Metabolism of the Systemic Insecticide *O,O*-Dimethyl 1-Carbomethoxy-1-propen-2-yl Phosphate (Phosdrin) in the Pea Plant

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This study was undertaken to define the degradation mechanism of the Phosdrin isomers in plants, because alkaline hydrolysis yields different degradation products with the two isomers. The two isomers were found to degrade in the same manner but at different rates in pea plants. By administering one metabolite, Phosdrin acid, to plants it was possible to recover a monodealkylated derivative. The enzymatic degradation of Phosdrin in pea plants differs from the alkaline hydrolysis in that at least two pathways are involved, the major one being via dimethyl phosphate directly.

A STUDY of the degradation of substituted vinyl phosphates which are insecticidal is usually complicated by the presence of geometrical isomers. In the case of some, such as *O,O*-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (Phosdrin) (I), the complexity is increased by the presence of another functional group—an aliphatic ester, attached to the vinyl group. The first problem can be solved by separation of the geometrical isomers on chromatographic columns (*1*) or by liquid-liquid countercurrent extraction (*8*). Where trace amounts of metabolites are produced, labeling of the parent material is essential, while fractionation and identification of the metabolic products may be accomplished by ion exchange (*6*) or paper chromatography with inert material as confirmatory markers as outlined in this paper.

Phosdrin exhibits systemic properties, as it is translocated readily throughout the plant. It has a much shorter life in the plant than most systemic insecticides and yet is moderately stable in vitro. Its half life at pH 8 is 26 days (*7*), while in the pea plant it is less than 24 hours for the cis isomer and 2 days for the trans (*2*). Studies of alkaline hydrolysis indicate that the two isomers do not yield the same end products (*8*). Dimethyl phosphate (III), *O,O*-dimethyl 1-carboxyl-1-propen-2-yl phosphate (Phosdrin acid) (II), and monomethyl phosphate are obtained from the cis isomer in a ratio of approximately 6 to 3 to 1. The trans isomer yields primarily dimethyl phosphate and a small amount of monomethyl phosphate. During the hydrolysis *O*-methyl 1-carbomethoxy-1-propen-2-yl phosphate (desmethyl Phosdrin) (V) was identified as one of the intermediates.

Casida *et al.* (*2*) found two components from Phosdrin metabolism in pea plants—dimethyl phosphate and an unidentified component partitioning equally between water and chloroform. This latter fraction was postulated as being Phosdrin acid (II). They suggested that degradation of Phosdrin in the plant proceeded via this intermediate. In view of the varied pathways of Phosdrin degradation in alkali and the speculative nature of its breakdown in the pea plant, the present study was undertaken in an attempt to help solve the problem.

$(MeO)_2P(O)OC(Me)=CHCOOMe$ I, cis and trans

$(MeO)_2P(O)OC(Me)=CHCOOH$. II

$(MeO)_2P(O)OH$ III

$(MeO)(HO)P(O)OC(Me)=CHCOOH$ IV

$(MeO)(HO)P(O)OC(Me)=CHCOOMe$ V

Methods and Materials

Radioactive Phosdrin. Casida and coworkers have reported briefly the synthesis of P^{32}-labeled Phosdrin which in one case contained 27% of an impurity (*2*, *3*). The method described here, similarly based upon the commercial process of Stiles (*9*), is essentially the same chemically; however, considerable modification in technical detail has provided a significant improvement in yield and purity of the final product as well as of the intermediate, trimethyl phosphite. The difficulty in procuring these labeled materials in good yield would seem to justify a detailed outline of the procedure here.

Trimethyl Phosphite-P^{32}. Pure phosphorus trichloride (approximately 1 gram) was sealed in a 9 × 30 mm. (outside diameter) quartz capsule and irradiated (by Atomic Energy of Canada, Ltd.) for 4 days at 6.6×10^{12} neutrons/sq. cm/sec. to attain a theoretical activity of 20 mc. The radioactive phosphorus trichloride was then transferred in vacuo into a flask containing 3 ml. of nonactive phosphorus trichloride, and the total was again distilled via the vacuum manifold. It was then redistilled at atmospheric pressure through a 15-cm. all-glass column into a weighed dropping funnel. The weight collected was 4.98 grams (36.2 mmoles).

