Synthesis of 3-Hydroxy-N,N-Dimethyl-Cis-Crotonamide Dimethyl Phosphate and its N-Methyl Analog Labeled with ^{32}P and ^{14}C

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SUMMARY

*Two vinyl phosphates, 3-hydroxy-*N,N-*dimethyl-*cis-*crotonamide dimethyl phosphate, and its* N-*monomethyl analog, 3-hydroxy-*N-*methyl-*cis-*crotonamide dimethyl phosphate, labeled with* ^{32}P *and* ^{14}C *in three positions, have been synthesized in milligram amounts. The radiosynthesis routes are reported and the effect of reduced scale preparation is discussed. As the compounds exist in geometric isomers, methods for their separation have been developed. The purity of the* cis-*isomers was determined to be 98 to 99 %. The yield of 3-hydroxy-*N,N-*dimethyl-*cis-*crotonamide dimethyl phosphate labeled with* ^{32}P *was 38 %, with* ^{14}C-O-*methyl 73 % and with* ^{14}C-N-*methyl 34 %. The yield of 3-hydroxy-*N-*methyl-*cis-*crotonamide dimethyl phosphate labeled with* ^{32}P *was 38 %, with* ^{14}C-O-*methyl 73 % and with* ^{14}C-N-*methyl 80 %.*

INTRODUCTION.

The reaction between trivalent phosphorus compounds and alpha-halo aldehydes or ketones yields vinyl phosphates, many of which are biologically active compounds. Bidrin insecticide (3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide dimethyl phosphate) and its *N*-methyl analog. Azodrin insecticide (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate), trademarks of Shell Chemical Co., Division of Shell Oil Co., are members of this group of vinyl phosphates whose insecticidal activity has been studied intensively during the past few years. Bidrin is highly toxic to a wide spectrum of species of insects and mites, and has the unique ability to control bark beetles [1]. Azodrin has been found to control many agricultural insect pests, including the bollworm and the boll weevil on cotton [2].

To facilitate the development and evaluation of these insecticides, radioactive samples of these two compounds were prepared for use in tracer studies. Each compounds has been labeled with radioactivity in three positions to more readily aid in certain studies involving metabolism, toxicology, and the mode of biological action. The present paper is a report of this work. Only the reactions involving the labeled compounds will be discussed as the precursors may be handled on a macro scale in the usual manner. The reactions used in the synthesis of the ^{32}P and the ^{14}C methoxy labeled compounds are summarized in Figure 1.

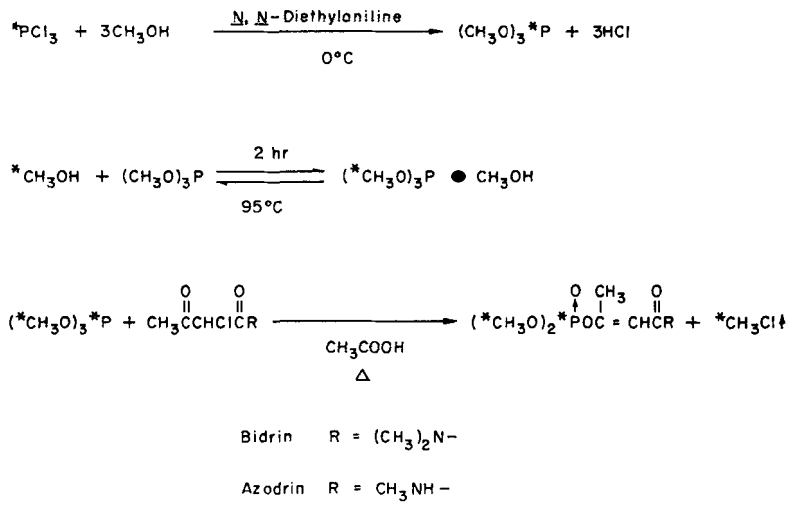


FIG. 1. Outline of ^{32}P and Methoxy-^{14}C Synthesis of Azodrin and Bidrin.

EXPERIMENTAL.

The first step in the preparation of ^{32}P Bidrin or Azodrin was the synthesis of trimethyl phosphite-^{32}P from ^{32}P-phosphorus trichloride and methanol. The procedure used was a modification of one previously published [3].

