

Synthesis of 2,2-Dichlorovinyl Dimethyl Phosphate Labeled with ^{14}C , ^{36}Cl , and ^{32}P

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Numerous radioactive samples of dichlorvos (2,2-dichlorovinyl dimethyl phosphate) have been synthesized for use in tracer studies to facilitate the evaluation and development of this biologically active vinyl phosphate. It has been labeled in four positions of the molecule with phosphorus-32, chlorine-36, and carbon-14 to aid investigations involving metabolism, toxicology, and the mode of biological action. The radiosynthesis routes are described. Methods for its complete separation from

side reaction products have been developed. The radiochemical purities of the various preparations were determined by chromatography employing a number of different systems. The chemical purities were assayed by infrared spectroscopy and bioassay methods. The yields and specific activities were determined by conventional radiometric procedures. The yields of purified dichlorvos ranged from 60 to 80% depending on the position of the radiolabel.

The reaction between trivalent phosphorus compounds and α -halo aldehydes yields vinyl phosphates, some of which are biologically active compounds. Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is a member of this group of organic phosphates whose biological activity has been studied extensively during the past several years. Dichlorvos has been labeled with radioactivity in four positions to aid in studies involving absorption, detoxification, distribution, excretion, and release from formulated products. The present paper is a report of this work. Only the reactions involving the labeled material will be discussed as the precursors may be handled on a macro scale in the usual manner.

EXPERIMENTAL

The reactions used in the synthesis of radioactive dichlorvos are shown in Figure 1. The first step in the preparation of ^{32}P or O^{14}CH_3 labeled dichlorvos was the synthesis of trimethyl phosphite (TMP) from labeled $^{32}\text{PCl}_3$ and/or $^{14}\text{CH}_3\text{OH}$. A previously published method (Marshall, 1958) was modified by the use of a tertiary organic base as an acid acceptor in the reaction.

An alternate but superior route to prepare O^{14}CH_3 labeled TMP was an exchange reaction, previously reported by Potter and Burton (1964). After preparation of labeled TMP by either method it was reacted with chloral, to yield dichlorvos. To prepare ^{36}Cl or ^{14}C -1-vinyl dichlorvos, labeled chloral was first prepared followed by reaction with TMP. The ^{36}Cl chloral was obtained by the exchange of a chlorine-36 labeled salt with chloral and by a procedure supplied in confidence by Amersham/Searle Corp. The ^{14}C chloral was prepared by the chlorination of ethanol-1- ^{14}C . The 2 position could also be labeled in the chloral, yielding ^{14}C -2-vinyl dichlorvos by use of the appropriate tagged ethanol.

Chemicals. The solvents used were distilled and the reaction glassware was oven dried at 105°C for 2 hr. The *N,N*-diethylaniline was freshly purified using the method described by Fieser (1941). The methanol was dried by the method of Riddich and Toops (1955). The methanol ^{14}C was purchased from New England Nuclear Corp., Boston, Mass. It had a specific activity of about 6.0 mCi/mmol and was used without further purification. The phosphorus trichloride used in the pilot experiments was purified by distillation through a 12-in. Vigreux column at a reflux ratio of 5 to 1. The ^{32}P phosphorus trichloride was purchased from New England Nuclear Corp., Boston, Mass.,

and Amersham/Searle, Arlington Heights, Ill. The $^{32}\text{PCl}_3$ was used as received without further purification, and the specific activity varied from 2 to 6 mCi/mmol. The trimethyl phosphite was purified by distillation through a 20-plate bubble column from metallic potassium at a reflux ratio of 10 to 1. The purity by infrared analysis and titration methods was determined to be 98%. The ^{14}C trimethyl phosphite, if not prepared in this laboratory, was purchased from Dhom Products Ltd., N. Hollywood, Calif., specific activity 2 mCi/mmol, and was purified by distillation from potassium. The chloral obtained from Hooker Chemical Co. was distilled through a 15-cm Vigreux column and the fraction, bp 95°C at 760 mm, was collected and used. By infrared analysis it was found to be over 95% pure and to contain no chloral hydrate or foreign impurity bands. The ^{14}C chloral labeled in the 1 position, specific activity from 2.7 to 5.9 mCi/mmol, was purchased from Mallinckrodt Nuclear, St. Louis, Mo. It was found to be of high chemical purity and was used without further purification. The ^{36}Cl as hydrochloric acid, 1.115 N, 4 mCi/g Cl, was purchased from the Radiochemical Center, Amersham, England. The Li^{36}Cl was purchased from Mallinckrodt Nuclear, St. Louis, Mo. Other chemicals used were analytical grade.

