Determination of Dasanit and Three of Its Metabolites in Corn, Grass, and Milk

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Dasanit {*O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl]-phosphorothioate} and three of its metabolites were determined in corn, grass, and milk. Extracts were separated by liquid chromatography on silica gel into three fractions which were concentrated and injected into a gas chromatograph equipped with a flame photometric detector sensitive to phosphorus. No additional cleanup was required, since no interference from crop or milk extract was encountered. Recoveries were 95 to 102% for all four compounds from corn and grass at the 0.05 ppm level and from milk at the 0.01 ppm level. Response was linear with concentration and sensitivity for each of the four compounds was 0.005 ppm or better. Retention times are given for the four compounds on five liquid phases and also *p*-values are given in six solvent systems. The conditions for partially separating the four compounds by liquid chromatography prior to gas chromatographic determination are described.

Dasanit, Bay 25141, *O,O*-diethyl *O*-[*p*-(methylsulfinyl)-phenyl]phosphorothioate, is a broad spectrum systemic insecticide which has shown promise in controlling a variety of forage insect pests. A method was needed to determine residues of this chemical and its major metabolites in corn and grass to be used as forage for livestock, and in milk from cows fed forage containing such residues.

Dasanit and the three metabolites that may form from it are shown in Figure 1. Dasanit differs from most organophosphorus insecticides containing an alkylthioether linkage in that this linkage in the parent compound is oxidized to a sulfoxide. Therefore, further oxidation results only in the sulfone or the oxygen analog (P=O) of its sulfone. The formation of *S*-ethyl metabolites has been reported (Benjamin *et al.*, 1959) but a recent reexamination of these metabolic studies using P^{32} labeled Dasanit in cotton plants (Katague and Anderson, 1967) has indicated that no *S*-ethyl metabolites are formed.

A method of determining Dasanit and its metabolites in pasture grass by the colorimetric measurement of total phosphorus was reported by Brewerton *et al.* (1968). Sensitivity of the method is 0.2 ppm. They also report that attempts to utilize a CsBr thermionic detector in a gas chromatographic method were not successful due to lack of sensitivity of the detector to Dasanit compounds. The persistence of Dasanit in soils was determined by bioassay (Read, 1969), and qualitative identification of the compound and its metabolites in cotton plants was accomplished by thin-layer chromatography (Katague and Anderson, 1967). Retention times relative to parathion were given for Dasanit and its metabolites on three different gas chromatographic columns by Watts and Storherr (1969), and for Dasanit and its *O*-analog on four different columns by Bowman and Beroza (1970).

A method sensitive to 0.005 ppm or less of each metabolite has been devised to determine all four compounds. The procedures used are essentially the same as those described by Bowman and Beroza (1968) for the determination of the residues of fenthion and its five metabolites. After the feed or forage is extracted, the extract is fractionated on a silica gel column into three fractions, and aliquots of each are then subjected to gas chromatography on an instrument equipped with a flame photometric detector (Brody and Chaney, 1966) operated in the phosphorus sensitive mode. Recoveries are 95 to 102% for all compounds.

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EXPERIMENTAL

Reagents and Solvents. Silica gel, J. T. Baker Chemical Co., No. 3405, was used as received. When heated overnight at 110° C it lost 3.70% of its weight.

Dasanit and its metabolites were kindly supplied by the Chemagro Corp., Kansas City, Mo., in the following purities: (I) P=S, SO—94.5%; (II) P=O, SO—100%; (III) P=S, SO_2—91%; and (IV) P=O, SO_2—96%.

Acetone, benzene, hexane, acetonitrile, and methylene chloride were C.P. grade solvents redistilled. Sodium sulfate was the anhydrous reagent grade chemical.

Equipment. A Hewlett-Packard Co. Model 5750 gas chromatograph was used. The instrument was equipped with a Melpar flame photometric detector (Tracor Inc., Austin, Texas) fitted with a 526-mμ interference filter which is highly selective for phosphorus.

Sample Preparation and Extraction of Corn and Grass. Transfer 20 g of the finely chopped and well mixed plant material to a Soxhlet extraction apparatus (Fisher Scientific Co., No. 9-556B) containing a plug of glass wool to prevent insoluble plant material from siphoning over during solvent exchanges. Extract the sample under nitrogen for 6 hr with 150 ml of chloroform-methanol, 9 to 1 by volume, at the rate of about six solvent exchanges per hr. Allow the extract to cool and percolate it through a plug of anhydrous sodium sulfate, about 25 nm in diameter by 30 mm thick; then wash the container and plug with 10 ml of chloroform. Evaporate the extract to dryness on a 50° C water bath under water pump vacuum (*ca.* 35 mm of Hg). (Presence of chloroform, water, or methanol can cause difficulty in the subsequent liquid chromatography.) Take up the residue in 10 ml of benzene for the liquid chromatography.

