Synthesis of Radiolabeled Polychlorophenyl Vinyl Phosphates

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Several radioactive samples of three polychlorophenyl vinyl phosphates have been synthesized for use in tracer studies to facilitate the evaluation and development of this valuable class of biologically active compounds. Radiosynthesis methods, which position radionuclides in three locations of the molecules, are described. As these compounds exist in two geometric isomers, they were separated by liquid–liquid partition chromatography. The chemical and radiochemical purities of the isolated isomers were established by proven techniques. The average overall yield of the polychlorophenyl vinyl phosphates labeled with ^{14}C in the 1,2 position was 22% (based on ^{14}C-trichloroethylene), in the *O*-^{14}C-alkyl group was 87% (based on ^{14}C-alcohol), and with ^{32}P was 42% (based on $^{32}PCl_3$).

Certain vinyl phosphates have the important characteristic of a very favorable toxicity ratio between mammals and insects. A number of these compounds possessing a polychlorophenyl group are highly toxic to insects but have an extremely low mammalian toxicity. Among those in which we have a particular interest are 2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate (Supona insecticide) (I), 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate (Gardona insecticide) (II), and 2-chloro-1-(2,5-dichlorophenyl)-vinyl dimethyl phosphate (III), an experimental compound. Supona (I) has found application in animal ectoparasite control and as a soil treatment for control of the maggot complex. The insecticide Gardona (II) has been found to control the corn earworm, fruit tree insects, and a number of important insect pests in dairies. The experimental compound III has a similar broad scope of biological activity. Radioactive samples of each of these bioactive compounds were prepared for tracer studies to aid in their development and evaluation. Metabolic fate investigations in particular are facilitated by the use of radioactive materials with the label located at different sites of the molecule. The vinyl moiety was selected for labeling, since it should survive in the metabolic process and be retained in the major skeleton of the molecule. The presence of ^{14}C in both the 1 and 2 positions was preferred for the proposed investigations, and also was more expeditious than ^{14}C-label restricted to either the 1 or 2 position. In addition, two chemically labile groupings, the ^{14}C-alkoxy and the ^{32}P-phosphate ester, were also selected for labeling. Consequently, with three differently tagged compounds, a metabolic route will be more easily identified. Synthesis routes were developed which specifically positioned radionuclides in three locations of the molecule, the phosphorus atom, ^{14}C in the alkoxy group, and 1,2-^{14}C in the vinyl position. The present paper is a report of this work.

EXPERIMENTAL SECTION

The synthesis path followed known methods and involved the preparation of trialkyl phosphite and polychloroacetophenone as precursors which, when reacted together, form polychlorophenyl vinyl phosphate. The reactions used in the radiosynthesis of these compounds are shown in Figure 1. The first step in the preparation of ^{32}P or ^{14}C-alkoxy-labeled polychlorophenyl vinyl phosphates was the synthesis of labeled trialkyl phosphite from $^{32}PCl_3$ and/or ^{14}C-ethanol or methanol. An alternate but superior route to prepare O$^{14}CH_3$-labeled trimethyl phosphite (TMP) was an exchange reaction previously reported by Potter and Burton (1964). After the preparation of labeled trialkyl phosphite by either procedure it was reacted, after the method of Phillips (1963), with polychloroacetophenone to yield polychlorophenyl vinyl phosphates. To prepare labeled polychloroacetophenone, 1,2-^{14}C-trichloroethylene was oxidized with oxygen in the presence of a catalyst, using a modification of the method of Gaertner and Ramey (1970), to yield 1,2-^{14}C-dichloroacetyl chloride, which was then reacted with di- or trichlorobenzene. These 1,2-^{14}C-polychloroacetophenones were subsequently reacted with the appropriate trialkyl phosphite to form labeled I, II, and III.

