Determination of Residues of Dasanit and Three Metabolites by Gas Chromatography with Flame Photometric Detection

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Dasanit {*O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl]-phosphorothioate} and three metabolites, Dasanit sulfone, Dasanit oxygen analog, and Dasanit oxygen analog sulfone, were extracted from plant material, separated by liquid chromatography on a silica gel column, and determined by gas chromatography on a 76-cm, 3% XE60 column. A flame photometric detector in the phosphorus mode was used. The response was such that Dasanit oxygen analog sulfone, the least sensitive of the four compounds, could be determined at the 0.01 ppm level. Recoveries averaged 90% when carrots, cauliflower, and potatoes were fortified at levels ranging from 0.1 to 4.0 ppm. Field-treated carrots showed the presence of Dasanit, Dasanit sulfone, and in some cases Dasanit oxygen analog. No Dasanit oxygen analog sulfone was detected. Interference from plant co-extractives was negligible.

Dasanit {*O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl]phosphorothioate (I)} is a potentially useful insecticide (Finlayson *et al.*, 1966; Harris, 1969) and nematocide (O'Bannon and Taylor, 1967). In studying its metabolism in cotton plants, Katague and Anderson (1967) detected three metabolites. Two of them, Dasanit oxygen analog (III) and Dasanit sulfone (II), were present in significant amounts, while a third, Dasanit oxygen analog sulfone (IV), was present in trace amounts. Structural formulas for Dasanit and these three metabolites are shown in Figure 1.

No residue method for the separate determination of all four compounds appears in the literature. This paper describes such a method.

The gas chromatographic determination of these compounds without prior separation proved to be impractical, but by using liquid chromatography with a silica gel column and appropriate solvents, three fractions were obtained which provided the desired separations. The first fraction contained compound II, the second, compounds I and IV, and the third, compound III. A 76-cm, 3% XE60 gas chromatographic column was used in the determinative step, and good separation of the two compounds in the second fraction was achieved.

EXPERIMENTAL

Solvents and Reagents. Solvents were all redistilled from reagent grade material. Silica gel, Davidson Grade 923 (Davidson Chemical, Baltimore, Md.) was used as received. Loss on heating this material overnight was 1.34% at 110° C or 1.89% at 200° C. Norit A, Alkaline, (Fisher Scientific) was used as received. Eluting solution A was a 20:80 v/v mixture of ethyl acetate and benzene. Eluting solution B was a 40:60 v/v mixture of acetone and benzene. Dasanit and its metabolites, analytical grade samples (Chemagro Corp., Kansas City, Mo.) were used as standards and for fortification. These were tested for purity by gas chromatography. Dasanit sulfone and Dasanit oxygen analog sulfone were both free of their precursors, but Dasanit and Dasanit oxygen analog both contained appreciable amounts of their respective sulfones. Further purification was therefore carried out on a silica gel column as described in the procedure.

Apparatus. The gas chromatograph was a Micro-Tek Model 220 equipped with a water-cooled Melpar flame photometric detector (Dale and Hughes, 1968) and a 526 mμ interference filter for phosphorus detection. The blender was an Omni-Mixer, with a 500-ml stainless steel cup fitted with a Teflon gasket (Ivan Sorvall Inc., Norwalk, Conn.). The concentrating flask consisted of a 1000-ml Kuderna-Danish flask with the standard tapered joints reversed, and a 10-ml concentrating tube on the tapered end, allowing the larger end to be fitted to a rotary evaporator.

Sample Preparation and Extraction. Wash and shred plant material and store frozen until required. Transfer a 50-g sample to the Omni-Mixer, add 100 ml of ethyl acetate and 0.5 g of filter pulp as a filtering aid, and blend for 3 min in an ice bath. Filter under suction through a Büchner funnel fitted with a glass filter paper into a 1000-ml separatory funnel. Return the plant material to the blender and extract twice more with 50-ml portions of ethyl acetate, combining all three extracts. Discard any water separating from the ethyl acetate extract, then filter through anhydrous sodium sulfate into a concentrating flask. Concentrate to approximately 2 ml on a rotary evaporator using an infrared lamp as a heat source.

Fractionation and Cleanup. Prepare two 2-cm i.d. liquid chromatography columns, the first with, from the bottom upward, 2 cm of anhydrous sodium sulfate, 10 g of silica gel, and 1 cm of anhydrous sodium sulfate; the second with 2 cm of anhydrous sodium sulfate, 6 g of 1:5 w/w mixture of Norit A Alkaline and Celite 545, and 1 cm of anhydrous sodium sulfate.

