Thin-layer Chromatographic Separation of Vernolate, Suspected Metabolites and Derivatives

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Introduction

The use of vernolate (S-propyl N,N-dipropylthiol carbamate) has become established in the production of soybeans, sweet potatoes, tobacco, and peanuts. It is incorporated with the soil as either a preplant, preemergence, or postemergence treatment. An advantage of this chemical is that its activity is largely confined to one growing season. It is lost by volitilization and chemical degradation. Kaufman's work (1) indicates that vernolate residues may include 1-propanethiol, dipropylamine, and 1-propanol. Propylamine is, also, likely to occur.

Vernolate and the mercaptan can be extracted from soil and analyzed (2) by gas-liquid chromatography (GLC). The other suspected metabolites can be analyzed (3) by GLC but interferences are likely with soil or biological sample extracts. Additional purification techniques are needed 1) to extend the usefulness of the second method, 2) to isolate vernolate from radiolysis products, 3) to check the purity of technical preparations of vernolate, and 4) to follow the degradation of radioactive vernolate in soil. The purpose of this report is to describe some thin-layer chromatographic techniques useful for the resolution and identification of vernolate, its suspected metabolites, and their derivatives.

Reagents

1) Acetic acid, acetone, benzene, butanol, diethyl ether, and ethanol; reagent grade or equivalent, redistilled.

2) Chromatographic solvent systems
   a) benzene
   b) 1:1:3, acetic acid-butanol-benzene by volume
   c) 3:10, diethyl ether-hexane
   d) 1:10, water-acetone
   e) butanol
   f) 1:10, acetic acid-ethanol

3) Visualizers
   a) iodine; place iodine crystals in a closed vessel
   b) Rimini's test
      A) 1% aqueous sodium nitroprusside
      B) 50% aqueous acetaldehyde

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c) Mitchell's test; Dissolve 1.7 g $AgNO_3$ in 5 ml of water, add 20 ml 2-phenoxyethanol, dilute with acetone to 200 ml, add 1 drop 30% H_2O_2 and age overnight
d) Congo red; A. Dissolve 0.4% Congo red in 50% (v/v) aqueous ethanol. B. Dissolve a concentrated bromine solution in CCl_4 and place in a closed vessel

4) Standards - Dilute with acetone to contain 1 mg/ml
  a) Vernolate (Stauffer Chemical Company)
  b) Dipropylamine
  c) Propylamine
  d) Propanethiol
  e) N,N-di-n-propyl 3,5-dinitrobenzamide
  f) N-n-propyl 3,5-dinitrobenzamide

Equipment

1) Eastman Chromatographic Sheets No. 6061

2) Development tank for Eastman Sheets

3) Disposable micropipets, 1 and 5 ul (Drummond Scientific Co.)

4) Chromatogram sprayer and source of air pressure

5) Ultraviolet light source, Uvi-Arc Model 420-U 1 Raymaster (George W. Gates and Co.)

Method

The general practices of thin-layer chromatography are widely used (4).

Absorbent. The absorbent has been discussed as type K301R2 silica gel (5). This 300 micron thick sheet has the advantage of being adapted for use in some radioactive paper chromatogram scanners.

Development. The ascending chromatography development method is applied. About 50 ml developer is deposited in the bottom of the tank. Allow the developer to ascend 10 cm above the solvent surface. The compounds of interest migrate to the R_f values given in Table I.

Visualization. Four visualizers have proven useful. Iodine is widely used as a general reagent (6) These compounds along with most organic substances yield an unstable brown color in this test. Silver nitrate spray reagent and exposure to ultra-violet light produced (7) brown spots for all the compounds.

Use of Rimini's test provides a means to distinguish between propylamine and dipropylamine (8). Sodium nitroprusside spray reagent on propylamine furnishes a purple color while dipropylamine gives none. If the chromatogram is, in addition, sprayed with acetone and then with 50% acetaldehyde, dipropylamine gives a bright blue spot which changes to bluish-purple after 20 minutes drying. At this stage the propylamine shows as a bluish-gray spot.

Congo red (9) spray reagent and exposure to bromine vapor produces blue spots against a pink background for vernolate and Eptam (S-ethyl N,N-dipropylthiolcarbamate).

Discussion

Thin layer chromatographic systems are given for the resolution of vernolate, suspected metabolites, and their derivatives. Four developers are especially useful for vernolate and should be considered for use when purifying either unlabelled or radioactive isotope labelled vernolate. Two solvent systems satisfactorily resolve propylamine and dipropylamine. Some visualizers are recommended. The iodine test reveals the presence of nonvernolate components in certain technical quality herbicide preparations.

TABLE 1

Effect of developer on the R_f values for vernolate, suspected metabolites, and their derivatives

Chemical	R_f for solvent mixture					
	a	b	c	d	e	f
vernolate	0.4	0.8	S.F.[1]	0.7	0.7	0.6
dipropylamine	0[2]	0.3	0	0	0.4	0.4
propylamine	0	0	0	0.4	0.4	0.5
propanethiol	S.F.	0.8	S.F.	-	-	-
N,N-di-*n*-propyl 3,5-dinitrobenzamide	-	-	0.7	-	-	-
N-*n*-propyl 3,5-dinitrobenzamide	-	-	0.6	-	-	-

1. "S.F." indicates spots which are so close to the solvent front as to have a R_f larger than 0.9.

2. "0" indicates spots which are so close to the origin as to have a R_f smaller than 0.1.

Among the developers tried are acetone, ammonium hydroxide: acetone (1:4), water: acetic acid: acetone (1:1:20 and 1:1:10), benzene: acetic acid: butanol (4:1:1), benzene: acetic acid (1:4), and ethanol: acetic acid (3:1). These either failed to place vernolate away from the chromatogram extremes or do not resolve vernolate from the amines and their contaminates. A benzene: isopropanol (100:1) solvent mixture in a thick layer chromatographic system accomplished purification of C-14 labelled vernolate.

The derivatives, N-phenyl N-*n*-propylthiourea and *p*-toluenesulfonic acid, *n*-propylamine salt, remain at the origin when benzene is used. When water: acetic acid: acetone (1:1:3) was used they migrated with the solvent front.

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