In a 250-ml. three-necked flask, fitted with a wire stirrer, were placed 100 ml. of dry, peroxide-free ether, 4.4 ml. (109 mmoles) of methanol, and 18.3 ml. (114 mmoles) of *N,N*-diethylaniline. The solution was maintained at 0° to 5° C. with ice water and, under anhydrous conditions, phosphorus trichloride was added dropwise with vigorous stirring. On completion, the heavy suspension was kept stirred for an additional hour at room temperature; the flask was then stored in the cold while the precipitate settled.

The diethylaniline hydrochloride was filtered off and washed with three 25-ml. portions of cold ether. Most of the ether was then distilled away from the filtrate, using a 15-cm. column and holding the distillation temperature at 35° C. or less. When the volume was reduced to 8 to 10 ml., the column was replaced by a trap cooled in dry ice–acetone. At a moderate vacuum (12 mm.) at room temperature, the residual ether and all of the trimethyl phosphite soon condensed in the trap, leaving the excess diethylaniline and residual hydrochloride in the flask. The ether–trimethyl phosphite solution (5 to 8 ml.) was fractionated through a 15-cm.

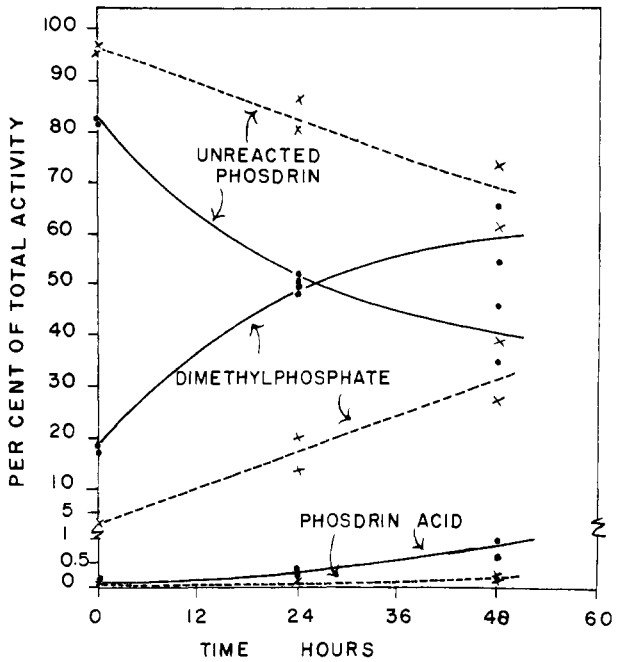

Figure 1. Pea metabolites of *cis*- and *trans*-Phosdrin

● cis isomer and metabolites
× trans isomer and metabolites

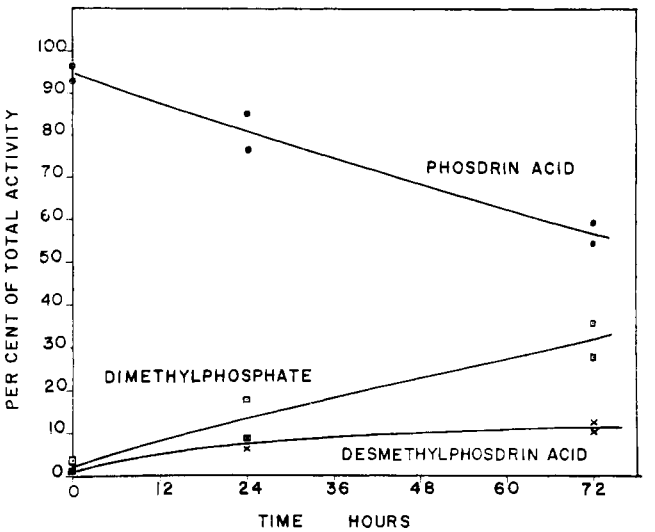

Figure 2. Pea metabolites of *cis*-Phosdrin acid

column at atmospheric pressure, with 1 ml. of bromobenzene as a chaser. The product weighed 3.85 grams (85%) (boiling point 110–112°, n_D^{24} 1.4067). The solvent and all reagents were carefully purified, dried, and distilled immediately prior to use.