Extraction of Milk. Shake the sample to disperse the cream uniformly and add 100 g to a Waring Blendor. Add 300 ml of acetone, blend for 3 min, and filter through Whatman No. 1 paper on a Buchner funnel. Wash the blender and filter funnel with an additional 25 ml of acetone. Extract the filtrate with 200 and then 100 ml of methylene chloride, and percolate each methylene chloride extract successively through a plug of sodium sulfate about 4 cm in diameter and 5 cm thick. Evaporate the percolate almost to dryness under a Snyder column on a steam bath, and then just to dryness at room temperature under water pump vacuum. Add 10 ml of benzene to dissolve the fatty residue, and reserve for the liquid chromatographic separation.

Liquid Chromatographic Separation. Prepare a silica gel column by adding successively to a 10-mm i.d. × 23-cm long glass column (Tudor Scientific Glass Co., Belvedere,

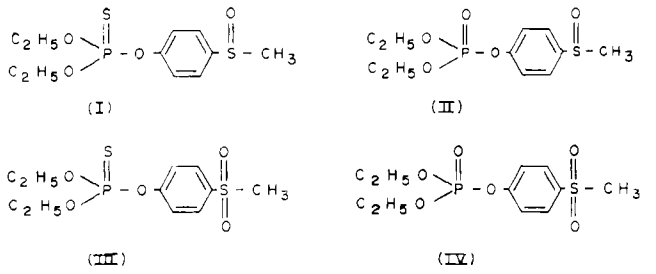

Figure 1. Dasanit (I) and three of its metabolites: I P=S, SO; II P=O, SO; III P=S, SO_2; IV P=O, SO_2

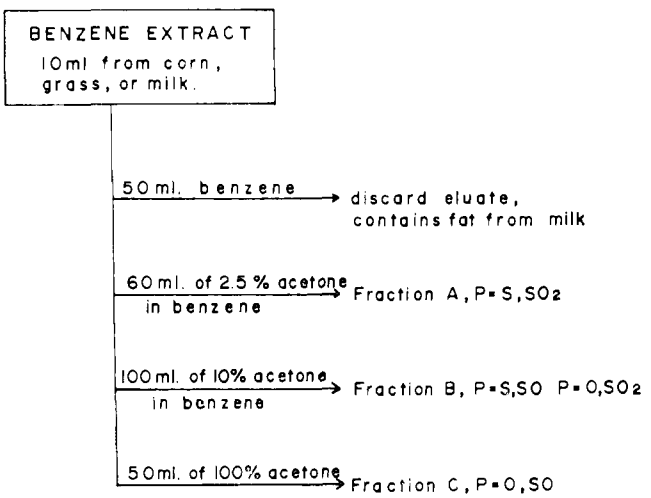

Figure 2. Summary of operations in liquid chromatography on silica gel; I P=S, SO; II P=O, SO; III P=S, SO_2; IV P=O, SO_2

S.C.) a plug of glass wool, 2 g of sodium sulfate, 5 g of silica gel, and 2 g of sodium sulfate. Prewash the column with 25 ml of benzene and discard the eluate. Add the corn or grass extract (10 ml, equivalent to 20 g of product) or the milk extract in benzene (10 ml, equivalent to 100 g of milk). Allow the extract to percolate into the column, then wash the container and column three times with 5-ml portions of benzene, allowing each portion to percolate into the adsorbent. Elute the column with 25 ml more of benzene (total—50 ml) and discard the filtrate.

Change receivers and elute the column with 60 ml of 2.5% acetone in benzene. The eluate (Fraction A) contains the sulfone of Dasanit (III).

Change receivers and elute with 100 ml of 10% acetone in benzene. The eluate (Fraction B) contains Dasanit (I) and its *O*-analog sulfone (IV).

Finally, change receivers and elute with 50 ml of 100% acetone. The eluate (Fraction C) contains the *O*-analog of Dasanit (II). Discard the contents of the column.

These liquid chromatographic operations are summarized in Figure 2.