The radioactive polychlorophenyl vinyl phosphates, as described, are essentially mixtures of two geometric isomers plus products resulting from side reactions. Since these isomers differ markedly in their biological properties, separation is necessary. In each case the Z isomer is the biologically active isomer, and in this configuration the chlorine and phosphate groups have the cis configuration about the double bond. The structure assigned to these isomers has been deduced by a combination of physical and chemical methods. The purification of I, II, and III can be accomplished and the isomers are separated by liquid–liquid partitioning operations, including chromatography. The partition coefficients in various solvent systems are given in Table I. Most samples were purified by liquid–liquid chromatography (llc). The radiochemical purities were determined in general by thin-layer chromatography (tlc), with radioautography employing a number of different solvent systems. The chemical purities were assessed by infrared spectroscopy, bioassay, and other conventional procedures. The yields and specific activities were determined by usual radiometric methods.

CHEMICALS

The solvents used were distilled and all glassware was oven-dried at 105° for 2 hr. The *N,N*-diethylaniline was freshly distilled. The methanol was dried by fractional distillation. The methanol-^{14}C was purchased from Bio-Rad Laboratories, Richmond, Calif. It had a specific activity of 10 mCi/mmol and was used without further purification. The phosphorus trichloride used in the pilot experiments was purified using the procedure described by Burton (1971). The ^{32}P-phosphorus trichloride, specific activity 29.6 mCi/g, was purchased from Nuclear Chicago, Chicago, Ill., and was used as received without further purification. The trimethyl phosphite was purified by distillation through a 20-plate bubble column from potassium at a reflux ratio of 10:1, the portion bp 95° (100

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Table I. Some Partition Coefficients of Radiolabeled Polychlorophenyl Vinyl Phosphates

Solvent system		Partition coefficient[a] of compound		
Polar	Hydrocarbon	I	II	III
Water	Chloroform	0.37	38	650
Water	Hexane	0.63	8	29
Water	Benzene	0.45	26	306
Acetonitrile	Hexane	86.0	0.1	<0.1

[a] Partition coefficient = concentration in hydrocarbon solvent/concentration in polar solvent.

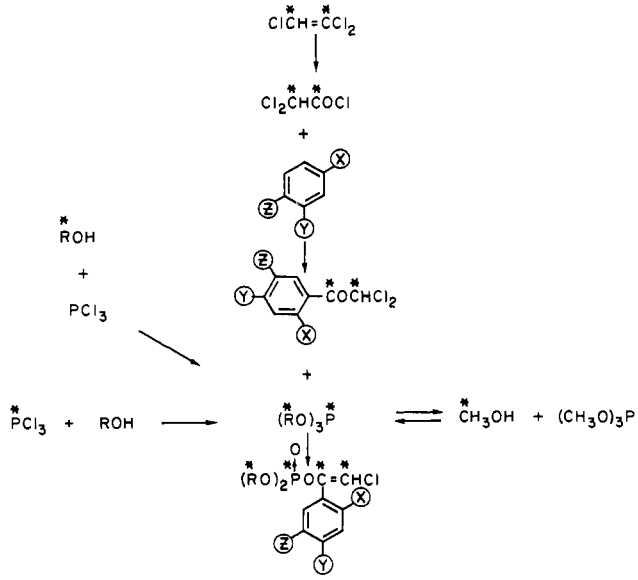
mm) being collected and used. The purity by infrared analysis and titration methods was determined to be greater than 97%.