Phosdrin-P^{32}. To the trimethyl phosphite, with vigorous stirring, was added 5.5 grams (36.5 mmoles) of methyl 2-chloroacetoacetate. The mixture was stirred and kept at 80° to 90° for 2 hours, while a quantitative yield of methyl chloride distilled off. The residue was fractionated through a 15-cm. column at reduced pressure. The product weighed 4.46 grams (55% based upon phosphorus trichloride) (boiling point 155–58°/10 mm., n_D^{24} 1.4492).

The material was resolved into its cis and trans isomers by countercurrent distribution, with water and ether as solvents (*8*). Each isomer was recovered and washed with 5% aqueous sodium carbonate to remove dimethyl phosphate, then extracted with chloroform and dried over sodium sulfate. On evaporation of the filtered solutions the cis and trans isomers were recovered. Paper chromatograms showed the materials to be radiochemically pure; the activity of the isomers was about 1.5×10^8 counts per minute per millimole, recorded with an end-window counter and corrected for decay and background.

***cis*-Phosdrin Acid-P^{32}.** Radioactive *cis*-Phosdrin acid was prepared by treating the radioactive Phosdrin with sodium carbonate as outlined for nonradioactive material (*8*).

Metabolic Experiments. Alaska variety pea plants about 4 inches high, grown in gravel, were placed in two replicates of five plants each in 0.1% aqueous solution of the labeled material for 15 hours and then transferred to water. At three time intervals two replicates were taken, cut into small pieces, and macerated in an Omni-Mixer with 5 ml. of water. The homogenate was filtered through glass wool and 5 times the volume of acetone was added. After coagulation of the precipitate the mixture was filtered through filter paper and the filtrate concentrated to 5 ml. The residue from the plants and the acetone precipitate were discarded. The unreacted Phosdrin was removed by extraction three times with chloroform. As the R_f of Phosdrin acid is rather close to that of dimethyl phosphate and desmethyl Phosdrin (*8*), Phosdrin acid was effectively removed by acidifying the aqueous residue to less than pH 1, followed by extracting six times with chloroform. Aliquots of the three fractions were then chromatographed on Whatman No. 1 paper using ascending chromatography with isopropyl alcohol–water–ammonium hydroxide (specific gravity 0.90) (75:24:1) as the developing solvent in preference to that used previously (*8*). Because extractable material in some cases affected the R_f values, sufficient nonradioactive material of the possible degradation products of Phosdrin was applied along with the radioactive fractions in quantities that could be located by use of the molybdate spray (*5*). The developed chromatograms were then scanned with an end-window Geiger tube and any concentrated radioactivity was identified from the nonradioactive markers. The radioactive spots were cut out and the activity was counted using a scaler.

The experiment with Phosdrin acid was carried out as in the isomer study, except that the last sampling was done at 72 instead of 48 hours.

Results and Discussion

The rates of degradation of the cis and trans isomers of Phosdrin and the appearance of the significant degradation products over a 48-hour period are shown in Figure 1, where each radioactive component is plotted as a percentage of the total activity of the components isolated. The faster rate of decomposition of the cis isomer is in agreement with previous work (*2*). Although dimethyl phosphate is the chief end product from both isomers, a very small but significant amount of Phosdrin acid was identified. This is in contrast to a much larger amount from alkaline hydrolysis of *cis*-Phosdrin (*8*). No significant amount of desmethyl Phosdrin (V) or desmethyl Phosdrin acid (IV) could be detected from either isomer.