Preparation of Trimethyl Phosphite- ^{32}P . In a typical preparation, 5 ml of phenylcyclohexane was placed in an anhydrous reaction flask equipped with a spiral water condenser and a rubber septum inlet. Anhydrous methanol, 1.003 g, 31.32 mmol, and 4.910 g, 32.88 mmol freshly redistilled *N,N*-diethylaniline, and 14 ml of phenylcyclohexane were added. With stirring, the reaction mixture was cooled to -10°C in a methanol ice bath, and 1.38 g, 10 mmol ^{32}P phosphorus trichloride in 2 ml of phenylcyclohexane was introduced by means of a shielded hypodermic syringe during a period of 45 min. After the addition of the ^{32}P phosphorus trichloride, the reaction mixture was further stirred for 30 min at 0° , followed by 30 min at room temperature. The ^{32}P TMP was distilled from the reaction mixture via a vacuum manifold into a receiver with a side arm. The ^{32}P TMP maintained at ice temperature was treated with several small pieces of freshly cut potassium during a period of 1 hr. The ^{32}P TMP was distilled into a graduated tube for measurement. The yield of purified ^{32}P TMP was 620 mg, 50% yield. The yields of other preparations have ranged between 41 and 84% with an average of approximately 50%. Infrared data on pilot batches indicated an average purity of over 95%.

An alternate procedure for larger amounts of labeled TMP was developed. The reaction apparatus consisted of a 100-ml round-bottomed three-necked flask equipped with a coil-type water condenser with drying tube, a side arm with a rubber septum, and a closed system magnetic stirring shaft

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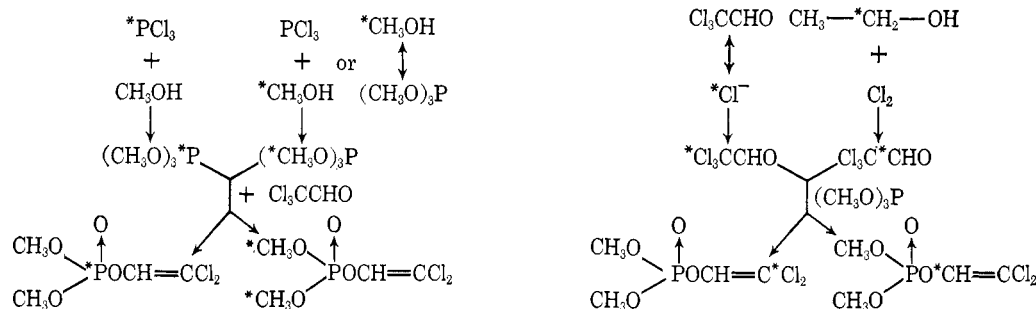


Figure 1. Reaction scheme for syntheses of labeled dichlorvos

and blade. Into the predried flask were introduced 158 mmol, 29.80 g of tributylamine, and 150 mmol, 4.805 g of anhydrous methanol. The reagent flasks were rinsed with nitrobenzene and the combined rinsings (9 ml) added to the reaction mixture. The $^{32}\text{PCl}_3$, 6.9 g, 50 mmol, was added by means of a shielded syringe during the period of 1 hr, the reaction being kept at 0° . After the reaction was complete, the TMP was distilled from the mixture under reduced pressure. The TMP was distilled through a micro distillation apparatus with a 15-cm column and the fraction, bp $110\text{--}112^\circ\text{C}$, collected. The weight of distillate was 2.846 g, 22.95 mmol, a 46% yield. The purity was comparable to micro and pilot batches.

Preparation of O^{14}CH_3 -Labeled Trimethyl Phosphite. TMP 4.0 g, 33 mmol, freshly distilled from metallic potassium was treated with 265 mg, 8.3 mmol of anhydrous ^{14}C methanol. The mixture was heated to reflux for several hours and the product isolated. The unchanged methanol was destroyed by the addition of metallic potassium and the purified product isolated by distillation. The product weighed 3.5 g, yield 86%. The amount of carbon-14 exchanged was 83%; this is 90% of the equilibrium value.

Preparation of ^{36}Cl Chloral. Lithium ^{36}Cl chloride was placed in a 5-ml continental-shaped flask equipped with a magnetic stirring bar, 15-cm vacuum jacketed distilling head fitted to receiving equipment, and a connection to a vacuum manifold. The reaction flask was surrounded by a controlled heating stage. Chloral was distilled into the LiCl in solvent and reacted for various periods of time and then the ^{36}Cl chloral was distilled under vacuum. Typically 1 g of purified chloral was stirred with 23 mg Li ^{36}Cl in tetramethylene sulfone at room temperature for 24 hr under anhydrous conditions. The yield of ^{36}Cl chloral having a purity of over 95% was 0.6 g, a chemical yield of 60% with an exchange of 19% of the radioactivity. Similar yields and conversion were obtained in 2 hr using refluxing chloroform as the solvent.