Support the columns so that the first is directly above the

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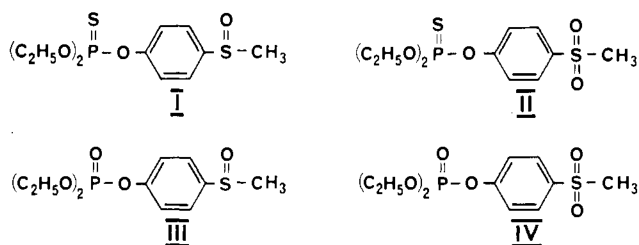

Figure 1. Structural formulas for Dasanit and three of its metabolites

second. Place a 150-ml beaker under the second column, transfer the concentrated plant extract to the first column, and elute through both columns with 75 ml of eluting solution A. This is Fraction 1. Remove the second column. Add 100 ml of eluting solution B to the first column and collect as Fraction 2. Follow this with 100 ml of acetone and collect as Fraction 3.

Evaporate each fraction almost to dryness by gently heating in a current of clean air or dry nitrogen. Transfer the eluates to 10-ml volumetric flasks and make up to volume with ethyl acetate.

Gas Chromatographic Procedure. Inject 5 to 10 μl aliquots of the sample or standard into the gas chromatograph under the following conditions. Temperature: Column 205° C, injection port 225° C, detector 215° C. Carrier gas: Nitrogen, 110 ml per min. Hydrogen Flow: Approximately 200 ml per min. Oxygen Flow: Approximately 20 ml per min. Air Flow: Approximately 20 ml per min. Column: A 76 cm × 2 mm i.d. glass column packed with 3% XE60 on 60/80 mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa.) which had been preconditioned for 48 hr at 240° C and further conditioned before use by repeated injections of 100 ng amounts of all four compounds until peak heights were reproducible.

Calculate the residue amounts present by comparing sample peak heights with those of appropriate standards.

Recovery Experiments. To test the procedure, carrots, cauliflower, and potatoes fortified prior to blending with all four compounds at concentration levels of 0.1, 0.4, 1.0, and 4.0 ppm were analyzed, and recoveries calculated. A number of field-treated carrots were also analyzed.

RESULTS AND DISCUSSION

Recovery of Dasanit and its three metabolites from fortified samples is given in Table I. Typical chromatograms for the three fractions from a fortified carrot sample, showing recoveries of Dasanit and metabolites, appear in Figure 2. In Table II data for a number of field-treated carrots are presented.

Because Dasanit sulfone was found in the field-treated carrots, it was desireable to determine the amount present in the material applied. Since both spray and granular applications had been made, both materials were analyzed. In

Table I. Recovery of Dasanit and Metabolites from Fortified Crop Samples

Fortification level (ppm)	% Recovery			
	I	II	III	IV
Carrot				
0.0	0.0	0.0	0.0	0.0
0.1	100	100	100	100
0.4	100	103	93	100
1.0	99	93	85	100
4.0	89	99	101	101
Cauliflower				
0.0	0.0	0.0	0.0	0.0
0.1	88	96	95	109
0.4	100	100	100	100
1.0	92	94	92	92
4.0	96	101	98	96
Potato				
0.0	0.0	0.0	0.0	0.0
0.1	99	93	88	75
0.4	92	100	86	88
1.0	90	95	100	100
4.0	88	100	85	112

I = Dasanit; II = Dasanit sulfone; III = Dasanit oxygen analog; IV = Dasanit oxygen analog sulfone.

Table II. Recovery of Dasanit and Metabolites from Field-Treated Carrots

Treatment[a]	Harvest[b]	Recovery (ppm)		
		I	II	III
Untreated	...	ND[c]	ND	ND
40–70	10	0.26	0.04	ND
40–70	30	0.25	0.06	ND
Untreated	...	ND	ND	ND
30–50–70	10	0.31	0.05	ND
30–50–70	30	0.30	0.06	ND
Untreated	...	ND	ND	ND
30–50–70–90	10	0.43	0.08	0.01
30–50–70–90	30	0.36	0.09	ND

[a] In furrow application at 1 oz active/1000 row ft (approx. 2 lb/acre), plus 2, 3, and 4 sprays at the days after seeding indicated, at 1 lb active/acre. [b] Days after final treatment. [c] None detected = < 0.01 ppm. I = Dasanit; II = Dasanit sulfone; III = Dasanit oxygen analog.