The 2,2,2′,4′-tetrachloroacetophenone was commercially purified by distillation, bp 97–99° (0.02 mm). The 2,2,2′,4′-tetrachloroacetophenone-^{14}C, specific activity 1.0 mCi/mmol, was prepared by the New England Nuclear Corp., Boston, Mass. It was found to be 96% chemically pure by infrared analysis. Using Whatman No. 1 paper treated with 5% silicone, and developing with ethanol–diethyl ether–chloroform in a ratio of 55:45:2, the material migrated to R_f 0.22. Only one spot was obtained, by tlc radioautography; its radiochemical purity was determined to be over 98%. It was used without further purification. The 2,2,2′,5′-tetrachloroacetophenone was purified by distillation, with the fraction bp 106–107° (0.02 mm) being collected and used. The 2,2,2′,5′-tetrachloroacetophenone-^{14}C, having a specific activity of 1.32 mCi/mmol, was prepared by the New England Nuclear Corp. Cochromatography with authentic material by tlc on Eastman silica gel sheets using hexane–acetone in a ratio of 95:5 gave an R_f of 0.63. Impurities were noted at R_f 0.0 (3.7%), R_f 0.27 (1.4%), and R_f 0.94 (1.1%). It was used as received without further purification. The 2,2,2′,4′,5′-pentachloroacetophenone was purified by fractional distillation. The fraction bp 113–115° (0.3 mm) was collected and used. The ^{14}C-2,2,2′,4′,5′-pentachloroacetophenone, specific activity 0.9 mCi/mmol, was prepared by the New England Nuclear Corp. Using Eastman silica gel sheets and hexane–acetone in a ratio of 19:1 as the developing solvent, the R_f value was 0.80. Impurities were observed at R_f 0.0 (2.3%) and R_f 0.97 (2.9%). The infrared spectrum was identical to that reported for the authentic compound and indicated a chemical purity of 98%. The product was used without further purification. Other chemicals used were analytical grade.

Preparation of Dichloroacetyl Chloride-^{14}C. The dichloroacetyl chloride-^{14}C was synthesized by the liquid phase oxidation of 1.57 g of ^{14}C-trichloroethylene (12 mmol), specific activity 4 mCi/mmol, in the presence of 0.001% benzyl peroxide and 100 ppm of triethylamine. Oxygen was supplied to the system at 45 psi, while the mixture was irradiated by uv light. These conditions were maintained for 12 hr at a temperature of 75°. Upon isolation and purification by distillation, dichloroacetyl chloride-^{14}C was obtained in a yield of 60%.

Preparation of Polychloroacetophenones-1,2-^{14}C. For 2,2,-2′,4′-tetrachloracetophenone-1,2-^{14}C, to a mixture of 2.0 g of anhydrous $AlCl_3$ (15 mmol) and 1.91 g of *m*-dichlorobenzene (13 mmol) was added with stirring over a 10-min period 1.92 g of dichloroacetyl chloride-1,2-^{14}C (13 mmol). The reaction was stirred at 90–100° for 4 hr, cooled, and poured into a separatory funnel containing 100 cm^3 of dilute HCl, 10 g of ice, and 100 cm^3 of petroleum ether (30–60°).

The petroleum ether extract was washed successively with water, aqueous $NaNCO_3$, and water, and was then dried over



* Position of radioactivity

Compound	R	Ⓧ	Ⓨ	Ⓩ
I	C_2H_5	Cl	Cl	H
II	CH_3	Cl	Cl	Cl
III	CH_3	Cl	H	Cl

Figure 1. Reaction schemes for radiolabeled vinyl phosphates

Na_2SO_4 at −20°. Removal of the solvent *in vacuo* at 35° resulted in a reddish-brown oil which was distilled at 95° (0.02 mm) to yield 1.34 g of 2,2,2′,4′-tetrachloracetophenone-1,2-^{14}C (5.2 mmol), representing a 40% yield.

Thin-layer chromatography on silica gel G using petroleum ether–diethyl ether (4:1 v/v), followed by scanning of the plate, indicated only one radiochemical spot which coincided with authentic 2,2,2′,4′-tetrachloroacetophenone when stained with I_2 vapors. Gas–liquid chromatography (glc) on a 6-ft 10% SE-30 column at 183° indicated a chemical purity of greater than 98%.

The specific activity was determined to be 1.0 mCi/mmol.