An alternate route was used to prepare the bulk of the labeled chloral. It was prepared from ^{36}Cl HCl by a modification of a method supplied in confidence by Amersham/Searle Corp., Arlington Heights, Ill. The specific activity of the ^{36}Cl chloral varied in activity from 4 to 15 mCi/mmol.

Preparation of ^{14}C , ^{36}Cl , and ^{32}P Dichlorvos. In a typical synthesis, trimethyl phosphite (TMP), 800 mg, was placed in a flask and treated with metallic potassium, a piece *ca.* 1 mm diameter. The TMP was distilled *via* a vacuum manifold into a reaction flask. The reaction system consisted of a 10-ml pear-shaped flask equipped with a magnetic stirring bar, a side arm fitted with a serum cap, a coil-type water condenser, and a connection to the vacuum manifold. The reaction flask was surrounded by a cooling bath.

The ^{14}C -1-chloral, 20 mCi, *ca.* 3.4 mmol, was distilled from the storage breakseal *via* the vacuum manifold into a small tube. Under anhydrous conditions the ^{14}C chloral was loaded in a 1-ml shielded hypodermic syringe. The ^{14}C chloral was added dropwise *via* the serum cap to the TMP at 0°C during the period of 1 hr. The mixture was stirred for 1 hr at room temperature and then allowed to stand overnight under anhydrous conditions.

The product was stripped on a vacuum system to remove excess TMP and volatile impurities. The yield of dichlorvos was 88%, 17.5 mCi. The yields of 10 trials averaged 86%.

Dichlorvos labeled with chlorine-36, phosphorus-32, or carbon-14 in the methoxy position was prepared in a similar manner by incorporating the appropriate labeled intermediate in the reactions.

Purification. The purification of technical dichlorvos, usually contaminated with minor amounts of side reaction product, can be accomplished in several ways such as distillation and liquid-liquid partition chromatography (llc). Most samples were purified by llc. Typically, crushed firebrick (Johns-Manville Silicel G-22 firebrick 60 to 80 mesh) was weighed and 1 ml of ethylene glycol added per gram of firebrick. The mixture was tumbled for 1 hr on a tilting table tumbler, and then added in small portions with tamping to a glass column (2-cm i.d.) fitted with a Teflon needle valve. A column 2-m long was prepared. The column was connected to a pump and the mobile phase (hexane-methylene chloride in a ratio of 9 to 1) passed through until all traces of air had been removed. The radioactive dichlorvos, about 3 mmol in a small amount of solvent, was charged to the column. Product was eluted and fractions of 12 ml were collected every 20 min with an automatic fraction collector.

Aliquots of the fractions were removed and counted with a liquid scintillation counter. A profile of radioactivity *vs.* volume eluant was prepared. The labeled dichlorvos was eluted in the calculated retention volume, with its peak at

Table I. Typical R_f Values of Radiolabeled Dichlorvos on Silica Gel

Solvents	Ratio v/v	R_f Value
Hexane-acetone	7 to 3	0.46
Cyclohexane-diethyl ether	4 to 1	0.20
Benzene-methanol	9 to 1	0.64
Benzene-chloroform	1 to 1	0.35
Benzene		0.24

Table II. Typical Preparations of Radiolabeled Dichlorvos

Batch	mmol		Reaction time at 0 → 20° C, min	Specific activity mCi/mmol	Position of label	Amount prepared		% purity ^a	% Yield, di-chlorvos	% Overall radio-chemical yield	Radio-chemical yield based on
	TMP	Chloral				g	mCi				
1	2.17	2.17	75	3.0	³² P	0.48	6.5	95	95	47	<i>b</i>
6	3.00	3.00	120	1.2	O ¹⁴ CH ₃	0.60	3.2	86	78	48	<i>c</i>
8	4.00	2.50	120	3.2	¹⁴ C-1-vinyl	0.55	8.0	88	88	44	<i>d</i>
14	30.00	27.70	180	0.01	³⁶ Cl	5.50	0.2	96	86	13	<i>e</i>

^a % dichlorvos contained in product prior to llc. ^b ³²P phosphorus trichloride. ^c ¹⁴C methanol. ^d ¹⁴C ethanol. ^e ³⁶Cl lithium chloride.

590 ml. The fractions within the radioactive dichlorvos profile were analyzed by thin-layer chromatography (tlc), and those fractions which contained only dichlorvos were combined. After removal of the solvent with a rotary evaporator, the yield of dichlorvos was 12.5 mCi, 63% of theory.