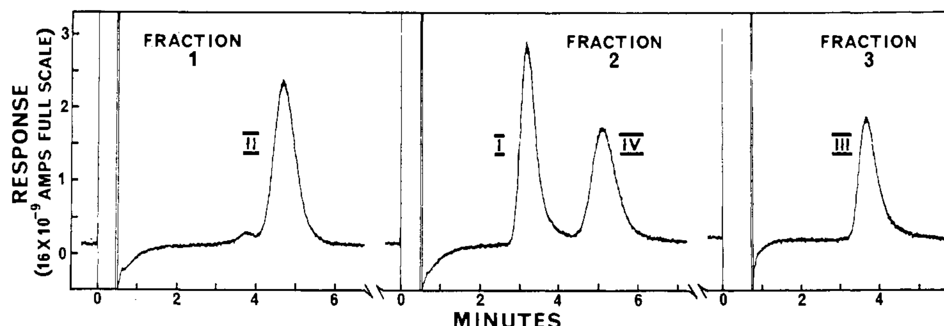

Figure 2. Chromatograms of Dasanit and three metabolites recovered from a fortified carrot sample. Sample fortified at 0.1 ppm. (I) Dasanit, (II) Dasanit sulfone, (III) Dasanit oxygen analog, and (IV) Dasanit oxygen analog sulfone. Ordinate figures ×10 denote percentage of full scale

the spray concentrate, 3.4% of the active insecticide was Dasanit sulfone; in the granular material the amount was 5.0%. In the carrots Dasanit sulfone averaged 12% of the total insecticide recovered, indicating some metabolic conversion.

Detector Response. Gas chromatographic response to all four compounds was linear to at least 80 ng. Relative sensitivity decreased in the following order: Dasanit sulfone, Dasanit, Dasanit oxygen analog, and Dasanit oxygen analog sulfone. The minimum detectable limit of Dasanit and its sulfone was 0.3 ng (twice noise level), while that of the oxygen analog and its sulfone was 0.5 ng.

Retention Times. Under the conditions described, retention times for Dasanit and its metabolites were as follows: Dasanit—1.6 min; Dasanit oxygen analog—1.8 min; Dasanit sulfone—2.3 min; and Dasanit oxygen analog sulfone—2.5 min. Lengthening the column or reducing the temperature improved separation but caused considerable broadening of the peaks, with a resultant loss in sensitivity.

Cleanup. Some coextractives were present in each of the three fractions, particularly in the carrot extracts, but these did not result in extraneous peaks or otherwise interfere. However, after a large number of injections, some broadening of peaks did occur, indicating slight contamination. This was overcome by having a plug of silanized glass wool at the inlet end of the column and replacing it periodically or when contamination was suspected.

Norit A, which aided considerably in the cleanup of the first fraction, could not be used for the other fractions, since it prevented quantitative elution of Dasanit oxygen analog and its sulfone. There was also some loss of these two compounds when sweep codistillation (Storherr and Watts, 1965) was tried as an additional cleanup step.

The liquid chromatography procedure described also proved suitable for separating diazinon from its oxygen analog. This and similar separations of other organophosphates and their metabolites (Bowman and Beroza, 1967, 1968) indicate that silica gel with appropriate solvents could provide a good general method for separating organophosphates from closely related metabolites.

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LITERATURE CITED

Bowman, M. C., Beroza, M., J. Agr. Food Chem. **15,** 651 (1967).
Bowman, M. C., Beroza, M., J. Agr. Food Chem. **16,** 399 (1968).
Dale, W. E., Hughes, C. C., *J. Gas Chromatogr.* **6,** 603 (1968).
Finlayson, D. G., Fulton, H. G., Noble, M. D., *J. Econ. Entomol.* **59,** 1082 (1966).
Harris, C. R., *J. Econ. Entomol.* **62,** 1437 (1969).
Katague, D. B., Anderson, C. A., *Bull. Environ. Contam. Toxicol.* **2,** 228 (1967).
O'Bannon, J. H., Taylor, A. L., *Plant Dis. Rept.* **51,** 995 (1967).
Storherr, R. W., Watts, R. R., *J. Ass. Offic. Agr. Chem.* **48,** 1154 (1965).

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