Metabolism of P^{32} Labeled Dasanit[R] in Cotton Plants

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Dasanit (formerly Bay 25141) is an insecticide and nematocide being developed by Chemagro Corporation under license. The chemical name is O,O-Diethyl-O-p-(methylsulfinyl)phenyl phosphorothioate. The metabolism of Dasanit in cotton plants was investigated by Benjamini and coworkers in 1959 (1). Using a paper chromatographic procedure, they tentatively identified 4 major components including the parent compound, the corresponding sulfone, the S-ethyl sulfoxide and the S-ethyl sulfone. Identification of the S-ethyl isomer sulfoxide and sulfone was based on the enhanced anticholinesterase activity observed and an apparent appearance of the P=O bond by infrared spectrometry.

[R] Reg. U.S. and Canadian Pat. Offs. by Farbenfabriken Bayer A.G., Chemagro Corporation licensee.

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A re-examination of the paper chromatographic system used by Benjamini and coworkers (1), showed that the relative mobilities of the S-ethyl compounds did not match those previously reported. The pure S-ethyl analogs were prepared by Regel and Botts (2). The purity of the standards were verified by thin layer chromatography. The identity of the S-ethyl analogs were confirmed by elemental analysis for phosphorus and sulfur as well as by infrared spectrometry. The availability of freshly prepared and pure S-ethyl analog standards and the need to include all metabolites in our residue method, prompted us to investigate further the metabolism of Dasanit in cotton plants.

METHOD

Apparatus and Reagents: All chemicals were reagent grade and reagent grade solvents were redistilled in an all glass system shortly before use. Aside from the normal laboratory glassware, the following were used: Blenders, Waring or equivalent; chromatographic column, borosilicate glass, 20 x 400 mm. with 300 ml. reservoir and Ultramax stropcock; rotary vacuum evaporator, Swissco or equivalent; thin layer chromatographic apparatus and a TLC Plate Scanner, Nuclear Chicago Model 1036. The silica gel used was Silica Gel GF (Silica AR™ TLC 7-GF, Mallinckrodt Chemical Works) 20 x 5 cm., 250 microns thick. The reagents were acetone, benzene (thiophene free), chloroform, Darco, 20 x 40 mesh (for refining grade) activated carbon,

2,6-dibromo-N-chloro-p-quinoneimine spray (DCQ, 1% w/v in acetone), ethyl acetate, pyrex glass wool, sodium sulfate (anhydrous), and superbrite beads (Minnesota Mining Company).

Treatment and Sampling of Plants: Five mg. of technical P^{32} labeled Dasanit (4.670 dpm./µg.) were applied to the stems of young cotton plants. In a companion experiment, the compound was allowed to be taken up by the plants by immersing the roots in 200 ml. of a water dispersion of technical grade P^{32} Dasanit for up to 9 days. In the root uptake studies, water was added to make up for any losses due to evaporation. The plants were placed under fluorescent lamps (about 600 foot candles at plant level), maintained on a 12 hours, on and off cycle. Leaves from treated and control plants were taken 4, 7 and 9 days after treatment.

Sample Preparation: Twenty-five grams of leaves were ground in a Waring blender in 200 ml. acetone, then reground with 200 ml. chloroform and 100 grams of anhydrous sodium sulfate. After filtering with suction, the extracts were concentrated to 30 ml. in a Swissco evaporator. They were then chromatographed (3) on a 20 x 400 mm. column containing 10 grams of activated carbon (Darco, 20 x 40 mesh, for refining grade). After eluting with 375 ml. of acetone, the eluate was evaporated to dryness in the Swissco. The residue was dissolved in 1.0 ml. of chloroform, a portion of which was applied to silica gel plates for chromatography.

Thin Layer Chromatography: It was observed that from 100 to 200 microliters of the extracts provided sufficient activity for detection without overloading the plates. The extracts were co-chromatographed with 10 micrograms each of the non-radioactive compounds suspected of being metabolites. The compounds tested included the S-ethyl analogs (2). The plates were developed with ethyl acetate for a distance of 15 cm. from origin to solvent front, air dried for a few minutes and developed a second time with benzene/acetone (10/90). In the above systems, Dasanit and all its possible metabolites were well separated. It was easy to visually distinguish between the P=S and P=O analogs, since the latter show up as yellow spots and the former pink to light red spots upon spraying with DCQ and heating the plate from 3 to 5 minutes at 110° C. The minimum limit of detectability was 2.0 micrograms for the S-ethyl analogs and 4.0 micrograms for the O-ethyl analogs.

Radioactive Counting: Prior to spraying with DCQ reagent, the plates were scanned for radioactivity using the Nuclear Chicago Model 1036 Thin Layer Plate scanner. The following settings were used: 975 volts, 1/16" slit opening, 10 seconds suppression, 260 ml./minute gas flow (98.7% helium, 1.3% butane) and a scanning speed of 12 inches per hour on linear scale. The attenuations varied from 300 to 30 K. The identification of the various compounds was made by comparing the R_f values

of non-radioactive standards to those of the radioactive peaks observed. In addition, the color of the spots in the unknown were compared with those observed for the standards.