Bidrin and Azodrin labeled with ^{14}C in the methoxy group may be prepared from methanol-^{14}C by the series of reactions indicated in Figure 1. In these laboratories ^{14}C-tagged trimethyl phosphite has been prepared by this route. However, because the yields obtained by this method were not completely predictable, a direct transesterification reaction was used. The methoxy groups of trimethyl phosphite were exchanged with similar labeled groups in methanol-^{14}C. The trimethyl phosphite-^{32}P or ^{14}C was reacted with 2-chloro-*N*,*N*-dimethylacetoacetamide in the presence of a catalyst at moderate temperatures to form Bidrin. To produce labeled Azodrin the radioactive

trimethyl phosphite was reacted in the presence of an inert solvent with 2-chloro-*N*-methylacetoacetamide.

Azodrin labeled with ^{14}C in the *N*-methyl position was prepared from methylamine-^{14}C (Fig. 2). To prepare Azodrin multilabeled in the same synthesis radioactive *alpha*-methylbenzyl 3-(dimethoxyphosphinyloxy) crotonate (^{32}P and ^{14}C-methoxy labeled) [4] was prepared (Fig. 2), proceeding then to the 3-hydroxycrotonoyl chloride dimethyl phosphate via the corresponding acid prior to the reaction with tagged methylamine.

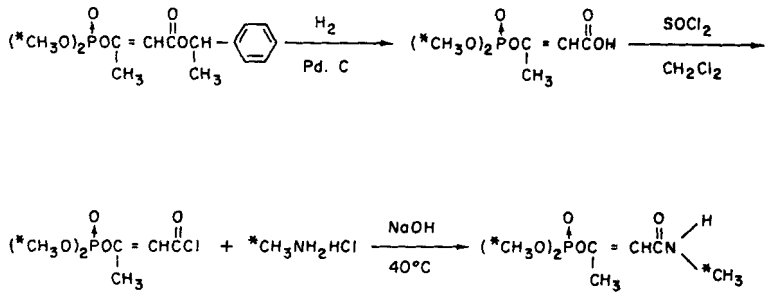


FIG. 2. Reaction Scheme for Synthesis of N,Methyl-^{14}C Azodrin.

Bidrin labeled with ^{14}C in the *N*-methyl position may also be prepared from 3-hydroxycrotonoyl chloride dimethyl phosphate. It was, however, synthesized from dimethylamine-^{14}C by the series of steps shown in Figure 3, with the possibility of using radioactive diketene, which would result in a label in the carbon chain.

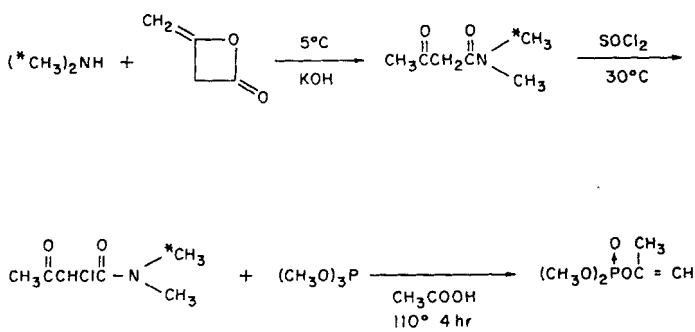
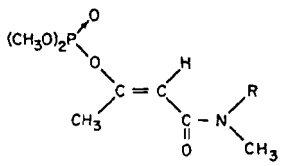


FIG. 3. Reaction Sequence of Synthesis of N-Methyl-^{14}C Bidrin.

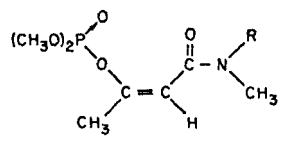
To carry out a multilabeled preparation the trimethyl phosphite could be either ^{14}C or ^{32}P labeled or both.

Crude Bidrin and Azodrin prepared as described are essentially mixtures of two geometric isomers. Since these isomers differ markedly in their insec-

ticidal properties, separation is important. In each case the *cis*-crotonamide is the biologically active isomer and is designated *alpha*, (Fig. 4). The *trans*-isomer, described as the *beta*-isomer, is biologically inactive. The structures assigned to the two isomers have been deduced by a combination of physical and chemical methods and by analogy with other known vinyl phosphates [5].



cis – Crotonamide
α – isomer



trans – Crotonamide
β – isomer

Bidrin R = CH_3

Azodrin R = H

FIG. 4. *Cis*- and *Trans*-Isomers.

