Thin Layer Chromatographic Separation of Linuron and Diuron and Some of Their Known or Suspected Soil Metabolites

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Linuron, 3-(3,4 dichlorophenyl)-1-methoxy-1-methyl urea and diuron, 3-(3,4 dichlorophenyl)-1,1-dimethylurea are used for the control of many weeds. Linuron is used for the selective control of weed seedlings in field corn and soybeans, while diuron is used as a selective herbicide on alfalfa, cotton, corn, potatoes, sugar cane and citrus fruits.

Hill and co-workers (4) indicated that the most probable mechanism for the disappearance of substituted urea herbicides from soils was the microbial degradation of the herbicides. Sheets (5) in reviewing the disappearance of substituted ureas for soils concluded that soil microorganisms played a major role in the disappearance of these compounds from soils. Geissbühler and co-workers (3) showed from their studies that soil microorganisms degraded tenoran by successive demethlation followed by hydrolysis of the urea to aniline. Dalton et al (2) confirmed the mechanism of degradation found that diuron degraded in soils by the successive removal of methyl groups followed by hydrolysis of the urea to

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to the corresponding aniline.

It is the intent of the communication to report a thin-layer chromatographic procedure that can be used in laboratory studies,in conjunction with the Bartha and Pramer (1) procedure,to follow the mechanism of linuron and diuron degradation in soils. This report also presents methods of separation of other possible degradation products which might occur during microbial destruction of these herbicides.

Reagents

(a) Acetone, chloroform, methanol and pyridine- reagent grade or equivalent redistilled

(b) Chromatographic Solvent System -100:5:1 chloroform-methanol-pyridine by volume.

(c) Ninhydrin Spray Reagent - Weigh 0.5 g ninhydrin into a 100 ml. volumetric flask. Dissolve with 95 ml. of n-butanol and bring to volume with 10% aqueous acetic acid.

(d) Silver Nitrate Spray Reagent - Dissolve 1.7 g silver nitrate in a small amount of water in a 200-ml. volumetric flask. Add 10 ml. of 2-phenoxyethanol. Add 50 ml. of 95% ethanol and bring to volume with water. If cloudy add 1 ml. increments of 95% ethanol until clear.

(e) Phenol Spray Reagent

1- Buffer solution pH 8: Equal volumes of solution a and solution b.

a - Ammonium hydroxide 0.5 N

b - Dissolve 104.5 g K_2HPO_4 and 72.3 g KH_2PO_4 per liter of distilled water

2- Spray Reagent A - 0.5 g 4-aminoantipyrine in 100 ml pH 8 buffer

3- Spray Reagent B - 2.0 g $K_3FE(CN)_6$ in ml. distilled water. Spray Reagent A followed by Reagent B.

(f) Standards - Prepared in acetone to contain 1 mg./ml.

(1) Linuron - 3-(3,4 dichlorophenyl)-1-methoxy-1-methyl urea

(2) Diuron - 3-(3,4 dichlorophenyl)-1,1 dimethylurea

(3) 3-(3,4 dichlorophenyl) urea

(4) 3-(3,4 dichlorophenyl)-1-methyl urea

(5) 3,4 dichloroaniline

(6) 3,4 dichlorophenol

(7) 1-methoxy-1-methyl urea

(8) 1-1 dimethylurea

(9) 3-(3,4 dichlorophenyl) 1-methoxy urea

Equipment

(a) Eastman Chromatographic Sheets K 301 R2 or 250 u Silica Gel G thin layer plates

(b) Apparatus capable of allowing chromatographic development of Eastman Sheets and thin-layer glass plates

(c) UV light source capable of developing spots after

spraying with $AgNO_3$ spray solution, such as Uvi-Arc Model 420 - U 1 Raymaster

(d) Drummond microcaps, 1 ul, 5 ul capacity - Drummond Scientific Company, Broomall, Pennsylvania

Procedure

Prepare three 100 g. soil samples containing 3,5, and 10 ppm each of linuron and its known or suspected metabolites, 3-(3,4 dichlorophenyl)-1-methyl urea, 3-(3,4 dichlorophenyl-1-methoxyurea, 3-(3,4 dichlorophenyl)urea, 3,4 dichloroaniline, 3,4 dichlorophenol, 1-methoxy-1-methylurea and diuron and its known or suspected metabolites, 3(3,4 dichlorophenyl)-1-methyl urea, 3-(3,4 dichlorophenyl)urea, 3,4 dichloroaniline, 3,4 dichlorophenol, and 1,1 dimethyl urea. This is accomplished by adding 3,5, and 10 µg. of each herbicide or metabolite per gram of soil by placing appropriate quantities of the standards in acetone in a test tube. Reduce the volume of acetone to less than 1 ml. Dilute with water to 20 ml. and add the aqueous solution to the soil and mix thoroughly. Allow the soil to stand in a closed container for 5 days. Air-dry the soil samples. Also air-dry 100-g. samples of untreated soils. After drying, grind the samples by means of a mortar and pestle.