The syntheses of 2,2,2′,4′,5′-pentachloroacetophenone-1,2-^{14}C (specific activity of 0.9 mCi/mmol) and 2,2,2′,5′-tetrachloroacetophenone-1,2-^{14}C (specific activity of 1.34 mCi/mmol) were conducted in a similar fashion, employing dichloroacetyl-1,2-^{14}C chloride and 1,2,4-trichlorobenzene and *p*-dichlorobenzene, respectively. Yields of 42 and 50% were obtained. Both products were analyzed by tlc and glc procedures described previously and a chemical and radiochemical purity of greater than 98% was observed. Each product behaved identically to the nonlabeled standard.

Preparation of O^{14}CH$_3$-Labeled Trimethyl Phosphite. Trimethyl phosphite (TMP), 563 mg (4.5 mmol) freshly distilled from metallic potassium, was treated with 16 mg of anhydrous ^{14}C-methanol (0.5 mmol). The mixture was heated to 95° for 2 hr, then to 105° for 2 hr, and the product was isolated. The unchanged methanol was destroyed by the addition of metallic potassium and the product was isolated by distillation. The product weighed 490 mg (3.9 mmol), a yield of 87%.

Preparation of Labeled Trialkyl Phosphite-^{14}C and -^{32}P. Typically, 0.935 g of anhydrous methanol (29.13 mmol) and 4.6 g of freshly distilled *N,N*-diethylaniline (30.6 mmol), and

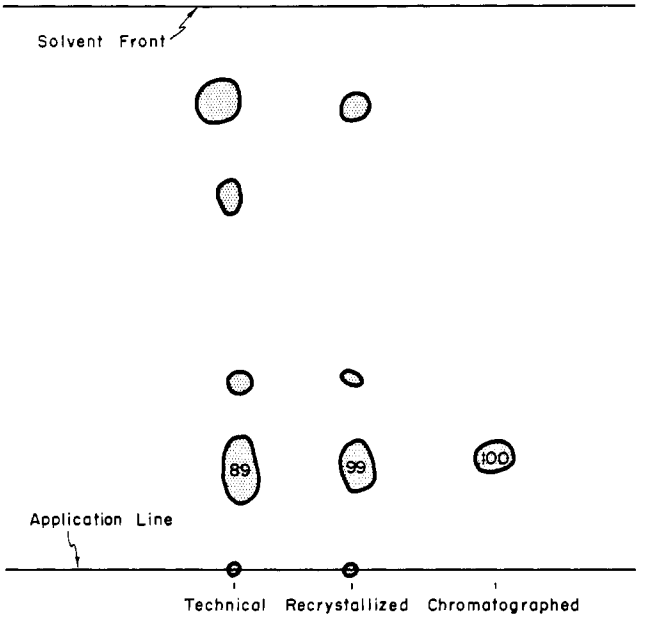


Figure 2. Trace of radioautogram of thin-layer chromatogram of III-^{14}C

3 ml of phenylcyclohexane were combined. The stirred reaction mixture was cooled to 0°, and 1.33 g of ^{32}P-phosphorus trichloride (9.71 mmol) in 2 ml of phenylcyclohexane was introduced during 60 min. The mixture was stirred further for 30 min and kept at room temperature for 30 min. The ^{32}P-TMP was distilled from the mixture *via* a vacuum manifold and purified by treatment with metallic potassium at 0° followed by distillation. The yield of ^{32}P-TMP was 840 mg (6.4 mmol), a 66% yield. Complete details of the reaction conditions and apparatus have been reported by Burton (1971). To prepare ^{32}P-triethyl phosphite, 0.316 g of anhydrous ethanol (6.9 mmol) in 1 ml of phenylcyclohexane and 1.07 g of *N,N*-diethylaniline (7.2 mmol) were added to 0.315 g of $^{32}PCl_3$ (2.3 mmol) at 5° during a period of 1 hr. Purification was carried out in the manner described for labeled TMP. The yield of ^{32}P-triethyl phosphite was 309 mg (1.9 mmol), a yield of 83%. Triethyl phosphite-^{14}C is prepared in a similar manner using ^{14}C-ethanol.