The separation of the two isomers of Bidrin and Azodrin can be accomplished in several ways such as liquid-liquid partition chromatography [6]. The partition coefficients in various solvent systems are given in Table 1. The best separation of the isomers and also the side products was effected by using a stationary phase of water and a mobile phase of benzene for Bidrin, and water-chloroform-benzene mixture for Azodrin. Compounds in the fractions were detected by liquid scintillation counting of aliquots of the radioactive effluent. Samples containing 40 to 80 % of *alpha*-isomer could be purified to materials having a high chemical and radiochemical purity. The radiochemical purity of the preparations was determined by paper chromatography with radioautography employing a number of different solvent combinations, including reverse phase systems. Liquid-liquid partition chromatography and thin layer chromatography were also used as analytical systems. The chemical purity was assessed by infrared spectroscopy and bioassay, as well as conventional procedures. The yields and specific activities were determined by usual radiometric procedures.

CHEMICALS.

The solvents used were distilled, and all glassware was oven-dried at 105° C for 2 hours. The *N*,*N*-diethylaniline was purified using the method described by Fieser [7]. The methanol was dried by the method of Riddich

TABLE 1. Partition Coefficients of the *Cis*-Isomers of Bidrin and Azodrin With Water and Various Organic Solvents.

Organic Solvent	Partition Coefficient[a]	
	Bidrin	Azodrin
CH_2Cl_2	7.8	1.3
CH_2Cl_2-hexane, 3 to 1	3.4	0.5
CH_2Cl_2-hexane, 1 to 1	1.0	0.1
CH_2Cl_2-hexane, 1 to 3	0.1	0.001
$CHCl_3$	18.0	2.3
$CHCl_3$-hexane, 3 to 1	5.9	0.9
$CHCl_3$-hexane, 1 to 1	2.3	0.2
$CHCl_3$-hexane, 1 to 3	0.17	—
CCl_4	0.1	0.02
Benzene	0.26	0.06
Hexane	—	0.01

[a] Partition Coefficient = $\frac{\text{Concentration in Organic Phase.}}{\text{Concentration in Polar Phase}}$

and Toops [8]. The methanol-^{14}C was purchased from suppliers 1, 2 and 3 *. The specific activity varied from 1 to 10 millicuries per millimole. It was used as received without further purification. The phosphorus trichloride used in the trial experiments was purified by distillation through a 12-inch Vigreaux column at a reflux ratio of 5 to 1, The phosphorus-^{32}P trichloride was purchased from suppliers 1, 3 and 4. The phosphorus-^{32}P trichloride was used as received without further purification and varied in specific activity from 2 to 5 mCi/mmole. The trimethyl phosphite was purified by distillation through a 20-plate bubble column from potassium at a reflux ratio of 10 to 1. The trimethyl phosphite (TMP)-^{32}P, if not prepared in these laboratories; was purchased from suppliers 3 and 4, and purified by the procedure described in Experimental for TMP. The specific activity varied from 2 to 4 mCi/mmole. The diketene was fractionated through a 10-inch glass helices packed column. The *N,N*-dimethylacetoacetamide was fractionated through an 8-inch Vigreaux column. The fractionated product was collected and recrystallized from ether. The ^{14}C-labeled methylamine · HCl, specific activity 11 mCi/mmole, was purchased from supplier 4, and was used without further purification. The

* (1) New England Nuclear Corp., Boston, Mass.
(2) Bio-Rad Co., Richmond, Calif.
(3) Volk Radiochemical Co., Skokie, Ill.
(4) Nuclear Chicago, Des Plaines, Ill.
(5) Nuclear Research Chemicals, Orlando, Flo.

N,*N*-dimethylamine-^{14}C, specific activity 4 mCi/mmole, was purchased from supplier 5, and it was used without further purification. To minimize the presence of the dichloro analog, 2-chloro-*N*,*N*-dimethylacetoacetamide and 2-chloro-*N*-methylacetoacetamide were especially prepared to reduce the dichloro content and purified by chromatography, fractional distillation or recrystallization, or a combination of these methods. However, the most satisfactory purification took advantage of the preferential distribution of the dichloro acetoacetamides into carbon tetrachloride from water; the amide and monochloro acetoacetamide distribute preferentially into the aqueous phase. The monochloro acetoacetamide was extracted from the aqueous mixture with methylene chloride. 3-Hydroxycrotonoyl chloride dimethyl phosphate, was prepared from the corresponding acid and purified by distillation. Other chemicals used were analytical grade.

Partition chromatography.