Preparation of Fractions for Gas Chromatography. Evaporate all fractions just to dryness using a water aspirator vacuum and a 50° C water bath. Take up the residue in a few ml of benzene, transfer to a calibrated tube, and adjust the volume to exactly 2 ml by evaporating the excess benzene with a jet of dry nitrogen. For corn and grass, 5 μl of this solution equals 50 mg of crop on a wet basis. For milk, 5 μl equals 250 mg of whole milk.

Gas Chromatographic Analysis. Use the following conditions:

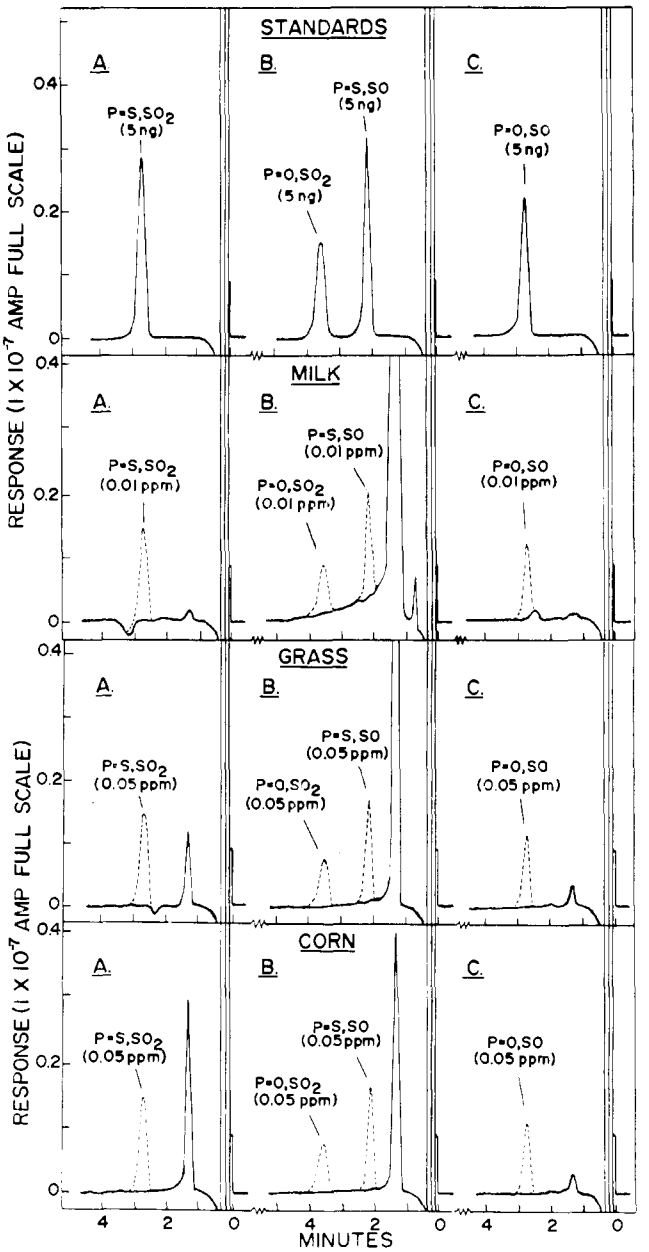

Figure 3. Gas chromatograms of Dasanit and three of its metabolites in fractions A, B, and C obtained by liquid chromatography on silica gel. Column length 100 cm in gas chromatography of all fractions. Solid line peaks are standards of unfortified controls. Broken line peaks are fortified milk, grass, and corn extracts

COLUMN.—Glass, 100 cm long × 4 mm i.d. (6 mm o.d.).

PACKING.—OV-210, 5% (w/w) on 80- to 100-mesh Gas-Chrom Q (Applied Science Laboratories, State College, Pa.).

CARRIER GAS.—Nitrogen at 160 ml per min.

OTHER GASES.—Oxygen at 40 ml per min and hydrogen at 200 ml per min.

TEMPERATURES.—Column 220° C; injection port 240° C; detector (external) 240° C; transfer line to detector 230° C.

Condition the column overnight at 230° C; then condition further with the gas chromatograph operated as described above by injecting 250 ng amounts of insecticide in extract equivalent to 50 or 100 mg of plant or milk until several successive injections of 5 ng amounts produce a constant response.