Synthesis of ^{32}P- and ^{14}C-Gardona Insecticide (II). Into a small reaction flask fitted with a water-cooled cold finger and magnetic stirring bar was weighed 1.75 g of 2,2,2′,4′,5′-pentachloroacetophenone (6.0 mmol). By means of a vacuum manifold, 840 mg of ^{32}P-TMP (6.4 mmol) was distilled into the reaction system. Predried air was admitted to the apparatus and the stirred mixture was heated at 90° for 2 hr on a controlled heating stage. After 2 hr the mixture was stripped of volatiles at room temperature at 0.1 μ pressure. The product, 1.294 g, was over 95% Z isomer (II). The product was purified in the manner described under the section on Purification. The yield was 63% based on TMP with a specific activity of 2 mCi/mmol.

As described above, 490 mg of ^{14}C-TMP (3.9 mmol) was reacted with 1.08 g of 2,2,2′,4′,5′-pentachloroacetophenone (3.7 mmol). The product, 1.372 g (3.7 mmol), had a Z isomer content of >95%. The yield based on ^{14}C-TMP was 95%.

As previously described, 575 mg of TMP (4.37 mmol) was reacted with 1.275 g of 2,2,2′,4′,5′-pentachloroacetophenone-1,2-^{14}C (4.37 mmol), yielding 1.56 g of II (4.27 mmol). The product was purified as described before, yielding 1.233 g (3.4 mmol), a 79% yield having a mp of 92–93°.

Table II. Liquid–Liquid Chromatographic Systems for Radiolabeled Polychlorophenyl Vinyl Phosphates

Radiolabeled compound	System: Stationary phase	System: Mobile phase	Column[a] in cm	Elution volume in ml[b]
I	EG[c]	Cyclohexane	1.5 × 94	200
II	EG	Hexane	2 × 110	400
III	EG	Hexane–carbon tetrachloride, 3:1 v/v	2 × 100	300
III	EG	Hexane–chloroform, 4:1 v/v	2 × 100	400

[a] Flow rate 1 ml/min. [b] Observed elution volume = volume of eluent collected between sample charge and peak maximum. [c] Ethylene glycol on Johns-Manville GC-22 super support crushed firebrick.

Radiolabeled 2-Chloro-1-(2,5-dichlorophenyl)vinyl Dimethyl Phosphate (III). Using the procedure described for the synthesis of II, 1.35 g (7.0 mCi) of 2,2,2′,5′-tetrachloroacetophenone-1,2-^{14}C (5.23 mmol) was treated with 744 mg of TMP (6 mmol) for 2 hr at 90°. The excess TMP was distilled and the product was extracted with hexane. The product, 1.5425 g (4.82 mmol) having a chemical purity of 97%, was obtained in a 92% yield. Its radiochemical purity was over 99%. Further purification to remove traces of dimethyl methyl phosphonate was carried out. The final purity was 100 ± 0.2%. Samples of ^{32}P-III and ^{14}C-methoxy-III are prepared in a similar manner by reacting 2,2,2′,5′-tetrachloroacetophenone with ^{32}P- or ^{14}C-TMP.

Synthesis of Radiolabeled Supona Insecticide (I). Following the same procedure as reported for II, 2.298 g of 2,2,2′,4′-tetrachloroacetophenone-1-2-^{14}C (8.90 mmol), specific activity 1 mCi/mmol, was reacted with 1.736 g of triethyl phosphite (13.1 mmol). The product, after isolation, weighed 3.1384 g (99%) with a purity of 95%. The material was purified by llc yielding I-1,2-^{14}C over 97% pure. Samples of ^{32}P-I and ^{14}C-ethoxy-I are prepared in this manner by reacting 2,2,2′,4′-tetrachloroacetophenone with ^{32}P- or ^{14}C-triethyl phosphite.

