Analysis of Phosdrin in Vegetables Using Gas-Liquid Chromatography

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A procedure has been described for determining the insecticide Phosdrin in the presence of two vegetable crops, green beans and artichokes. Extraction with chloroform followed by gas chromatography equipped with a cesium bromide thermionic flame detector provided a quick and sensitive means for detection, as well as separation of the two isomers of Phosdrin. The method was capable of detecting levels of the insecticide in crop extracts as low as 0.01 ppm without cleanup. The method was also compared with a cholinesterase inhibition procedure.

Phosdrin insecticide consists of two isomers of 2-carbomethoxy-1-methylvinyl dimethyl phosphate. The α-isomer (cis-crotonate isomer) is much more biologically active than the corresponding β-isomer (*trans*-crotonate isomer). The chemical is used on a great variety of crops for the control of a broad range of insects. Procedures for detecting Phosdrin are available using thin-layer chromatography, cholinesterase inhibition, and colorimetry for total phosphorus determination. These methods either are not particularly sensitive or require extensive or laborious cleanup procedures prior to detection. Also, with the exception of thin-layer chromatography, these methods are not capable of separating and detecting the two isomers.

Several investigators (Burke and Holswade, 1966; Egan *et al.*, 1964; Giuffrida, 1964; Hinden *et al.*, 1964; Watts and Storherr, 1965) have utilized gas chromatography for detecting Phosdrin in the presence of other organophosphates. However, few discuss the separation of the isomers or sensitivity, and only Watts and Storherr (1965) reported detecting Phosdrin in the presence of an agricultural commodity using gas chromatography. Peak shape, size, and sensitivities, as well as separation of the α and β isomers of Phosdrin, were not discussed.

The present paper describes a rapid, simple, and sensitive procedure that is capable of detecting the two isomers of Phosdrin independently. This method involves an extraction of the crop material with chloroform, followed by injection into a gas chromatograph equipped with a cesium bromide flame thermionic detector. The method is compared with a cholinesterase-inhibiting procedure and includes a method for the separation of natural cholinesterase inhibitors in artichokes and beans.

MATERIALS AND EQUIPMENT

Gas Chromatograph and Recorder. A Varian Aerograph Model 204 gas chromatograph equipped with a cesium bromide thermionic flame detector (Hartmann, 1966) was used for all analyses. The signal from the detector was supplied to a 1-mv Westronics recorder operating at a chart speed of 30 inches per hour.

Column and Operating Conditions. A 6-foot $\times$ $^1/_8$ in. o.d. borosilicate glass column was packed with 2% (w/w) diethylene glycol succinate (DEGS, Analabs. Inc. Hamden, Conn.) on 100- to 120-mesh Chromosorb G, AW, DMCS. The temperatures of the injection port, column oven, and detector oven were held to 210°, 190°, and 210° C, respectively. Flow rates of all gases were regulated by milliflow back reference controllers (Milliflow Div., Veriflo Corp., Richmond, Calif.) and the flow rates of the gases in ml per min were 45, 180, and 18, respectively, for nitrogen (carrier), oxygen, and hydrogen.

Evaporator. A rotary evaporator, Model 5155 (California Laboratory Equipment Company, Oakland, Calif.) was used for all evaporation procedures.

Reagents. Reagents included redistilled reagent grade chloroform; redistilled reagent grade hexane; redistilled acetonitrile; coagulating solution prepared by dissolving 1.0 g of ammonium chloride in 800 ml of water containing 2.0 ml of 85% phosphoric acid; Phosdrin standard (Shell Development, Modesto, Calif.) containing 60% of the α or cis-crotonate isomer and 40% of the β or *trans*-crotonate isomer.

PROCEDURE

Extraction from Crop Material. Approximately 500 g of sample was chopped in a Hobart food cutter in the presence of a small quantity of solid carbon dioxide. The presence of solid carbon dioxide keeps the sample frozen and permits a more efficient chopping operation. The sample was chopped until a powdery composition was obtained. The carbon dioxide was allowed to evaporate and 200 g of the chopped sample was transferred to a 1-quart Waring Blendor cup along with 400 ml of chloroform. The contents were blended for at least 1 min at high speed. The solvent was decanted into a 2-liter heavy duty Erlenmeyer flask, and again 400 ml of chloroform was added to the sample in the blendor cup, followed by reblending. The contents from the blending operations were combined in the Erlenmeyer flask and mixed on a Gyrotory shaker (New Brunswick Scientific Co.) for one-half hour at 180 rpm. The extract was filtered through a 24-cm Whatman No. 1 fluted filter paper containing sodium sulfate and stored in a tightly sealed glass bottle until analyzed.

