Measurement of Residues of Phorate and Its Oxygen Analog Sulfone in Plant Tissues by Total Phosphorus Determination

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A method is described in which residues of phorate and its oxygen analog sulfone in plant tissue are measured by total phosphorus determination. The phorate or its metabolites are extracted from the plant tissue with benzene. The extract is purified by passage through a Darco–sodium carbonate column, and the phorate is then oxidized to its oxygen analog sulfone with *m*-chloroperbenzoic acid. The mixture is chromatographed on a silicic acid column, and the phorate oxygen analog sulfone in the chromatography fractions is oxidized to orthophosphoric acid, and measured by the molybdenum blue method.

Phorate (*O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate) has been recommended for the control of insects on various crops. When phorate is present as a residue on, and in, plants, the occurrence of five metabolites has been demonstrated (Bowman and Casida, 1957, 1958; Metcalf *et al.*, 1957). To use this as an insecticide, determination of residues of this compound and its metabolites in plant tissue was necessary. Two methods of residue measurement, each dependent on conversion of phorate to its oxygen analog sulfone, have been published. One is the cholinesterase inhibition procedure (Archer *et al.*, 1963; Curry, 1961; Miskus and Hassan, 1959), and the other involves the colorimetric determination of the hydrolytic product, formaldehyde, with chromotropic acid (Giang and Schechter, 1960; Waldron *et al.*, 1963). Laws and Webley (1961) determined phorate and its oxygen analog sulfone separately by the molybdenum blue method of phosphorus determination. In the method described here, phorate and its metabolites are determined as total phosphorus by a modification of the method of Steller and Curry (1964). Since, for the purpose of residue measurement, a total residue only is required, the author decided to determine this as the final toxic metabolite, the oxygen analog sulfone. Therefore, the phorate and other metabolites in the plant extract were converted by oxidation to this compound. Blinn (1964) found that the oxidizing reagent of choice for this conversion was *m*-chloroperbenzoic acid. The method was tested successfully on several plant tissues fortified with phorate and phorate oxygen analog sulfone. Recovery data are presented in Table I. This method is sensitive to 0.1 p.p.m.

APPARATUS

All equipment must be free of even trace amounts of phosphorus. Phosphate-containing detergents should be avoided in washing any glassware used, and all glassware should be rinsed thoroughly with distilled water.

Rinco rotating film evaporator, or equivalent.

Aluminum heating block (Steller and Curry, 1964).

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REAGENTS

All reagents must be free of any trace of phosphorus, and should be tested for this in a blank determination.

***m*-Chloroperbenzoic Acid** (Food Machinery and Chemical Corp., Carteret, N. J.).

1-Amino-2-naphthol-4-sulfonic Acid (Eastman Organic Chemicals, Rochester, N. Y.). Add 0.5 gram of 1-amino-2-naphthol-4-sulfonic acid, with mechanical stirring, to 200 ml. of freshly prepared 15% sodium bisulfite, and then add 1.0 gram of anhydrous sodium sulfite. Stir for 15 minutes and filter. Store in a low actinic flask, and prepare a fresh solution every week.

Standard Phorate and Phorate Oxygen Analog Sulfone (Agricultural Division, American Cyanamid Co., Princeton, N. J.).

Table I. Recovery of Phorate and Phorate Oxygen Analog Sulfone from Plant Tissue

Crop[a]	Apparent P.P.M. in Control Tissue	No. of Runs	Range of Phorate Added, P.P.M.	Av. Recovery, %
Alfalfa	0.02	5	0.05 to 1.00	84.2
Cantaloupe	0.01	2	0.10 to 0.50	82.5
Green tomatoes	0.05	2	0.50 to 1.00	96.6
Milo	0.04	5	0.05 to 1.00	89.9
Potatoes	0.01	5	0.05 to 1.00	95.8
Rice	0.02	3	0.20 to 1.00	93.5

	No. of Runs	Range of Phorate Oxygen Analog Sulfone Added, P.P.M.	
Alfalfa	4	0.05 to 1.00	86.8
Cantaloupe	2	0.10 to 0.50	111.2
Green tomatoes	2	0.50 to 1.00	75.6
Milo	5	0.05 to 1.00	94.9
Potatoes	5	0.05 to 1.00	86.8
Rice	3	0.20 to 1.00	74.4

[a] 100-gram samples used.