PURIFICATION

Typically, crushed firebrick (Johns-Manville Silocel G-22 firebrick 60–80 mesh) was weighed and 1 ml of ethylene glycol was thoroughly admixed per gram of firebrick. The mixture was used to prepare a glass pipe column fitted with a stainless steel needle valve or small bore stainless hypodermic tubing coupled to an automatic fraction collector. The column was connected to a mechanical pump and the mobile phase was passed through until all traces of air had been removed. The radioactive sample, dissolved in a few milliliters of the eluting solvent, was charged to the column. Product was eluted and fractions of various sizes were collected. The chromatographic systems used for these compounds are presented in Table II.

Aliquots of the fractions were removed and counted with a liquid scintillation counter. A profile of the radioactivity *vs.* volume eluent was prepared. The appropriate fractions containing the Z isomer were analyzed by tlc and those fractions which contained only pure compound were combined. After removal of the solvent with a rotary evaporator, the sample was recrystallized, and the yield was determined.

Samples containing 89–95% polychlorophenyl vinyl phosphates were purified to material having a high chemical and radiochemical purity. Such enhancement of purity is illustrated for III in Figure 2.

Table III. Typical R_f Values of Radiolabeled Polychlorophenyl Vinyl Phosphates

Stationary system	Developing phase Solvents	Ratio, v/v	R_f value I	II	III
A[a]	ETOH–H_2O–$CHCl_3$	60:40:2	0.61	0.58	0.67
A	ETOH–H_2O–$CHCl_3$	55:45:2	0.48	0.47	0.61
B[b]	Hexane–acetone	3:2	0.61	0.60	0.56
B	Hexane–acetone	3:1	0.35	0.36	0.33
B	Benzene–ETOAc	9:1	0.31	0.31	0.27
B	Benzene–ETOAc	3:1	0.51	0.55	0.49
B	Cyclohexane–CH_3OH–acetone	80:20:25	0.43	0.39	0.38
B	Hexane–$CHCl_3$–acetone	70:15:15	0.46	0.39	0.32
B	Hexane–acetone	2:1	0.48	0.46	0.42

[a] Whatman No. 1 paper with 5% silicone. [b] Silica gel G.

The fractions accounting for the shoulder peaks, slopes, and tailings were combined for further purification. For example, the fractions surrounding the main peak obtained from the llc of II-1,2-^{14}C were combined and further purified by dry column chromatography after the method of Loev and Snailer (1965). Experimentally, about 85 mg of 80% pure II was charged to a dry silica gel column 60 × 1.5 cm. It was developed with hexane–acetone in a ratio of 3:1. The product at the calculated R_f value (determined by tlc) was isolated and extracted. Purity of II-^{14}C obtained was 95%.

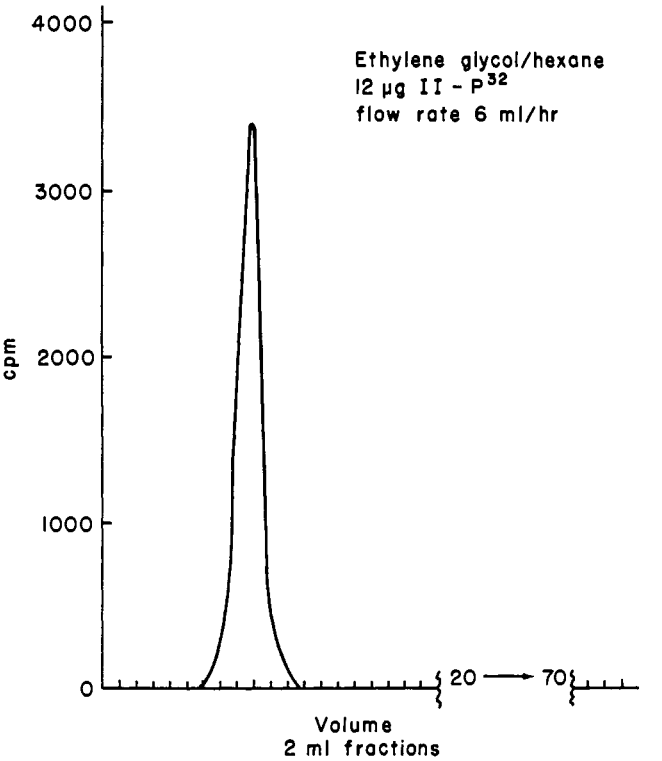


Figure 3. Partition chromatography of II-^{32}P

ANALYTICAL CHROMATOGRAPHY

The presence of labeled polychlorophenyl vinyl phosphates, radioactive impurities, and their relative amounts were in general determined by paper and thin-layer chromatography (tlc), including cochromatography with authentic samples.