Using the stated conditions, inject 5 μl of the extracts as prepared for gas chromatographic analysis or diluted as

Table I. Minimum Detectable Levels of Dasanit and Three of Its Metabolites[a]

Chemical	In Grass or Corn, ppm, 50 Mg Equiv/Analysis	In Milk, ppm, 100 Mg Equiv/Analysis
P=S, SO	0.003	0.0006
P=S, SO_2	0.003	0.0006
P=O, SO	0.004	0.0008
P=O, SO_2	0.005	0.001

[a] Based on a signal to noise ratio of 2; $2 \times (5 \times 10^{-10}$ amp) $= 10^{-9}$ amp.

appropriate. Base response on peak height. This response of all four compounds is linear over the entire range of the electrometer.

Recovery Experiments. Prior to the 6 hr Soxhlet extractions, duplicate 20 g samples of corn and grass were separately fortified with 1 ml solutions of the four compounds in benzene at the 5.0 ppm level and with mixtures of all four compounds at 0.1 ppm (except P=O, SO—5.0 ppm) and 0.05 ppm. Duplicate 100 g samples of milk were fortified prior to the addition of acetone with 1 ml. Used were acetone solutions of the four compounds at the 0.5 ppm level and with mixtures of all four compounds at the 0.05 ppm and 0.01 ppm levels. Unfortified controls were also run concurrently for corn, grass, and milk.

RESULTS AND DISCUSSION

Samples of corn, grass, and milk fortified as described were run through the entire analytical procedure. Recoveries were between 95–102% for all compounds in all substrates and at all levels. Controls contained nothing that interfered with the analysis. There was no evidence that any of the four compounds were converted to any of the others in the course of analysis. However, when fortification was carried out with a large amount of a compound, the impurities contained in it were detectable. Analysis of each of the standards for the other metabolites revealed that P=S, SO contained 1.97% of P=S, SO_2; P=S, SO_2 contained 0.32% of P=S, SO; P=O, SO contained 0.044% of P=S, SO and 2.31% of P=O, SO_2; and P=O, SO_2 contained 0.056% of P=S, SO_2 and 0.096% of P=S, SO.

Chromatograms of fractions A, B, and C obtained in the liquid chromatographic separation on silica gel are shown for standards milk, corn, and grass in Figure 3. In the case of milk, grass, and corn, chromatograms of control samples fortified with 0.01 or 0.05 ppm of the compounds are shown as dotted lines superimposed on chromatograms of unfortified controls (solid lines). The peak occurring at about 1.6 min in the milk, grass, and corn samples is the result of an impurity in the acetone (even though it was C.P. grade, redistilled in glass); however, it does not interfere with the analysis. The A fractions of milk and grass have small negative peaks. These are sometimes encountered in gas chromatography using a flame photometric detector, especially when injecting highly concentrated extracts. It appears to be caused by a material emerging in the gas chromatographic effluent that has strong properties for quenching the background photoemission.

The method has high sensitivity, as shown by the minimum detectable levels based on twice the noise level presented in

Table II. Retention Times of Dasanit and Three of Its Metabolites on Five Gas Chromatographic Columns

Gas Chromatographic Column[a]	Column Temp. °C	Retention Time, Min, for Indicated Compound			
		P=S, SO	P=S, SO_2	P=O, SO	P=O, SO_2
5% OV-225 (methyl silicone with 25% phenyl and 25% cyanopropyl groups)	250	2.50	3.15	2.60	3.30
5% OV-25 (phenyl methyl silicone; 75% phenyl)	220	3.80	4.00	3.40	3.50
10% OV-17 (phenyl methyl silicone; 50% phenyl)	230	3.15	3.20	2.65	2.70
10% OV-101 (dimethyl silicone)	220	3.00	3.25	2.50	2.60
5% OV-210 (methyl silicone with 50% trifluoropropyl groups)	220	2.10	2.75	2.80	3.60

[a] All columns are borosilicate glass, 6 mm o.d. (4 mm i.d.) × 100 cm long; Gas Chrom Q (80–100 mesh) was used to prepare all packings; columns conditioned overnight at 250° C prior to use.