PROCEDURE

Preparation of Standard Curve. Prepare standard solutions of phorate and phorate oxygen analog sulfone in chloroform at a concentration of 10 μg. per ml. Pipet 1-, 2-, 3-, 4-, 5-, 6-, 7-, and 8-ml. aliquots of each solution into 18 × 150 mm. test tubes which have been previously marked at the 10-ml. level. Add 1 drop of propylene glycol to each solution, and evaporate the chloroform under an air jet.

Add 1.5 ml. of 6*N* nitric acid, and chill in an ice bath. Add 0.5 ml. of 70% perchloric acid, and allow the tubes to warm to room temperature for 5 minutes. Place the tubes in the aluminum heating block on a cold hot plate. Raise the temperature of the block gradually to 225° C. Hold the block at 225° to 245° C. for 30 minutes and then turn off the hot plate. Allow the block to cool to below 150° C. before removing the tubes with clean tongs.

Rinse down the sides of the cooled tubes with 5 ml. of water. Add, in the following order, 1 ml. of 6*N* sulfuric acid, 0.4 ml. of 4% ammonium molybdate, and 0.4 ml. of the 1-amino-2-naphthol-4-sulfonic acid reagent. Dilute to between 9.8 and 10 ml. with water, and mix well. Heat the tubes in a boiling water bath for 12 minutes, and then immediately cool them in a cold water bath to room temperature. Dilute to the 10-ml. mark with water, and mix. Measure the absorbance of each sample at 820 mμ in a 1-cm. cell using a Beckman DU or equivalent spectrophotometer with water as a reference blank. Correct the absorbances for a similarly run reagent blank, and plot the corrected absorbance for each compound against the number of micrograms present.

Determinations of Residues of Phorate and Its Oxygen Analog Sulfone in Plant Tissue. Weigh 100 grams of the finely ground sample of the plant tissue in a 32-ounce wide-mouthed jar. Add enough anhydrous sodium sulfate to bind the water present in the sample so that the tissue does not become lumpy in the benzene slurry. This will vary from 50 to 200 grams, depending upon the moisture content of the plan ttissue. (A dry crop such as alfalfa requires 50 grams, while a crop with a high moisture content such as potatoes requires 200 grams.) Add 500 ml. of benzene, cap with a foil-lined screw cap, and shake on a reciprocating laboratory shaker for 1 hour. Filter the extract through a piece of glass fiber filter paper and a bed of 10 to 15 grams of anhydrous sodium sulfate into a 500-ml. graduated cylinder. Record the volume of filtrate. Add 1 drop of propylene glycol, and concentrate the extract under vacuum at 45° to 55° C. to 20 to 30 ml. in a 1-liter round-bottomed evaporating flask. Prepare a reagent blank of 400 ml. of benzene, and carry it through the processing with the plant tissue extract.

Prepare a chromatography column as follows: Place 10 grams of sodium sulfate in the bottom of a 20 × 400 mm. column fitted with a coarse fritted disk. Add, as a slurry in benzene, 40 grams of a mixture of 60 grams of sodium carbonate, 20 grams of Celite 545, and 10 grams of Darco G-60 activated carbon. When the level of the benzene is down to the top of the absorbent bed, add 10 grams of sodium sulfate. Pour the concentrated sample extract into the column, and start to collect the column effluent at this point in a 500-ml. evaporating flask. Rinse the evaporating flask with 10 ml. of benzene, and add this to the column. Maintain a percolation rate of 1 drop per second, using light air pressure if necessary. When the level of the concentrated extract has reached the top of the adsorbent bed, add 200 ml. of benzene and continue the elution. Collect all of the eluate as long as it flows from the column. Add 1 drop of propylene glycol, and evaporate under vacuum almost to dryness.