A number of different solvent combinations, including reverse phase systems, were used. The radioactive materials and their corresponding unlabeled reference standards had the same R_f value when chromatographed together or separately. The presence of radioactive spots on the chromatograms and the amounts of radioactivity were determined by radioautography. By placing the radioautogram and chromatogram in coincidence, the areas of radioautogram containing the radioactive isotope were determined, isolated, and counted with a Packard 3003 liquid scintillation counter. In other instances, the chromatograms were counted and graphed directly on a Vanguard strip scanner. Nonradiolabeled materials were determined by chromogenic procedures. Some typical R_f values and development systems are listed in Table III.

Liquid–liquid chromatography was also used as an analytical method. The results of a typical analytical columnar chromatographic determination where only micrograms of material were analyzed are shown in Figure 3. Cochromatography with authentic samples for purity and characterization was also used in the llc system as well as previously described in the tlc method.

Infrared Measurements. Infrared analyses were performed to determine the chemical purity of these compounds, as well as for identification purposes. Standard spectra of chromatographically pure samples were obtained in carbon disulfide solution (*ca.* 1%) in a 1.0-mm sodium chloride cell using a Beckman IR-4 spectrophotometer with normal instrument settings. The spectrum of the radioactive sample was obtained in the same manner and the absorbance was determined by a baseline procedure.

The following band assignments can be made for I: 1300 cm^{-1} assigned to P → O absorption band; 1230 cm^{-1} assigned to C=C absorption band; 920 cm^{-1} assigned to C=C absorption band; 1040 cm^{-1} assigned to POC absorption band; and 1230 cm^{-1}, specific for Z isomer.

The following band assignments were found for II: 1299 cm^{-1} assigned to P → O absorption band; 1281 cm^{-1} assigned to P → O absorption band; 1221 cm^{-1} assigned to C=C absorption band; 940 cm^{-1} assigned to C=C absorption band; 1040 cm^{-1} assigned to POC absorption band; and 1221 cm^{-1}, specific for Z isomer.

The band assignments for III are as follows: 1298 cm^{-1} assigned to P → O absorption band; 1228 cm^{-1} assigned to

Table IV. Typical Preparations of Radiolabeled Polychlorophenyl Vinyl Phosphates

Compound	Batch	mmol Trialkyl phosphites	mmol Polychloroacetophenones	Reaction time, 90–100° (min)	Specific activity, mCi/mmol	Position of label	Yield g	Yield %	% Purity Chemical[a]	% Purity Radiochemical[b]
I	6	13.3	8.9	120	1.0	^{14}C-vinyl	3.0	99	>95	>97
II	1	2.0	1.9	60	4.8	^{32}P	0.6	88	95	98
II	4	3.7	3.7	120	1.0	O$^{14}CH_3$	1.3	75	95	98
II	7	1.5	1.0	120	0.9	^{14}C-vinyl	0.3[c]	91	>95	>98
III	9	6.0	5.8	120	1.3	^{14}C-vinyl	1.5[d]	90	>97	>99

[a] Determined by infrared analysis and bioassay. [b] Determined by tlc and dilution analysis. [c] ^{14}C-II, mp. 94–96° [d] ^{14}C-III, mp 100–101°.

C=C absorption band; 940 cm^{-1} assigned to C=C absorption band; 1040 cm^{-1} assigned to POC absorption band; and 1228 cm^{-1}, specific for Z isomer.

No significant differences in absorptivities were seen between the solution of the standard and respective radioactive preparation.

