.94 mmol), *p*-nitrophenol (0.75 g, 5.4 mmol) and anhydrous potassium carbonate (0.74 g, 5.4 mmol) in anhydrous toluene (10 ml) was refluxed under stirring for 7 hr. Working up in the similar manner described above, a residue obtained was chromatographed on silica gel with hexane-benzene (2:1 v/v) to give (+)-O-ethyl O-*p*-nitrophenyl phenyl-$^{14}C_6$-phosphonothioate (2a)(4.22 mCi, 0.27 g, 4.96 mCi/mmol); $[\alpha]_D^{20}$ +36.6° (c=2.81). Treatment of (+)-O-ethyl phenyl-$^{14}C_6$-phosphonochloridothioate (4b) in the similar manner gave (-)-O-ethyl O-*p*-nitrophenyl phenyl-$^{14}C_6$-phosphonothioate (2b)(5.47 mCi, 0.35 g, 5.02 mCi/mmol, 94.8% yield); $[\alpha]_D^{20}$ -36.2° (c=3.54). The radiochemical and chemical purities of both products were 99% on RGC and RTLC [(i) hexane/benzene=1/1 v/v ($R_f$-value 0.15), (ii) hexane/acetone=5/2 v/v ($R_f$-value 0.45), (iii) toluene/acetic acid=7/1 ($R_f$-value 0.64)]. Both products showed similar spectra; IR spectrum ($cm^{-1}$, $CHCl_3$): 1525 ($NO_2$); NMR spectrum (δ, $CDCl_3$): 1.37 (3H,t, J=6 Hz, $-CH_2\underline{CH_3}$), 4.00-4.59 (2H,

m, -$CH_2$-), 7.08-8.21 (9H, m, aromatic H). Both products were identical in every respect with the unlabelled authentic samples[7], respectively.

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