Table III. *p*-Values of Dasanit and Three of Its Metabolites in Various Solvent Systems

Compound	Hexane-Water	Hexane-20% MeCN (80% Water)	Hexane-40% MeCN (60% Water)	Hexane-60% MeCN (40% Water)	Hexane-80% MeCN (20% Water)	Benzene-Water
Dasanit P=S, SO	0.75	0.34	0.056	0.012	0.006	1.00
Dasanit sulfone P=S, SO_2	0.98	0.72	0.18	0.027	0.008	1.00
Dasanit oxygen analog P=O, SO	0.002	0.001	0.001	<0.001	<0.001	0.62
Dasanit oxygen analog sulfone P=O, SO_2	0.021	0.018	0.009	0.003	0.002	0.96

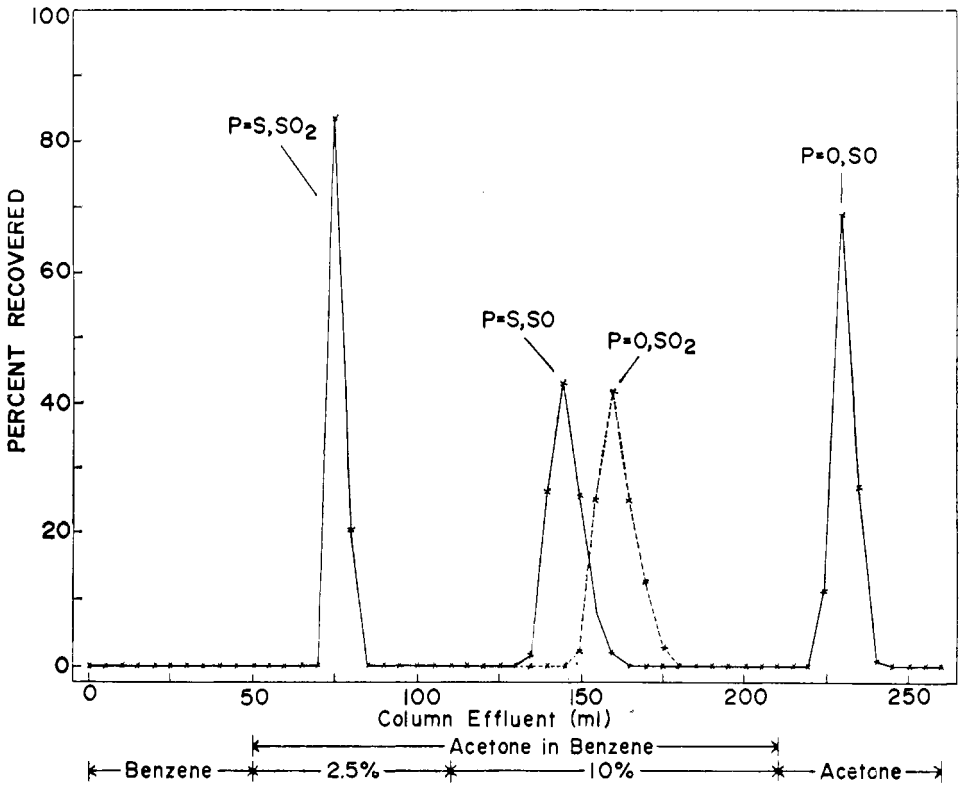

Figure 4. Separation of Dasanit and three of its metabolites by liquid chromatography on silica gel

Table I. Retention times of the compounds at specific temperatures are given in Table II for the 100 cm long OV-210 column and for four other columns: OV-17, OV-25, OV-101, and OV-225. These additional retention times and column temperatures may prove useful for analysis in circumstances where unexpected interferences occur or where additional confirmation of identity is desired. As a further aid in confirming identities at the ng level, *p*-values (Beroza and Bowman, 1965; Beroza *et al.*, 1969) were determined in six solvent systems, representing a wide range of partition coefficients. These data are given in Table III.

The separation of the four compounds by liquid chromatography on silica gel was investigated as the principle on which the method is based. A 1 ml solution containing 100 μg of each of the four compounds was placed on the column and washed into the absorbent with a few ml of benzene. The eluate was then analyzed by gas chromatography of 5 ml aliquots as the column was sequentially eluted with 50 ml of benzene; 60 ml of 2.5% acetone in benzene; 100 ml of 10% acetone in benzene; and finally 50 ml of pure acetone. The results are shown in Figure 4. The Dasanit sulfone was completely eluted by the 2.5% acetone mixture. Dasanit and the *O*-analog sulfone are completely eluted by the 10% acetone mixture, but are only partially resolved. This creates no difficulty, since these two compounds differ in retention time by 1.5 min on the OV-210 column used. The *O*-analog is completely eluted by the pure acetone. In addition to illustrating the basic analytical scheme used here, these results may be useful for metabolic studies or for adaptation of the method to other substrates. A more complete separation of Dasanit and the *O*-analog sulfone might be achieved by eluting with a 5 or 7% acetone in benzene solution, however much larger volumes of solvent would be required.

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