When *cis*-Phosdrin acid was metabolized by pea plants, the degradation was not as rapid as that of *cis*-Phosdrin but was comparable to that of the trans isomer, as shown by a comparison of the rates in Figures 1 and 2. However, in addition to the main hydrolysis product, dimethyl phosphate, desmethyl Phosdrin acid (IV) was also identified. Failure to detect any from the degradation of *cis*- or *trans*-Phosdrin is presumably due to the insignificant amount that would be formed from the very small amount of Phosdrin acid produced.

Casida *et al.* (*2*) suggested that

Phosdrin acid is the first step in the degradation of Phosdrin in plants, followed by subsequent cleavage to dimethyl phosphate. The results shown in Figure 1 might support this hypothesis. However, those in Figure 2 cast doubt on the single pathway. If Phosdrin acid were an essential step in Phosdrin degradation in the plant, desmethyl Phosdrin acid should have been isolated in Phosdrin degradation and been present in the same proportion to dimethyl phosphate as indicated in Figure 2. Since none was detected, there are at least two pathways for Phosdrin degradation, the main one being directly to dimethyl phosphate and the other via Phosdrin acid.

Monodealkylation of organophosphorus insecticides by plants had not been reported until recently. Under conditions of alkaline hydrolysis some occurred with *cis*-Phosdrin (*8*), while in the plant some was shown with Dimethoate (*4*). Although no desmethyl Phosdrin was found in this study, some monodealkylation was shown with the identification of desmethyl Phosdrin acid (IV) from the metabolism of Phosdrin acid as shown in Figure 2.

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HERBICIDE UPTAKE FROM SOILS

Uptake of Radioactive Ethyl-*N*,*N*-di-*n*-propylthio carbamate (EPTC-S^{35}) and Translocation of Sulfur-35 in Various Crops

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A study of the absorption of radioactive EPTC by crops in pre-emergence application indicated an uptake of this chemical from soil. By use of radioautographic technique the differences in accumulation patterns of sulfur-35 from EPTC-S^{35} among crops were demonstrated; above-ground portions of beans, peas, and corn contained slightly more sulfur-35 than the roots, while above-ground portions of radishes, carrots, and other plants contained 70 to 94% of the sulfur-35 from the absorbed EPTC-S^{35}. Total absorption by individual crops at various stages of growth was determined. Generally, an increase in applied EPTC-S^{35} increased absorption, but not in proportion to the increase in rate of application.

AN EARLIER STUDY (*1*) showed that less than 3% of the ethyl-*N*,*N*-di-*n*-propylthiolcarbamate (EPTC) absorbed from soils was left as a residue in a variety of plant tissues. This investigation has been extended to include the total uptake of radioactive EPTC and the distribution of sulfur-35 by various crops, when a pre-emergence application of EPTC-S^{35} is made to soil.

Experimental

The crops used were kidney beans, sweet corn, garden peas, radishes, carrots, cabbage, mustard, swiss chard, table beets, Mung beans, and cucumbers. Beans, peas, and corn were planted in

Table I. Absorption of EPTC-S^{35} from Soil and Translocation of Sulfur-35 by Kidney Bean Plants

Appl. Rate, Lb./Acre	Interval,[a] Days	No. of Plants	Accumulation of S^{35}, %					Total EPTC-S^{35} Absorption, γ/Plant
			Root	Stem	First leaf	Trifoliate	Flower and pod	
1	10	5	48	26	26	..	..	5.0
	17	5	33	32	35	..	..	7.2
	24	5	53	22	16	8	..	11.6
	38	5	48	22	19	8	3	15.7
	52	5	42	14	14	19	11	22.4
4	10	5	35	33	32	..	..	18.8
	17	5	33	40	27	..	..	16.7
	24	5	38	32	30	..	..	33.2
	38	5	39	29	26	4	3	40.7

[a] Interval between treatment and harvest.