One hundred grams of crushed firebrick (Johns Manville Silocel C-22 firebrick 60 to 80 mesh) and 95 ml of 0.05 M potassium acid phthalate was used to prepare a glass pipe column 1 × 150 cm. The column was fitted with a stainless steel needle valve or small bore stainless hypodermic tubing immersed in a constant temperature bath and coupled to an automatic fraction collector. The sample of crude Azodrin, not exceeding 500 mg dissolved in a few milliliters of the solvent mixture, was charged to the column. The flow rate was maintained at 1 ml per minute, and 20 ml fractions were collected. Aliquots of the fractions were counted in a liquid scintillation counter (Packard 314A), or with some phosphorus samples, the entire fraction was counted with a rate meter. The appropriate fractions containing the *cis*-isomer were combined, and the solution was concentrated to a small volume; this was dried, and the remainder of the solvent was removed. The position of the elution of components did not change in the range of 50 to 500 mg of Azodrin or Bidrin. Bidrin was chromatographed by the above technique, using benzene as the elution solvent.

Paper chromatography.

The presence of radioactive impurities and their relative amounts in the preparations before and after partition chromatography were, in general, determined by paper chromatography. Isopropanol-concentrated ammonium hydroxide (3 to 1 v/v) as the mobile phase and Whatman No. 1 paper were used to separate the polar impurities from the *cis*- and *trans*-isomers. Other systems used include Whatman No. 3 paper with acetonitrile-water-concentrated ammonium hydroxide 80 : 18 : 2; Whatman No. 3 paper with benzene as the mobile phase; silicone-treated Whatman No. 1 paper as the stationary phase, and water or methanol or mixtures of these were used to separate the

isomers. Other reverse phase systems include : ethylene glycol-treated Whatman No. 1 paper with benzene as the mobile phase; mineral oil-treated Whatman No. 1 paper with water or methanol or mixtures of these as the mobile phase.

The areas of the paper containing ^{14}C or ^{32}P were determined, isolated, and counted with a Nuclear-Chicago gas flow GM counter. In other instances the strips were counted directly on a Vanguard paper strip scanner. Impurities were identified by cochromatographing mixtures of 1 to 10 μg of the radioactive samples and 50 to 100 μg of a known sample. Thin-layer chromatography was also used for identification purposes. Alumina employing carbon tetrachloride chloroform methanol 30 : 70 : 1 and silica gel using the same solvents in a ratio of 50 : 50 : 2 have effectively separated Azodrin and Bidrin.

Infrared analyses.

Infrared analyses were performed to determine the chemical purity as well as for identification purposes. Standard spectra of chromatographically pure samples were obtained on a carbon tetrachloride or methylene bromide solution (1/2 to 1 %) in a 1.0-mm sodium chloride cell using a Beckman IR-4 spectrophotometer with normal instrument settings. The spectrum of the sample was obtained in the same manner and the absorbence determined by a base line procedure.

Bioassay methods.

To determine if the radioactive Azodrin and Bidrin were biologically comparable to pure unlabeled standards the preparations were bioassayed. Certain of the trial experiments were also assayed to compare the active isomer content with that found by physical measurements. To determine if there was any isotopic effect between ^{14}C and ^{32}P preparations or the site of the radioactivity, bioassays were performed on the various labeled compounds. Normal house flies 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.

^{32}P-Labeled trimethyl phosphite.

In a typical preparation a small, two necked reaction flask, fitted with a rubber-sealed inlet, a magnetic stirrer and cold water condenser, was dried by continuous pumping on the vacuum manifold with a diffusion pump for several hours. Air was then admitted to the reaction flask through a drying chamber. Five milliliters of phenylcyclohexane was added to the reaction flask. Next, 589 mg (16.89 mmoles) of anhydrous methanol and 2.65 g (17.73 mmoles) of *N,N*-diethylaniline were weighed in a dry box and trans-

ferred to the reaction flask by means of hypodermic syringes. The mixture of methanol, *N,N*-diethylaniline and phenylcyclohexane was then cooled in an ice bath.

The ^{32}P phosphorus trichloride, 774 mg (5.63 mmoles), was diluted with 1 ml of phenylcyclohexane, and the solution was taken up in a shielded syringe under anhydrous conditions. With stirring, the ^{32}P phosphorous trichloride was added dropwise to the reaction mixture during a period of 45 minutes. The mixture was then stirred for 30 minutes at 0° C, warmed to room temperature and allowed to stand an additional 30 minutes. The reaction mixture was then cooled in liquid nitrogen, the reaction flask was evacuated, and the trimethyl phosphite-^{32}P (TMP-^{32}P) was distilled into a round-bottomed flask fitted with a side arm. An ice bath was then placed around the flask containing the TMP-^{32}P, and dry air was admitted to the flask. Several small pieces (25 mg each) of freshly cut potassium were added to the flask over a period of 1 1/4 hours. The TMP-^{32}P was then distilled into a graduated tube fitted with a stopcock, and the volume of TMP-^{32}P was measured. The weight of TMP-^{32}P was then calculated from its volume and the density (1.05 g/ml) of TMP. The yield of TMP-^{32}P was 367 mg (2.94 mmoles) 52 %. The yields with inactive trials were 37 to 86 %, as determined by infrared analyses, titration methods, and formation of derivatives.