Redissolve the residue in 25 ml. of chloroform, and add 2 ml. of a 10% solution of *m*-chloroperbenzoic acid in chloroform. Allow this mixture to stand for 15 minutes, and transfer it to a 125-ml. separatory funnel. Shake for 1 minute with 25 ml. of saturated sodium sulfite solution, and then for 1 minute with 25 ml. of saturated sodium bicarbonate solution. Draw off the chloroform layer, and pass it through a 10-mm. column, fitted with a coarse glass fritted disk, containing 10 grams of sodium sulfate. Collect the effluent in a 250-ml. acetylation flask. Wash the sulfite-bicarbonate layer twice with 25 ml. of chloroform each time, and pass these chloroform extracts through the sodium sulfate column into the acetylation flask. Wash the sodium sulfate column with 10 ml. of fresh chloroform, and collect this with the extracts. Add 1 drop of propylene glycol, and concentrate under vacuum to 5 to 7 ml.

Pour a slurry containing 2.5 grams of silicic acid in 10 ml. of chloroform into a 10-mm. column fitted with a coarse glass fritted disk. Use 2 to 4 pounds of air pressure to force the chloroform through the column. When the chloroform level is down to the top of the silicic acid bed, transfer, by pipet, the concentrated extract from the acetylation flask to the column. Allow the extract to percolate through the column under air pressure and collect the eluate in 5-ml. fractions. When the level of the concentrate is down to the top of the silicic acid bed, add fresh chloroform, and continue the elution. Collect five fractions of 5-ml. each in 18 × 150 mm. test tubes marked at the 10-ml. level. Add 1 drop of propylene glycol to each fraction, and evaporate the chloroform under an air jet. The tubes may be placed in a water bath at 40° C. to aid the evaporation.

Perform the perchloric acid digestion step as described under Preparation of the Standard Curve. Some of these fractions may contain a large amount of residual organic matter, and the temperature of the block will have to be raised slowly (over 2½ to 3 hours) to prevent spattering and loss of material. Develop the molybdenum blue color of each fraction, following the procedure used for the standard curve, and measure the absorbance with the Beckman Model DU spectrophotometer at 820 mμ. If any of the solutions are too dark to be read directly, dilute them with 1*N* sulfuric acid. Add the absorbances of the two richest consecutive fractions for both the sample and the reagent blank. Subtract this total absorbance obtained for the reagent blank from the total absorbance obtained for the sample. Calculate the amount of phorate present in the sample from the following formula:

$$\text{P.P.M. phorate} = \frac{\mu\text{g. read from standard curve}}{\text{ml. of benzene extract recovered}} \times \frac{500 \text{ ml. of benzene}}{100 \text{ grams of tissue}}$$

DISCUSSION

Chloroform also was tried as an extracting solvent for the plant tissue. While the recoveries were comparable with those obtained using benzene, the plant tissue blanks were higher.

The use of anhydrous sodium sulfate during the extraction is necessary to prevent either lumping or jelling caused by the water present in the plant tissue. This can be prevented by increasing the amount of sodium sulfate used.

The standard curve has been found to be linear within the range covered here. An absorbance of 1.0 is equivalent to 100 μg. of phorate, 103 μg. of the oxygen analog sulfone, or 12.5 μg. of phosphorus.

The main purification of the plant tissue extract appears to take place during the Darco–sodium carbonate column chromatography which was done according to the method of Archer *et al.* (1963). The silicic acid chromatography of Barette and Payfer (1961) effects a further purification and concentrates the phorate oxygen analog sulfone within a small volume of eluate. While it would be preferable to have the phorate oxygen analog sulfone always appear in the same two fractions of eluate, this has not been achieved. However, good recoveries along with low plant tissues blanks have been consistently obtained by adding the absorbances of the two richest consecutive cuts. If all of the metabolites were present during this chromatography, they would be eluted over a wider volume of effluent. This is prevented by oxidizing all of the metabolites to phorate oxygen analog sulfone with *m*-chloroperbenzoic acid before this step.

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