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Note

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

Gy. HUBER KOVÁCS
Research Institute for Heavy Chemical Industries, P.O. Box 160, H-8201 Veszprém (Hungary)
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Phosmethylan, O,O-dimethyl-S-[N-{(*o*-chlorophenyl)butyramido}methyl]dithiophosphate, a broad spectrum, non-systemic insecticide, can be used for controlling a large variety of pests in different crops; it is also an effective sheep dip insecticide[1]. Very little is known about the behaviour of this compound in the environment. The thermal instability of the parent compound, however, greatly limits the applicability of gas–liquid chromatography. On the basis of the functional groups of the parent compound and its main metabolites, thin-layer chromatography (TLC) with different chromogenic reagents[2–4] is a powerful tool for metabolic studies. In this paper we describe a simple TLC technique using hexane–dioxane–acetic acid (79:20:1) as developing system for the separation of phosmethylan and its metabolites on silica gel F_{254}, with three visualization techniques.

EXPERIMENTAL

Reagents for chromatography

Analytical-grade (>99%) phosmethylan, N-hydroxymethyl-*o*-chlorobutyroanilide, *o*-chlorobutyroanilide and *o*-hydroxybutyroanilide were synthetized. 2-Amino-3-chlorobutyrophenone and 4-amino-5-chlorobutyrophenone were prepared by TLC. *o*-Chloroaniline was supplied by EGA Chemie.

Solvent system and development of the plates

Silica gel F_{254} (0.25 mm thick) precoated plates (20 × 20 cm) were used. Phosmethylan and its major metabolites (1 μg each in 10 μl of hexane) 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 *ca.* 0.7 cm diameter. The spotted plates were developed in a glass tank saturated with the developing solvent. Four solvent systems were tested: (1) hexane–dioxane–acetic acid (79:20:1), (2) hexane–dioxane (80:20), (3) hexane–dichloromethane (30:70) and (4) chloroform–diethyl ether (80:20). The developed plates were removed from the tank after a 15-cm run, dried at room temperature and sprayed with chromogenic reagents for visualization.

Spot visualization

Three spot visualization techniques were used: (1) *o*-Tolidine plus potassium

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iodide spray[2]: 1 g of *o*-tolidine was dissolved in 10 ml of glacial acetic acid and 4 g of potassium iodide in 10 ml of water; the two solutions were mixed and diluted to 1 l with water; the air-dried plates were put into a tank saturated with chlorine vapour for 30 sec (10 g of $KMnO_4$ plus 5 ml of concentrated hydrochloric acid); residual chlorine was removed with air stream at room temperature and the plates were sprayed with the reagent; amides and amines appeared as blue spots. (2) Palladium chloride spray[3]: the air-dried plates were sprayed with palladium chloride solution (0.5% in 1 *M* hydrochloric acid) in the fume hood and heated in the oven at 80°C for 3–5 min; phosphorus compounds appeared as yellowish brown spots against a white background. (3) *p*-Dimethylaminobenzaldehyde spray[4]: the air-dried plates were sprayed with the reagent dissolved in a mixture of ethanol (35 ml) and concentrated hydrochloric acid (5 ml); primary aromatic amines appeared as intense yellow spots on a white background.

Application for metabolite separation and identification

The TLC method described above was applied to the separation and identification of phosmethylan and its metabolites in small amounts in natural water and

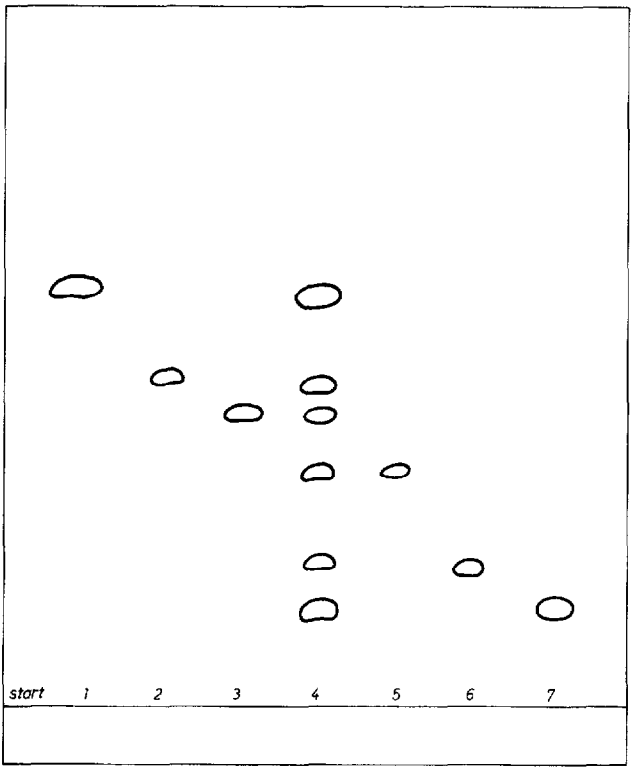

Fig. 1. Schematic representation of a thin-layer chromatogram of phosmethylan and its metabolites in natural water. Developing solvent system: hexane–dioxane–acetic acid. Spots 1, 2, 3, 5, 6 and 7: 2 μg of 2-amino-3-chlorobutyrophenone (R_F 0.7), *o*-chlorobutyroanilide (0.56), *o*-chloroaniline (R_F 0.5), phosmethylan (R_F 0.4), N-hydroxymethyl-*o*-chlorobutyroanilide (R_F 0.28) and *o*-hydroxybutyroanilide (R_F 0.2), respectively; spot 4: water extract.

soil, as well as for the detection of the parent compound and the main decomposition product, *o*-chlorobutyroanilide, in different crops (alfalfa, cabbages, beans, peaches, pears, apples, potatoes), ewe-milk and mutton.

RESULTS AND DISCUSSION

The best results were obtained with hexane–dioxane–acetic acid (79:20:1). *o*-Tolidine–potassium iodide is a common chromogenic reagent for the detection of secondary amines, but primary aromatic amines also react with it after chlorination. The colour reaction that took place on the layer with this chromogenic reagent was due to amines and amides (*o*-chlorobutyroanilide, *o*-hydroxybutyroanilide, 2-amino-3-chlorobutyrophenone, 4-amino-5-chlorobutyrophenone, *o*-chloroaniline) formed from phosmethylan. Reaction with *p*-dimethylaminobenzaldehyde is specific only for primary aromatic amines. Thus, a combination of these two visualization techniques offers a high degree of specificity for the detection of amines and amides together. The parent compound was detected with palladium chloride.

Photolysis of phosmethylan was followed in natural water exposed to UV light. The identities of phosmethylan and its metabolites were confirmed by TLC using the three visualization methods described above. A typical thin-layer chromatogram on silica gel F_{254} using hexane–dioxane–acetic acid (79:20:1) as developing system is shown in Fig. 1. The structures of the metabolites were confirmed by direct mass spectrometry after TLC preparation.

In summary, the TLC technique described in this paper gives good separation of phosmethylan and its major metabolites with a detection limit of 0.5 μg, and will be a useful technique for the detection and confirmation of phosmethylan and its decomposition products in environmental samples.

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