^{32}P-Labeled Bidrin.

An example of the preparation of labeled Bidrin is given : 2-chloro--*N,N*-dimethylacetoacetamide, 384 mg (3.4 mmoles) was weighed into a micro reaction flask, fitted with a water cooled cold finger, and 25 µl of acetic acid was added. The flask was connected to the vacuum manifold, and 426 mg (3.43 mmoles) of TMP-^{32}P was distilled into the flask. Dry air was admitted to the flask, and the mixtures was then heated at 95° C, increasing to 115° C during 4 hours with a Glas-Col heating mantle. The temperature was controlled with a Celect-Ray temperature controller. After 4 hours the mixture was stripped at room temperature at 0.1 µ pressure. The product weighed 799 mg (3.3 mmoles), 97 % yield. It contained 76 % α-isomer and 16 % β-isomer. In trial experiments, the yield of Bidrin obtained as mixed isomers was 67 to 99 %.

^{32}P-Labeled Azodrin.

A preparation typical of the ^{32}P-labeled Azodrin was carried out as follows : 2-chloro-*N*-methylacetoacetamide, 180 mg (1.2 mmoles), was weighed into a micro reaction flask, and 1 ml of benzene was added. The TMP-^{32}P was distilled into the flask, and the mixture was then heated at reflux for 4 hours in a liquid heat exchanger. After 4 hours, the mixture was stripped at room temperature at 0.1 µ pressure. The product weighed 261 mg

(1.1 mmoles), 92 % yield. The α-isomer content was 80 %, the β-isomer 10 %. The yield of the mixed isomers of Azodrin in the pilot experiments was 45 to 85 %.

^{14}C-Labeled trimethyl phosphite.

An example of the general reaction is as follows: trimethyl phosphite, 434 mg (3.5 mmoles), was weighed into a round-bottomed flask. The TMP was treated with a few pieces of freshly cut potassium at room temperature; the flask was then connected to the vacuum manifold, and the TMP was distilled into a two-necked reaction flask. Sixteen milligrams (0.5 mmole) of anhydrous methanol-^{14}C (specific activity 8.5 mCi/mmole) was distilled into the flask containing the TMP. After all the methanol-^{14}C had been added, the mixture was allowed to come to room temperature, and the flask was removed from the manifold. The flask was then fitted with a spiral water condenser, drying tube and cold trap followed by another drying tube. The reaction mixture was heated to 95° C with a Glas-Col heating mantle for 2 hours. The temperature was then increased to 105° C, and the mixture was heated at this temperature for an additional 4 hours. The temperature was controlled with a Celect-Ray temperature controller. The apparatus was connected to the vacuum manifold and the TMP-^{14}C and methanol-^{14}C were distilled into a flask with a side arm for potassium treatment. A slight residue remained in some cases from the distillation which was determined by infrared analysis to be trimethyl phosphate, dimethyl phosphite and dimethyl methyl phosphonate. The distilled TMP-^{14}C was then cooled in a water-ice mixture, and freshly cut potassium was added. One millimole of potassium was added per millimole of methanol-^{14}C. The TMP-^{14}C was then distilled into a tared receiver tube fitted with a stopcock and weighed. The product weighed 396 mg (3.2 mmoles), 90 % yield. Yields of TMP averaged about 90 % in the inactive trials.

^{14}C Methoxy-labeled Bidrin.

Trimethyl phosphite-^{14}C, 136 mg (1.1 mmoles) was reacted with 2-chloro-*N*,*N*-dimethylacetoacetamide, 164 mg (1.0 mmole), as described in the preparation of Bidrin labeled with ^{32}P. The product, consisting of mixed isomers, weighed 212 mg (0.9 mmole), 90 % yield. The α-isomer content was determined to be 90 %. The average yield of Bidrin in the inactive experiment was 88 %.

^{14}C Methoxy-labeled Azodrin.

