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Note

Detection of aminocarb and its major metabolites by thin-layer chromatography

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Aminocarb (Matacil®), 4-dimethylamino-*m*-tolyl N-methylcarbamate, a broad spectrum, non-systemic insecticide, has been used extensively for controlling the spruce budworm (*Choristoneura fumiferana* Clem.) in eastern Canada since 1976[1,2]. Very little is known about the metabolism of this pesticide in the environment. To study the fate of this chemical in the ecosystem, a sensitive method for the detection of aminocarb and its major metabolites is necessary. Strother[3] has used two-dimensional thin-layer chromatography (TLC) to isolate and identify the methylamino, amino and hydroxymethyl analogues from the *in vitro* metabolism of aminocarb by liver homogenates from humans and rats. Balba and Saha[4] obtained similar results with one-dimensional TLC using diethyl ether as the developing solvent. In this paper we describe a simple TLC technique using either (a) hexane–acetone (1:1, v/v) or (b) diethyl ether–hexane–ethanol (77:20:3, v/v) as the developing solvent for the separation of aminocarb and its major metabolites on silica gel G or silica gel F_{254}, along with two visualization techniques.

EXPERIMENTAL

Chemicals for chromatography

Analytical grade (>99%) aminocarb (4-dimethylamino-*m*-tolyl N-methylcarbamate), MA (4-methylamino-*m*-tolyl N-methylcarbamate), AM (4-amino-*m*-tolyl N-methylcarbamate), MFA (4-methylformamido-*m*-tolyl N-methylcarbamate) and FA (4-formamido-*m*-tolyl N-methylcarbamate) supplied by Chemagro (Mississauga, Canada) were used in this study.

Solvent system and development of the plates

Two types of thin-layer plates were used: (1) silica gel F_{254} (0.5 mm thick) precoated plate (20 cm × 20 cm) and (2) glass plate (20 cm × 20 cm) coated with silica gel G (0.5 mm thick). All thin-layer plates were heated in the oven at 110 °C for 1 h before use. Aminocarb and its metabolites (5 μg each in 100 μl acetone) were spotted on the plate, 1.5 cm above the lower edge, and dried under a gentle stream of nitrogen. The spot size was maintained at about 0.75 cm in diameter. The spotted plates were developed in a glass tank saturated with the developing solvent. The two

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solvent systems tested were: (1) hexane–acetone (1:1, v/v, pesticide-grade) and (2) diethyl ether–hexane–ethanol (77:20:3, v/v, pesticide-grade). The developed plates were removed from the tank when the solvent front was 15 cm from the origins. They were air dried, then sprayed with chromogenic reagents for visualization.

Spot visualization

Two spot visualization techniques were used. (1) Ninhydrin spray: the air dried plates were sprayed with sodium hydroxide solution (10% aq.) in the fume hood, heated in the oven at 60 °C for 3–5 min, then sprayed with ninhydrin (2% in ethanol), followed by heating in the oven at 60 °C for 30 min. Aminocarb and its metabolites appeared as pink spots. (2) Cholinesterase inhibition: cholinesterase was prepared from fresh pig liver[5]. The air dried plates were sprayed gradually and evenly with the pig liver homogenate until thoroughly wet. The plates were allowed to dry at room temperature for 30 min, then sprayed with the freshly prepared substrate spray in the same manner as the pig liver homogenate. The substrate spray consisted of a 20-ml mixture of two solutions as follows: solution A was prepared by dissolving 20 mg of 5-bromoindoxyl acetate in 5 ml absolute ethanol, ferrocyanide solution (0.416 g of potassium ferricyanide and 0.52 g potassium ferrocyanide in 25 ml distilled water) with 13 ml of 0.05 *M* Tris buffer. Aminocarb and its metabolites appeared as white spots on blue background 30 min after spraying.

Application in metabolite separation and identification

To evaluate the applicability of the above-described TLC method in metabolic study, an experiment to isolate and identify aminocarb and its metabolites was conducted. Rainbow trout, *Salmo gairdneri* Richardson, was exposed to 15.0 ppm of aminocarb in the aquarium at 10 °C for 144 h. At the end of exposure, aminocarb and its metabolites were extracted from fish tissues (whole fish) with ethyl acetate and analyzed by gas–liquid chromatography–alkali flame-ionization detection (GLC–AFID)[6]. The identities of aminocarb and its metabolites were also confirmed by TLC as described in this paper.

RESULTS AND DISCUSSION

The two developing solvent systems used in this study gave good separation of aminocarb and its metabolites, MA, AM, MFA, FA on both silica gel G and F_{254} (Table I). MFA and FA did not resolve completely on silica gel F_{254} using hexane–acetone (1:1, v/v) as the developing solvent. The R_F values for MFA and FA were 0.46 and 0.42, respectively. Silica gel G and F_{254} showed similar separation characteristics. In general, better resolution was obtained with hexane–acetone (1:1, v/v) as the developing solvent.

Ninhydrin is a common chromogenic reagent for the detection of amino acids, amines and amino sugars. The color reaction with the ninhydrin spray observed in this study was due to the amines formed from the alkaline hydrolysis of N-methylcarbamate esters. Consequently, this technique was specific for nitrogen. On the other hand, the cholinesterase inhibition technique was specific for the detection of cholinesterase inhibitors such as aminocarb and its metabolites that retained the carbamate moiety. Thus, a combination of both techniques will offer a high degree of specificity for detecting aminocarb and its major metabolites.