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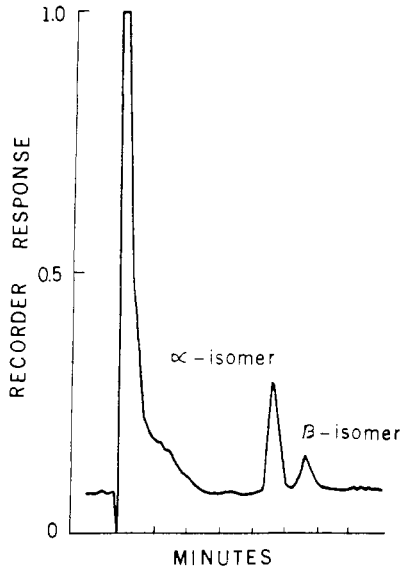

Figure 1. Gas chromatogram of Phosdrin standard

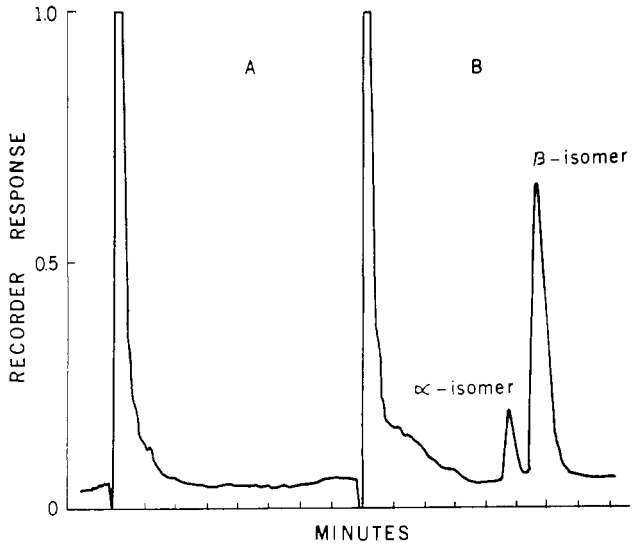

Figure 2. Gas chromatogram of (A) control sample of artichokes and (B) field treated artichoke sample containing 0.02 ppm of the α isomer and 0.20 ppm of the β isomer of Phosdrin

An alternate extraction procedure using a Soxhlet apparatus with 10% methanol in chloroform as recommended by Bowman *et al.*, 1968, was compared to the method just discussed. There was a slight decrease in residue found for both isomers of Phosdrin from treated samples using the Soxhlet extraction. However, the difference in residue found could be considered insignificant and, for all practical purposes, either extraction could be recommended. The blending procedure does have the advantage in handling and time saved for a particular analysis.

Recovery samples were fortified with the analytical standard prior to adding solvent in the blendor cup, and then processed in the same manner as the other samples. Fortification levels ranged from 0.01 to 1.0 ppm.

Separation from Crop Material. Samples of artichokes and snap beans did not require a cleanup procedure where gas chromatography was employed for detection of Phosdrin. However, for cholinesterase determination, separation of naturally occurring cholinesterase-inhibiting materials in the crop material from the pesticide was necessary. A portion of extract representing 10 g of crop was transferred to a round-bottomed flask and evaporated to dryness *in vacuo* on a rotating evaporator. The sample was transferred to a separatory funnel with 100 ml of hexane, followed by two 25-ml washings with acetonitrile. The sample was extracted with vigorous shaking for 30 sec. The acetonitrile fraction was transferred to a round-bottomed flask and the hexane fraction was again extracted two times with 25 ml of acetonitrile. The acetonitrile fractions were combined and the contents were evaporated to complete dryness on the rotating evaporator. The residue in the flask was dissolved in 10 ml of chloroform, and 5 ml of coagulating solution was added.