Results and Discussion

The extraction and clean-up procedure described removed enough of the extractives so that the mobilities of the standards were the same in the presence or absence of sample extract. In addition, it was shown by thin layer chromatography and also by total phosphorus analyses (3), that all the suspected metabolites listed in Table I are recovered from the above clean up procedure. Table I below lists the R_f values and behavior of all suspected metabolites to the DCQ spray.

TABLE I

R_f Values of Dasanit and Suspected Metabolites and Behavior with DCQ Spray Reagent*

	Compound	R_f Values	Color with DCQ
(I)	p-$CH_3SOC_6H_4OP(O)(OEt)_2$	0.24-0.26	light yellow
(II)	p-$CH_3SOC_6H_4OP(O)$ OEt, SEt	0.34-0.36	dark yellow
(III)	p-$CH_3SOC_6H_4OP(S)(OEt)_2$**	0.53-0.57	pink
(IV)	p-$CH_3SO_2C_6H_4OP(O)(OEt)_2$	0.64-0.68	light yellow
(V)	p-$CH_3SO_2C_6H_4OP(O)$ OEt, SEt	0.75-0.78	dark yellow
(VI)	p-$CH_3SO_2C_6H_4OP(S)(OEt)_2$	0.84-0.88	pink
(VII)	p-$CH_3SC_6H_4OP(S)(OEt)_2$	0.96-0.98	dark pink

*Developed first with ethyl acetate, then with benzene/acetone (10/90). Silica gel 7-GF, Mallinckrodt Chemicals, 250 microns thick.

**Dasanit (formerly Bay 25141), 0,0-diethyl-0-p-(methylsulfinyl) -phenyl phosphorothioate.

The results showed that in all of the stem application and root uptake samples about 80 to 89% of the radioactivity was in the form of the parent compound. A thin layer chromatographic scan of a 4-day root uptake sample showed peaks at R_f 0.25, 0.53 and 0.84 corresponding to the oxygen analog (I), Dasanit (III) and Dasanit sulfone (VI) respectively. A similar pattern was obtained for a 4-day stem application sample. Co-chromatography of the extracts with non-radioactive S-ethyl analogs (II and V), confirmed the absence of the two compounds. Table II shows the relative percent distribution of the organosoluble P^{32} at various intervals in the stem application samples.

TABLE II

Chemical Transformation of Dasanit, Following Stem Application to Cotton Plants

Compound	Percent of Total Organosoluble P^{32} at Indicated Days Following Application		
	4	7	9
(I) p-$CH_3SOC_6H_4OP(O)(OEt)_2$	3.5	4.6	7.2
(III) p-$CH_3SOC_6H_4OP(S)(OEt)_2$	88.3	86.8	80.8
(IV) p-$CH_3SO_2C_6H_4OP(O)(OEt)_2$	~	~	traces
(VI) p-$CH_3SO_2C_6H_4OP(S)(OEt)_2$	8.2	8.6	12.0

The amount of oxygen analog (I) and Dasanit sulfone (VI) increased slightly between the 4th and 9th days and there was a corresponding decrease in the amount of parent compound (III).

In the root uptake experiment a similar pattern was observed in the 4th and 7th day samples. Table III lists the relative percent distribution of the organosoluble P^{32} at 4, 7 and 9 days after starting the root uptake experiments.

TABLE III

Chemical Transformation of Dasanit, Following Root Uptake by Cotton Plants

Compound	Percent of Total Organosoluble P^{32} at Indicated Days Following Application 4	7	9
(I) p-$CH_3SOC_6H_4OP(O)(OEt)_2$	4.3	4.9	6.7
(III) p-$CH_3SOC_6H_4OP(S)(OEt)_2$	87.0	85.2	79.9
(IV) p-$CH_3SO_2C_6H_4OP(O)(OEt)_2$	-	-	4.7
(VI) p-$CH_3SO_2C_6H_4OP(S)(OEt)_2$	8.7	9.9	8.7

The percentage for Dasanit would probably have been considerably less, after 9 days, if the plants had not been allowed to remain in contact continuously with the radioactive dispersion. A measurable amount of the oxygen analog sulfone (IV) was detected in the 9 day sample. A comparison of Table II and III shows that the relative amounts of metabolites compared to the parent compound was slightly higher in the root uptake experiments than with the stem applied sample. In no case were either of the S-ethyl analogs detected.

SUMMARY

The results of this investigation showed that aside from the parent compound, there were two major metabolites of Dasanit in cotton plants. They were the oxygen analog (I) and the Dasanit sulfone (VI). A small amount of oxygen analog sulfone (IV) was also detected. No S-ethyl analogs (II and V) were found.

References

1. E. BENJAMINI, R. METCALF and T. FUKUTO. J. of Economic Entomology 52, 99 (1959).
2. E. K. REGEL and M. F. BOTTS, Chemagro Corporation Report No. 17,027, October 21, 1965
3. C. A. ANDERSON, Chemagro Corporation Report No. 8544, February 4, 1962.