BIOASSAY METHODS

To determine if the radioactive polychlorophenyl vinyl phosphates were biologically comparable to pure authentic unlabeled reference standards the samples were bioassayed. Houseflies were tested by the topical method. By plotting the dosage *vs.* the percent mortality, the LD_{50} was found and the toxicity index was calculated. No significant differences in the LD_{50} were seen between the solutions of I, II, and III and the corresponding reference standards.

RESULTS

The polychlorophenyl vinyl phosphate molecule has been successfully specifically labeled in three positions. Typical reaction conditions, yields, purities, and specific activities of some of the preparations are given in Table IV. The reaction of trialkyl phosphite and polychloroacetophenone, as herein reported, gives mainly the bioactive Z isomer in a ratio of 10 (or higher) to 1 of the E isomer.

The main impurities in the technical material before purification were the E isomers in each instance plus dimethyl methyl phosphonate in III, TMP in II, and an unidentified ketonic material in I.

As the data show, liquid–liquid partition chromatography resolved these vinyl phosphates and allowed pure isomers to be isolated. The results of a typical separation are illustrated in Figure 3.

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LITERATURE CITED

Burton, W. B., *J. Label. Compounds* **8,** 111 (1971).
Gaertner, G. W., Ramey, D. E., U. S. Patent 3,509,210 (Apr 28, 1970).
Loev, B., Snailer, K. M., *Chem. Ind. (London)* 15 (1965).
Phillips, D. D., U. S. Patent 3,102,842 (Sept 3, 1963).
Potter, J. C., Burton, W. B., J. AGR. FOOD CHEM. **12,** 439 (1964).

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Synthesis of ^{14}C-Benzenoid-Ring-Labeled Guthion

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A detailed method for synthesizing radiochemically pure Guthion (benzenoid-ring-UL-^{14}C) in good yield is described. The overall yield of labeled Guthion (specific activity 1.0 μCi/mmol) from the seven-step, three-trial synthesis was 80%. Each derivative of the synthesis was screened for composition and purity by conventional spectroscopic and radiometric procedures. This ring-labeled insecticide will be utilized as an aid in the elucidation of several currently reported unknown degradation products and will also undoubtedly prove to be a valuable aid in studying the metabolic patterns, distribution, and translocation of this compound in animals and plants.

Guthion {*O,O*-dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3-(4*H*)-yl methyl] phosphorodithioate} is a potent organophosphate insecticide toxic to insects and mammals by its ability to inhibit cholinesterase activity (Martin, 1966). Increasing amounts of Guthion have been used recently with the diminution in the use of chlorinated hydrocarbon insecticides (EPA, 1971).

Although two types of radiolabeled Guthion (^{32}P and ^{14}C at the methylene group) have been synthesized (Everett *et al.*, 1966), the synthesis of ring-labeled Guthion has been ignored and several metabolites still remain unidentified. Labeling of groups easily removed from the molecule by metabolic processes can be a great disadvantage in some cases. For this reason there is often justification for making the more difficult synthesis involved in the labeling of groups which are more central in the molecule, such as aromatic rings or ring substituents (Casida, 1969). This paper describes an extremely efficient method for synthesizing Guthion (benzenoid-ring-UL-^{14}C), of high chemical purity, that can be used directly in studies involving absorption, distribution, metabolism, excretion, and related physiochemical transformations.

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EXPERIMENTAL SECTION

Chemicals. Double-distilled reagent grade quality solvents were used throughout the synthesis (Figure 1). Technical *o*-nitroaniline (compound I) was purified by dissolution in hot ethanol–water (1 : 2) to which was added activated carbon, and the resultant slurry was clarified by vacuum filtration through a hot Büchner funnel dressed with 0.5 in. of Celite. The filtrate was chilled to 4°, seeded if necessary for crystallization, and then further chilled to −10°. Efficient agitation was used throughout the crystallization period. The fine bright orange-yellow crystals were collected by vacuum filtra-