Extract the air-dried soil samples with 250 ml. of acetone by means of a sohxlet extractor for 4 to 6 hours. Concentrate the acetone extract to a volume of 2 ml.

Spot 10 ul. to 20 ul. of soil extract on either an Eastman Chromatogram Sheet or a 250-μ silica gel G thin layer plate. Each plate should contain an equivalent spot of untreated soil as well as 1-ul spottings of standards.

Develop the chromatographic plates with the solvent system, chloroform-methanol-pyridine, 100:5:1 by volume, in a suitable developing chamber for a distance of 10 cm. Developing time is about 40 minutes. Remove the chromatographic plate from the chamber and remove the solvent with a hair drier.

Spray the plate with the ninhydrin spray reagent and place in an oven at 130° for 5 to 10 minutes. All compounds except 3,4 dichlorophenol are visualized. An additional plate is necessary for the determination of the presence of 3,4 dichlorophenol. Develop the additional plate in the same manner. Spray with phenol spray reagent A followed by phenol spray reagent B. A strawberry-red spot indicates the presence of a phenol. A spray using Mitchell's reagent (Reagent D) can be used on an additional plate to confirm those compounds having the 3,4 dichlorophenyl moiety.

Results and Discussion

The herbicides, linuron and diuron, and their known or suspected metabolites were equilibrated with soils for 5 days prior to air-drying. Since this was far in excess

of the 0.5 hr. equilibrium time found by Sherbourne and Freed (6) for monuron, it was assumed that the various entities reached absorptive equilibrium with the colloidal and/or organic fractions during this time.

The acetone extraction of the soils by means of a sohxlet extractor for 4 to 6 hours appears to be sufficient to remove the vast majority of each of the compounds added to the soil. If the extraction is less than quantitative, sufficient quantities are extracted to determine the presence of all at the 3 ppm level. If necessary, larger soil samples can be taken , extracted, and concentrated to determine levels below 3 ppm.

Blank soil samples examined by this procedure did not exhibit positive ninhydrin spots. Samples of a sandy soil and two loams, a silt loam and a sassafras loam, were used with similar results. In all the soils, a streaking of soil extract was noted after spraying, but no positive or extraneous ninhydrin spots were present. This was confirmed,using Mitchell's silver nitrate spray for those compounds having the 3,4 dichlorophenyl moiety and for the 3,4 dichlorophenol using the 4-amino anti-pyrine spray. Table 1 shows the compounds studied, the Rf values, lower limits of detectability,and spray used.

TABLE 1

Chromatographic Data for Linuron, Diuron and Their Known or Suspected Metabolites

Compound	Rf	Lower Limit of Detectability (ug.)	Spray
Linuron	0.95	0.3	(1), (2)
Diuron	0.80	0.5	(1), (2)
3-(3,4 dichlorophenyl) urea	0.22	0.3	(1), (2)
3-(3,4 dichlorophenyl) -1-methyl urea	0.51	0.3	(1), (2)
3,4 dichloroaniline	0.85	0.1	(1), (2)
3,4 dichlorophenol	0.85	0.1	(3), (2)
1-methoxy-1-methyl urea	0.35	0.5	(1)
1-1 dimethylurea	0.13	0.2	(1)
3-(3,4 dichlorophenyl) 1-methoxy-1-methyl urea	0.75	0.3	(1), (2)

(1) ninhydrin (2) $AgNO_3$ (3) 4-aminoantipyrine

It is necessary to use two plates to determine the presence of the 3,4 dichlorophenol and possibly three plates to determine all the mixture. The 3,4 dichlorophenol is ninhydrin negative while all the other materials are 4-aminoantipyrine negative.

By using a preparative silica gel technique, it is possible to isolate many of the metabolites and confirm their presence by infrared techniques.

It should be noted that this procedure can be used to follow the biodegradation of these herbicides in laboratory studies but is not intended for use in determining the presence or absence of either herbicide or their metabolites in soil samples of unknown origin or treatment.

References

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