Trimethyl phosphite-^{14}C, 273 mg (2.2 mmoles), was reacted with 2-chloro-*N*-methylacetoacetamide, 298 mg (2.0 mmoles), as described in the preparation of Azodrin labeled with ^{32}P. The product weighed 423 mg (1.9 mmoles),

95 % yield. Its α-isomer content was 87 %, β-isomer content 5 %. The results of the trials with inactive materials gave an average yield of 77 %.

^{14}C Bidrin-labeled in the N-methyl position.

Into a small two-necked reaction flask, fitted with a serum cap, magnetic stirrer and cold water condenser, was transferred 408 mg (5.0 mmoles) of ^{14}C dimethylamine hydrochloride in 1 ml of water. The reaction flask was surrounded by an ice bath, and 280 mg (5.0 mmoles) of potassium hydroxide in 1 ml of water was introduced by a syringe during a period of 30 minutes. Diketene, 420 mg (5.0 mmoles) was added during 2 hours to the reaction mixture which was then held at 25° C for 2 hours. The excess diketene and water were distilled under vacuum. The residual acetoacetate-^{14}C was dissolved in 4 ml of methylene chloride. The organic phase was filtered using a modified Skau apparatus. The solvent was distilled from the filtrate. Weight of the acetoacetate-^{14}C was 653 mg (5.0 mmoles), 100 % yield. The yields of inactive preparations were 76 to 96 % as determined by infrared analysis. The acetoacetate was diluted with 10 ml of methylene chloride, and the reaction flask was fitted with a dropping funnel. The temperature was kept at 30° C, and with stirring 675 mg (5.0 mmoles) of thionyl chloride in 10 ml of methylene chloride was added dropwise during 2 hours. After the addition of thionyl chloride was complete the reaction mixture was heated under reflux for 2 hours. The crude reaction mixture was extracted with aqueous sodium bicarbonate, followed by water. The organic phase was dried over sodium sulfate and filtered by means of a filter stick. The filtrate was stripped of solvent under vacuum. The product weighed 548 mg (3.3 mmoles), 67 % yield. Trial chlorinations were performed with yiels of 75 to 91 %. To the 2-chloro-*N*,*N*-dimethylacetoacetamide-^{14}C was added 868 mg (7 mmoles) of TMP and 30 μl of acetic acid. The reaction was heated to 95° C, and during 4 hours the reaction temperature was gradually increased to 110° C. All volatile material was distilled. The Bidrin-^{14}C weighed 680 mg (2.86 mmoles), 57 % yield. The α-isomer content was found to be 60 %.

^{14}C Azodrin-labeled in the N-methyl position.

Into a small two-necked flask equipped with a rubber-sealed inlet, magnetic stirrer and condenser was transferred 135 mg (2.0 mmoles) of methylamine-^{14}C hydrochloride. To this was added 547 mg (2.2 mmoles) of 3-hydroxycrotonoyl chloride dimethyl phosphate in 10 ml of methylene chloride. The flask was immersed in an ice bath and 4 mmoles of potassium hydroxide in 300 μl of water was introduced dropwise by means of a syringe through the serum cap during 1/2 hour. After addition was complete, the mixture was stirred for 1 hour at about 40° C. The organic phase was removed and washed twice with 1 ml portions of water. The organic phase was dried

and filtered, and solvent was distilled from filtrate. The Azodrin-^{14}C weighed 443 mg (2.0 mmoles), 99 % yield. The α-isomer content was determined to be 80 %.

RESULTS.

The experiments yielded from six to several hundred milligrams of labeled compound. Most trial experiments were carried out on a 1 to 2 mmole scale.

The yields, purities, and specific activities prior to chromatography are given in Table 2 for the radioactive preparations of Bidrin and Azodrin. The purity of the trimethyl phosphite was one of the greatest factors in increasing the yields of both Azodrin and Bidrin, and work on purification techniques of the TMP has continued. Reduced yields are thought to be in part to the incomplete distillation of the TMP-^{32}P from the reaction mixture. This could be improved by filtration; however, the decrease in yield is sacrificed

TABLE 2. Preparations of Radioactive Bidrin and Azodrin.