Numerous other llc columns were used to purify the labeled dichlorvos. For most, ethylene glycol on firebrick was used and the amount of dichlorvos charge varied from a few milligrams to several grams. The column sizes ranged from 1.8 cm × 32 cm to 40 cm × 2 m. The flow rates were maintained within the range of 0.1 ml to 2.0 ml/min. The eluting solvents used were hexane or hexane with 5 to 10% methylene chloride.

Distillation of some samples was accomplished in a vacuum jacketed 15-cm Vigreux distillation head. After the forerun was distilled (all material distilling below 98° C at 1 mm), a constant boiling center cut 98° C at 1 mm was collected. This distillate was dissolved in hexane and partitioned with water, phase separated, dried, and the solvent removed using a rotary evaporator. Samples containing 65 to 95% dichlorvos were purified to material having a high chemical and radio-chemical purity.

Infrared Measurements. Infrared analyses were performed to determine the chemical purity as well as for identification purposes. Standard spectra of pure dichlorvos were obtained in a carbon disulfide solution (ca. 8%) in a 1.0-mm sodium chloride cell using a Beckman IR-4 or IR-12 spectrophotometer with normal instrument settings. The spectrum of the radioactive dichlorvos was obtained in the same manner and the absorbance determined by a base line procedure. The chemical purity as determined by ir analysis was greater than 98%. No significant differences in adsorptivities were seen between the solution of the standard and the radioactive dichlorvos.

Thin-Layer Chromatography. Radioactive dichlorvos and its radioactive impurities or breakdown products may be separated by tlc. The presence of radiochemical impurities and their relative amounts in the preparation before and after partition chromatography were determined in several systems. Hexane-acetone in a ratio of 3 to 1 v/v as the mobile phase and Eastman silica gel "chromagram" sheets were used to separate impurities from dichlorvos. Other systems include cyclohexane-diethyl ether in a ratio of 4 to 1 v/v, benzene-methanol 9 to 1 v/v, benzene-chloroform 1 to 1 v/v, and benzene on silica gel as Eastman sheets or Brinkman plates. Some *R_f* values obtained are listed in Table I. The presence of radioactive areas on the tlc and the approximate proportions of activity in these areas can be determined by conventional radioautography.

Bioassay Methods. To determine if the radioactive dichlorvos was biologically comparable to pure authentic

unlabeled reference standards, certain of the preparations were bioassayed. Some of the trial experiments were also assayed to compare the percent active component with that found by physical measurements. Normal houseflies were tested by the topical method. By plotting the dosage *vs.* the percent mortality, the LD₅₀ was found and the toxicity index calculated. No significant differences in the LD₅₀ were seen between the solution of the reference dichlorvos and the radioactive dichlorvos preparations.

RESULTS

The dichlorvos molecule has been successfully labeled in four positions with three different radioisotopes. Typical reaction conditions, amount of reactants, yields, purities, and specific activities of some of the 16 preparations of dichlorvos prior to chromatography are given in Table II. The purity of the TMP was one of the major factors in increasing the yields. The advantage of the exchange reaction in the preparation of O¹⁴CH₃ TMP has been presented. When the isotope was located in the TMP, only a small excess of TMP was used in the reaction to conserve the isotope; therefore, the reaction was not driven to completion. When the isotope was located in the chloral, an excess of TMP was used to give a more complete reaction. Apparently only one chlorine atom is involved in the lithium chloride-chloral exchange. Increasing the ratio of Li³⁶Cl to chloral did not increase the specific activity to the extent expected; however, longer reaction times did tend to enhance the specific activity. The separation from side reaction products is complete and the dichlorvos is isolated in a state of high purity as shown by a number of proven methods. Portions of these radiolabeled samples have been used at the Biological Sciences Research Center; others have been used by collaborating laboratories.

ACKNOWLEDGMENT

The author wishes to thank J. C. Potter for certain chromatographic data, G. E. Pollard for infrared data and interpretations, E. R. Johnson for bioassay of compounds, T. D. Hoewing for technical assistance, and A. C. Page for helpful discussions.

LITERATURE CITED

- Fieser, L. F., "Experiments in Organic Chemistry," 2nd ed., Part II, Heath and Co., Boston, 1941, p 382.
- Marshall, W. W. (to Monsanto Chemical Co.), U.S. Patent 2,848,474 (Aug. 19, 1958).
- Potter, J. C., Burton, W. B., J. AGR. FOOD CHEM. **12**, 439 (1964).
- Riddich, J. A., Toops, E. E., Jr., "Techniques in Organic Chemistry," Vol. III, Organic Solvents, Physical Properties and Methods of Purification, 2nd ed., Interscience, New York, N.Y., 1955, p 335.

Received for review December 28, 1970. Accepted March 5, 1971.