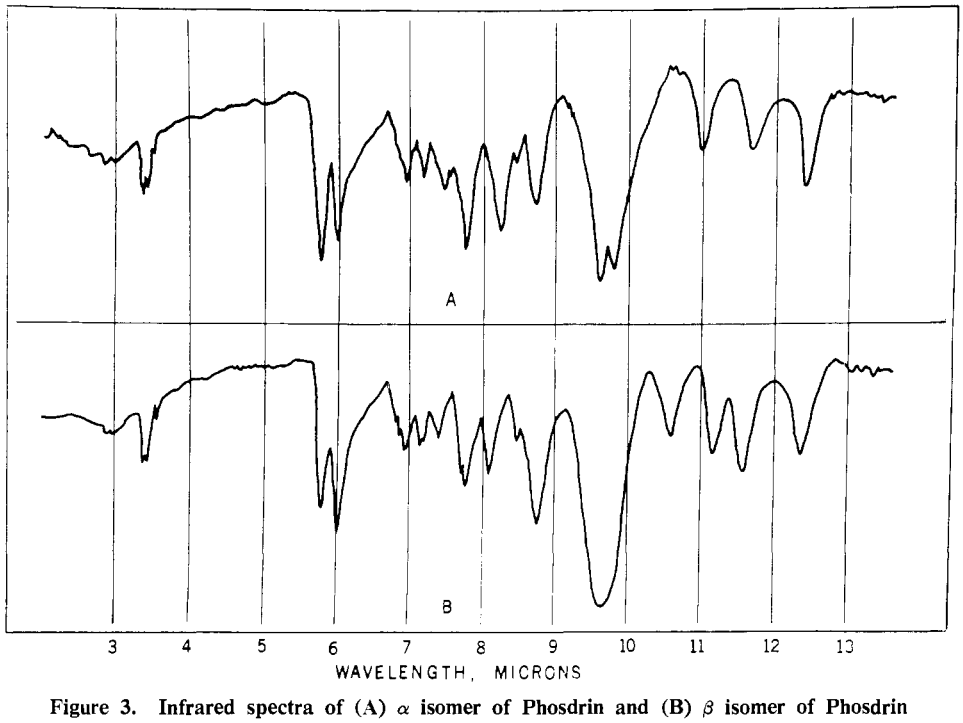

Figure 3. Infrared spectra of (A) α isomer of Phosdrin and (B) β isomer of Phosdrin

Table I. Comparison of Two Detection Systems and Residue Found for the Analysis of Phosdrin on Artichokes and Green Beans

Rate of Application (lb/A)	Days from Application to Sampling	Total Residue Found (ppm)		
		Gas Chromatography		Cholinesterase Inhibition
		α isomer	β isomer	$\alpha + \beta$ isomers
		Artichokes		
Control	0	<0.01	<0.02	<0.03
1	0	2.52	1.60	2.63
Control	2	<0.01	<0.02	<0.03
1	2	0.90	1.25	0.92
Control	5	<0.01	<0.02	<0.03
1	5	0.13	0.59	0.19
Control	7	<0.01	<0.02	<0.03
1	7	0.02	0.20	<0.03
		Green Beans		
Control	0	<0.01	<0.02	<0.03
0.5	0	0.05	0.22	0.06
Control	1	<0.01	<0.02	<0.03
0.5	1	0.02	0.22	0.04
Control	2	<0.01	<0.02	<0.03
0.5	2	<0.01	0.11	<0.03
Control	3	<0.01	<0.02	<0.03
0.5	3	<0.01	0.06	<0.03
Control	4	<0.01	<0.02	<0.03
0.5	4	<0.01	0.05	<0.03

The chloroform was carefully evaporated until there was absolutely no trace of the organic solvent remaining. The remaining aqueous solution was filtered through Whatman No. 1 filter paper into a separatory funnel, followed by at least five individual washings of 5 ml of 10% methanol solution. The aqueous solution was extracted three times with 25 ml of chloroform. The chloroform was filtered through anhydrous sodium sulfate into a round-bottomed flask and evaporated to a volume of 10 ml. The amount of Phosdrin present was determined using the potentiometric method of cholinesterase inhibition, according to the method described by Archer *et al.* (1963).

Detection by Gas Chromatography. An amount of extract equivalent to 33 g of sample was concentrated to 1 ml in a McNaught and McKay-Shevky-Stafford sedimentation tube by dry air with the tube immersed in a water bath held at 50° ± 5° C. As much as 10 μl of the chloroform extract, equivalent to 330 mg, was injected into the gas chromatograph. Peak area was measured using a planimeter and compared to a standard curve. Standard solutions of Phosdrin contained 60% of the α-isomer and 40% of the β-isomer. Therefore, a solution containing 3 ng per μl of the α-isomer contained 2 ng of the β-isomer. Standard solutions were prepared for on-column injections ranging from 3 to 300 ng for the α-isomer and 2 to 200 ng for the corresponding β-isomer. All solutions were in chloroform and ranged in concentration from 5 to 50 ng/μl of the technical product.