Preparation Number	Bidrin		Chemical Yield[a] of Mixed Isomers, %	α-isomer Content, %
	Position of Label	Specific Activity, μCi/mg		
1	^{14}C-Methoxy	2.5	89	90
2	^{14}C-Methoxy	5.0	100	44
3	^{14}C-*N*-Methyl	4.2	57[b]	60
4	^{32}P	20.0	101	60
5	^{32}P	12.0	97	76
6	^{32}P	19.3	93	45
7	^{32}P	12.6	100	50
	Azodrin			
8	^{14}C-Methoxy	1.0	95	87
9	^{14}C-*N*-Methyl	9.0	99[c]	80
10	^{32}P	4.0	96	42
11	^{32}P	5.3	97	43
12	^{32}P	9.5	102	47
13	^{32}P	6.9	100	80
14	^{32}P	7.2	88	70
15	^{32}P	6.9	92	80

[a] Based on trimethyl phosphite-^{32}P and ^{14}C as appropriate unless otherwise noted.
[b] Based on dimethylamine-^{14}C.
[c] Based on methylamine-^{14}C.

for ease of handling and higher purities of the product. The usual impurities found in TMP, dimethyl methylphosphonate, dimethyl phosphite, and trimethyl phosphate increase with higher specific radioactivities. Most reactions are thus carried out at less than 32 μCi/mg.

The yield of TMP from the exchange reaction is much superior to the conventional synthesis when it can be employed. One disadvantage is the reduced radiochemical yield of TMP-^{14}C due to the loss of radioactivity of non-exchanged MeOH. In the exchange reaction between tagged methanol and TMP, the amount of unlabeled TMP may be varied to alter the specific activity as desired. Ratios of 4 to 8 TMP to methanol have been quite satisfactory in this work. The formula for this calculation has been reported in an earlier paper [4].

The effects of several variables on the vinyl phosphate reaction have also been studied. After the preparation of TMP the toxification step to form Azodrin is identical whether TMP-^{32}P or ^{14}C is used. The size of the reaction had little effect on the yield of the desired α-isomer. The optimum reaction conditions of those tried were found to be 4 hours at 80° C. Dramatic effects on the *cis*-isomer content are caused by solvent changes on macro experiments. Hydroxylic solvents, particularly alcohols and acetic acid, gave the best *cis*-isomer enrichment. On a micro scale little if any increase of *cis*-isomer content was observed with acetic acid; however, in general, the *cis* content was usually higher on a micro scale than on macro preparations. This is probably due to the presence of a higher ratio of solvent.

When the isotope was located in the TMP, only a small excess of TMP was used to conserve the isotope; therefore, some unreacted starting amide usually remained. A 5 % excess of TMP could result in up to 20 % of the unreacted starting amide, whereas a 50 % excess left only 2 % of the chloroamide unreacted. In large-scale operations the TMP is used in excess (as solvent); thus the reaction may be driven more nearly to completion. The principal radioactive impurity in the crude preparations of Azodrin was dimethyl methylphosphonate. The major non-radioactive impurity was the intermediate chloroamide. In preparing the *N*-methyl-^{14}C Azodrin from 3-hydroxycrotonoyl chloride dimethyl phosphate and methylamine-^{14}C, considerable higher yields were obtained when the amide was liberated from its salt, *in situ*. Closely-controlled temperature and increased ratio of solvent to solute also enhanced yields.

The reaction between TMP and 2-chloro-*N*,*N*-dimethylacetoacetamide is the final toxification step in the preparation of Bidrin, whether the label is ^{32}P, ^{14}C in the methoxy group, or ^{14}C in the *N*-methyl position. The first step in the synthesis of *N*-methyl-^{14}C Bidrin was the formation of acetoacetate-^{14}C from dimethylamine-^{14}C and diketene. The yield in this step was high, and the reaction proceeded smoothly in a short time at 5° C with the formation of only 3 % impurities as determined by GLC. The chlorination reaction posed a problem. To completely chlorinate the acetoacetamide,

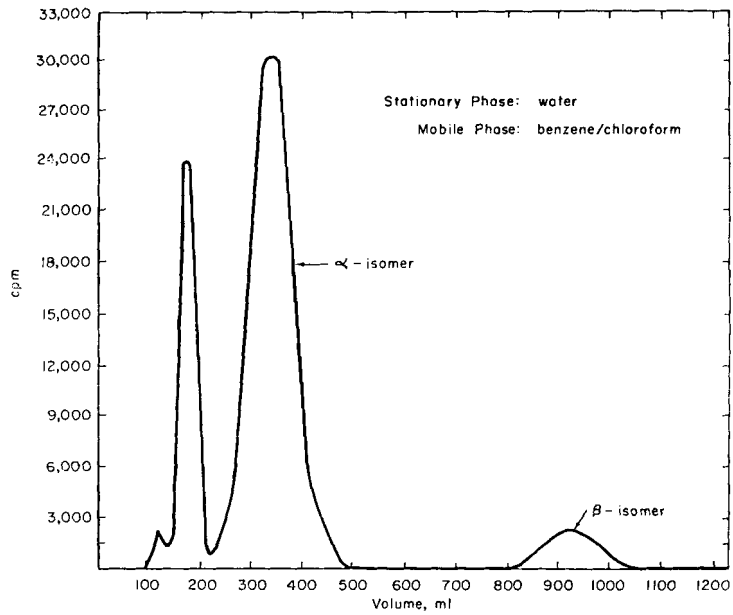


FIG. 5. Partition Chromatography of Azodrin-^{32}P.