TABLE I

R_F VALUES OF AMINOCARB AND ITS MAJOR METABOLITES RESOLVED WITH TWO DIFFERENT SOLVENT SYSTEMS ON SILICA GEL G AND SILICA GEL F_{254}

Compound	*R_F values*			
	Silica gel G		*Silica gel F_{254}*	
	Hexane–acetone (1:1, v/v)	*Diethyl ether–hexane–ethanol (77:20:3, v/v)*	*Hexane–acetone (1:1, v/v)*	*Diethyl ether–hexane–ethanol (77:20:3, v/v)*
Aminocarb	0.81	0.70	0.74	0.67
MA	0.70	0.57	0.66	0.53
AM	0.53	0.40	0.52	0.37
MFA	0.45	0.30	0.46	0.28
FA	0.39	0.22	0.42	0.20

Aminocarb and its metabolites, namely MA and AM were detected in rainbow trout exposed to aminocarb for 144 h by GLC–AFID. The column (183 cm × 2 mm I.D.) used in this investigation was 1.0% OV-17 + 1.0% OV-210 on Ultra-Bond 20 M, 80–100 mesh. The column temperature was 180 °C isothermal. A typical chromatogram is given in Fig. 1. The minor peaks appeared in the chromatogram were

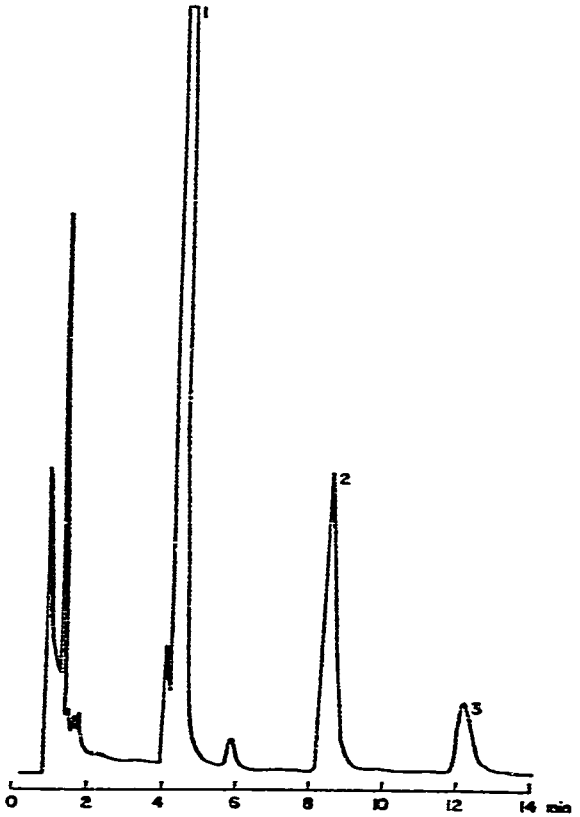

Fig. 1. Gas chromatogram of rainbow trout exposed to 15 ppm aminocarb for 144 h. Peaks: 1 = aminocarb; 2 = AM; 3 = MA.

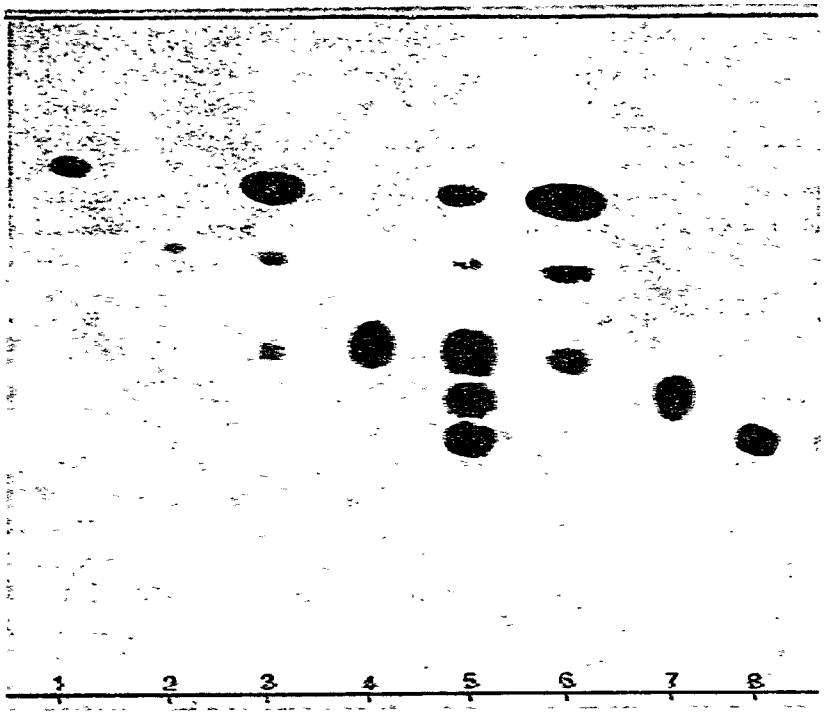

Fig. 2. Thin-layer chromatogram of rainbow trout exposed to 15 ppm aminocarb for 144 h. Spots 1, 2, 4, 7 and 8: 5 μg each of aminocarb, MA, AM, MFA and FA, respectively; spot 5: a mixture of all five; spots 3 and 6: rainbow trout tissue extracts.

also observed in fish from the control group indicating that they were naturally occurring compounds present in fish tissues. The identities of aminocarb and its 4-methylamino and 4-amino analogues were confirmed by TLC using either of the visualization techniques described in this paper. A typical thin-layer chromatogram on silica gel G using hexane–acetone (1:1, v/v) as the developing solvent and ninhydrin as the chromogenic agent is given in Fig. 2. Similar results were also obtained with cholinesterase inhibition.

In summary, the TLC technique described in this paper gives good separation of aminocarb and its metabolites and is very sensitive. Therefore, it will be a useful tool for the detection and confirmation of aminocarb and its major metabolites in environmental samples.

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