RESULTS AND DISCUSSION

The flame thermionic detector using a cesium bromide pellet mounted on a quartz tip gave specific and sensitive response to the isomers of Phosdrin. Figure 1 shows the response given by a 10-ng injection of the Phosdrin standard, and includes the separation of the α and β isomers. Figure 2A shows the response of a 330-mg injection of an untreated control sample of artichokes and Figure 2B a field treated artichoke sample containing 0.02 ppm of the α-isomer and 0.20 ppm of the β-isomer of Phosdrin. Several columns were investigated for separating and detecting the two isomers of Phosdrin. Only the DEGS and a 5% DC-11 on 100- to 120-mesh Chromosorb G, AW, DMCS gave satisfactory separation with sharp, symmetrical peak response.

To confirm the identity of the peaks, Figure 3 shows the infrared spectrum of both isomers separated and collected from a gas chromatograph. The separated isomers were rechromatographed to observe any change in molecular structure. Each isomer gave the same retention time both before and following collection, which was indicative of no detectable change in the structure, according to the method used for separation by gas chromatography. Figure 3A presents infrared spectra of the α-isomer of Phosdrin and Figure 3B the β-isomer. These data agree with those reported by Stiles *et al.* (1961).

The β-isomer of Phosdrin is known to be less active as a cholinesterase inhibitor. However, on the crops studied, it appeared to be more stable and therefore resisted decomposition, as compared with the α-isomer. Table I shows the amount of residue found in green beans and artichokes, using both the cholinesterase and gas chromatography procedures. Since cholinesterase inhibition was not capable of differentiating between the isomers, but rather gave a total inhibition of the combined isomers, one would expect a higher residue value than what actually existed for the biologically active α-isomer of Phosdrin. Therefore, a cholinesterase determination was made of the GLC-separated isomers to determine their relative activity as a cholinesterase inhibitor. The β-isomer was found to be about 6% as active as the α-isomer. Therefore, adding 6% of the amount of the β-isomer found to the α-isomer determined by gas chromatography explained the slightly higher residue found by cholinesterase inhibition. This indicated that analyses using cholinesterase inhibition were not greatly influenced by the presence of the β-isomer, since it was so much less active than the α-isomer. On the other hand, it did show that separation of the two isomers was necessary when employing gas chromatography, since the β-isomer appeared to have a much longer residual effect. The α-isomer was relatively unstable, and within a very few days there was no detectable

Table II. Recovery Study of Phosdrin from Green Beans and Artichokes as Detected by Gas Chromatography

Crop	ppm Added	ppm Found	Recovery %	Av. Rec. %
Beans	(1) 0.01	0.0099	99	97
	(2) 0.01	0.0095	95	
Artichokes	(1) 0.01	0.0101	101	96
	(2) 0.01	0.0091	91	
Beans	(1) 0.1	0.107	107	106
	(2) 0.1	0.105	105	
Artichokes	(1) 0.1	0.103	103	97
	(2) 0.1	0.091	91	
Beans	(1) 1.0	0.97	97	96
	(2) 1.0	0.94	94	
Artichokes	(1) 1.0	0.94	94	95
	(2) 1.0	0.96	96	
Overall avg				100

residue. The β isomer, on the other hand, still persisted after several days.

Table II illustrates a typical recovery study of samples freshly fortified and analyzed at various levels of Phosdrin to beams and artichokes. Recoveries ranged between 95 to 107%, with an overall average of 100%. However, when some extracts were permitted to remain in storage for several days prior to analysis, some of the fortified samples have been known to show an increase in recovery up to 25%, particularly when using the cholinesterase procedure. One explanation is that the β isomer is converting to the α isomer. However, this could not be verified by gas chromatography. Since the cause of high recoveries cannot be explained at this time, it is recommended that the samples be analyzed within a short time after their arrival or stored in subzero temperatures prior to analysis.

The cleanup procedure used for the cholinesterase inhibition study could also be used prior to detection by gas chromatography. However, it was not necessary for the plant extracts in this study. The cleanup procedure used by Watts and Storherr (1965) using "sweep codistillation" could possibly be used as a quick procedure if crop extracts should require preparation prior to detection by gas chromatography. Other detection devices, other than the flame thermionic detector, can also be utilized in conjunction with a gas chromatograph, as long as they are specific for phosphorus. For example, the Melpar flame photometric detector (available from Tracor, Inc., Analytical Instruments Division, Austin, Tex.) performs effectively for the detection of Phosdrin. This instrument was not employed, since it was not available at the time this study was conducted.

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