some dichloroacetoacetamide will be formed. This reacts with TMP to give a chlorodimethylcrotonamide. Ratios of 1 to 1 chlorinating agent to amide were used, resulting in some unreacted amide, which is generally better than over-chlorination which will produce a chloro compound in the end product. In the final reaction step, considerable excess of TMP could be used, which resulted in little or no unreacted starting chloroamide. In the synthesis of ^{32}P and ^{14}C-methoxy materials, closely-controlled reaction conditions gave reproducible results. The results were similar to those discussed in the Azodrin reaction, including the *cis*-isomer enrichment. The main radioactive impurity

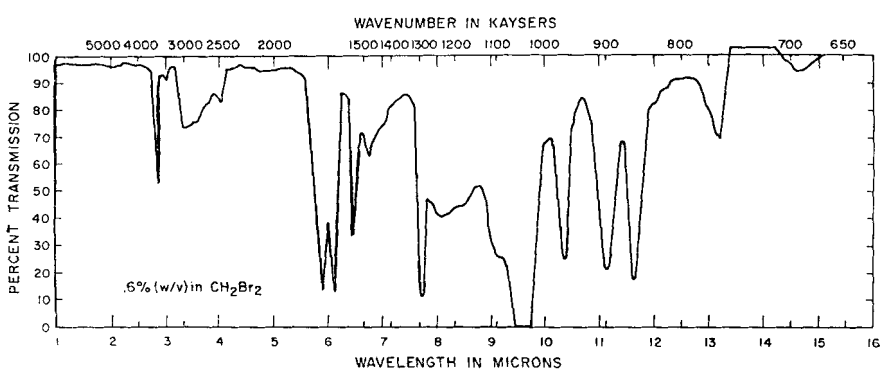


FIG. 6. Infrared Scan of the Azodrin-^{32}P.

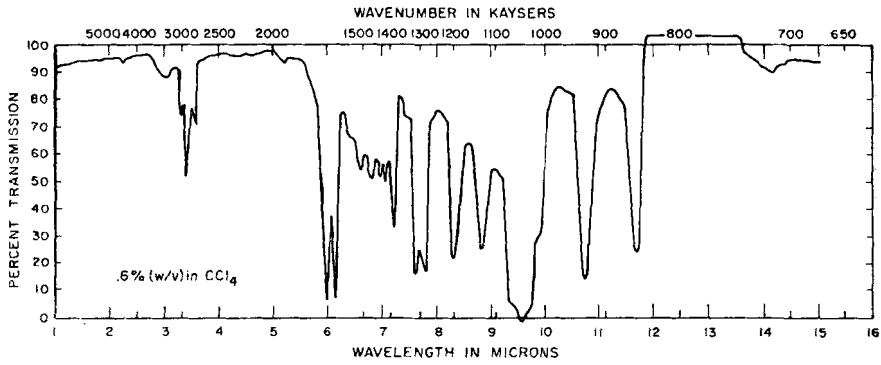


FIG. 7. Infrared Scan of the Bidrin-^{14}C.

was dimethyl methylphosphonate. Again, apparently for the same reasons, the average *cis*-isomer yield was less in the radioactive synthesis than in the inactive preparations.

The results of a typical columnar chromatographic separation are shown in Figure 5. The separation of the two isomers is complete. Typical infrared scans of chromatographed Azodrin and Bidrin are shown in Figures 6 and 7. In chromatographic separations yields of the *cis*-isomer were less than quantitative. The losses are believed to be caused by catalytic decomposition of the vinyl phosphate by the crushed firebrick support.

LLPC increased the purity of labeled Bidrin from about 45 to 99 %, and Azodrin from 80 to >99 %. The impurities in the samples before purification are mainly acetoacetamide, chloro derivative of methyl or dimethylcrotonamide, trimethyl phosphate and dimethyl methylphosphonate. There is good correlation between the toxicity index and *cis*-isomer content of the trials and radioactive preparations. Also, as expected, the nature of the radioactivity or its location in the molecule does not alter this agreement.

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