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THIN-LAYER CHROMATOGRAPHIC DETECTION OF MONOCROTOPHOS IN BIOLOGICAL MATERIALS

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Summary—An organophosphorus insecticide monocrotophos is increasingly being used in agriculture to control insects on a wide range of crops. Its ready access has resulted in misuse in many instances of homicidal and suicidal poisoning cases. This paper describes a chromogenic spray reagent for the detection of monocrotophos in biological materials by thin-layer chromatography. Monocrotophos on alkaline hydrolysis yields *N*-methylacetoacetamide which in turn reacts with diazotized sulphanilamide or sulphanilic acid to give a red colour. Other organophosphorus insecticides do not give a coloured spot. Moreover, organochlorine and synthetic pyrethroid insecticides and constituents of viscera (amino acids, peptides, proteins etc.) which are generally co-extracted with the insecticides, do not interfere. However, phenolic compounds and hydrolysed product of carbamate insecticides, may interfere and are differentiated from monocrotophos by R_F values. The limit of detection is *ca* 1 μg.

Monocrotophos (azodrin, nuvacron) an organophosphorus insecticide, is widely used in agriculture in India to control sucking, chewing and boring insects on a wide range of crops. Unfortunately, its ready access has resulted in an increased use in homicidal and suicidal poisoning cases. Consequently, characterization of this insecticide is necessary in forensic toxicology.

A number of spray reagents have been described for the detection of organophosphorus insecticides by thin-layer chromatography (TLC). The most common reagents include palladium (II) chloride,[1,2] bromine-fluorescein, silver nitrate,[3,4] congo red,[5] mercury (I) nitrate, 4-(*p*-nitrobenzyl) pyridine-tetraethylenpentamine and acidified potassium iodate–starch.[8] These reagents do not react with monocrotophos, and hence it was felt necessary to develop a spray reagent for the detection of monocrotophos in biological materials.

In this paper the use of diazotized sulphanilamide or sulphanilic acid is described for the detection of monocrotophos by TLC. Diazotized sulphanilamide or sulphanilic acid reacts with hydrolysed monocrotophos to produce a red colour. The reagent is selective for monocrotophos, among the organophosphorus group of insecticides.

EXPERIMENTAL

Reagents

All reagents were of analytical reagent grade. Distilled water was used throughout.

Silica gel G of particle size 15 μm with 13% $CaSO_4$ binder (Merck, Darmstadt, F.R.G.) was employed on prepared TLC plates.

A monocrotophos stock solution, (1 mg/ml) was prepared by dissolving 13.5 mg of 74% technical grade monocrotophos (Hindustan Ciba-Geigy, Bombay, India) in 10 ml of ethanol.

Sodium hydroxide solution (20%) was prepared by dissolving 20 g sodium hydroxide in distilled water and diluting the solution to 100 ml.

Diazotized sulphanilamide/sulphanilic acid reagent 0.5 was prepared by dissolving 0.5 g sulphanilamide or sulphanilic acid and 1 g sodium nitrite in 100 ml of 10% v/v hydrochloric acid (ice cold solution).

Procedure

A standard glass TLC plate was coated with a slurry of silica gel G in water (1:2) to a thickness of 0.25 mm. The plate was activated

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by heating it at 110°C for about 1 hr. Monocrotophos solution (10 μl) in ethanol equivalent to 10 μg of monocrotophos (1 mg/ml) was spotted onto the plate, which was then developed in a previously saturated TLC chamber using chloroform–acetone (7:3) as solvent up to a height of 10 cm.

The plate was removed from the chamber, dried in air and sprayed with 20% w/v sodium hydroxide solution. After waiting 5 min for complete hydrolysis it was sprayed with ice-cold diazotized sulphanilamide or sulphanilic acid reagent. A red spot was observed immediately on the TLC plate with an R_F value of 0.45.

Extraction of monocrotophos from biological materials

To portions of *ca* 50 g each of various types of visceral tissues (stomach, intestine, liver, spleen and kidney) containing the above insecticide, 10 g ammonium sulphate were dissolved and individually minced in an aqueous solution. Each sample was extracted in a separating funnel with 150 ml ethyl acetate, shaking the funnel for 2–3 min. The ethyl acetate extract was transferred into an evaporating dish and the aqueous phase re-extract two to three times with 50 ml of ethyl acetate. The extracts were combined and the solvent evaporated at room temperature. The residue is then dissolved in 1–2 ml ethanol. A known volume (10 μl) of the solution was spotted on an activated TLC plate together with the standard solution of insecticide. The plate was then developed as described under 'Procedure' and sprayed with sodium hydroxide solution followed by ice-cold diazotized sulphanilamide or sulphanilic acid reagent.

Recovery experiment

A 10 mg sample of monocrotophos in ethanol was added to 50 g of minced visceral tissue, mixed well and kept for a day. The insecticides were then extracted with ethyl acetate as described under 'Extraction', the solvent was evaporated at room temperature and the residue dissolved in 10 ml ethanol. A 10 μl volume of this solution was spotted on an activated thin-layer plate together with 10 μl each of standard technical monocrotophos solutions containing known concentrations, 6, 7, 8, 9, 10, 11 and 12 mg per 10 ml, in ethanol. The plate was then developed as described earlier and sprayed with 20% w/v sodium hydroxide solution followed by ice-cold diazotized sulphanilamide or sulphanilic acid reagent. The intensity of the red spot developed for the visceral extract was visually compared with those of 9 mg per 10 ml (average of three experiments). Hence the recovery was *ca.* 90%.

RESULTS AND DISCUSSION

On alkaline hydrolysis, monocrotophos yields *N*-methylacetoacetamide. The α-hydrogens of *N*-methylacetoacetamide are located alpha to two carbonyl groups, and hence increases the reactivity of hydrogens in a methylene group (—CH_2—). This active methylene group, further, coupled with diazotized sulphanilamide or sulphanilic acid in alkaline media to give a red coloured species (Fig. 1). The intense red spots remain stable, in alkaline media, for >30 min. Thus, to keep the media alkaline the lateral reagent should be sprayed so as just to obtain the red spot. If the developed red spot fades immediately due to heavy acidic spray of diazotized sulphanilamide or sulphanilic acid, it should be sprayed again with sodium hydroxide solution on the same plate to make the media alkaline and to stabilize the red spot. The limit of detection of the reagent was *ca.* 1 μg per spot observed after development.

This diazotized sulphanilamide or sulphanilic acid reagent also coupled with phenolic compounds (phenol, *o*-cresol, *m*-cresal, 1-naphthol, 2-naphthol, *etc.*) even without preceding alkaline hydrolysis. In alkaline medium the reactivity of the reagent increased with an increase in colour intensity which consequently increased the sensitivity of detection. The reagent also coupled with hydrolysed product of propoxur (baygon), carbofuran and carbaryl from carbamate insecticides. These carbamate insecticides on alkaline hydrolysis yields the respective phenolic compounds which further react with diazotized sulphanilamide or sulphanilic acid, giving orange-red and pink-violet coloured spots. The sensitivity of detection for these carbamate insecticides and phenolic compounds was *ca.* 1 μg per spot, observed after development. The colour of the spots of phenolic compounds, carbamate insecticides and monocrotophos, before and after hydrolysis, with their R_F values are given in Table 1, in two different solvent systems. However, monocrotophos can be differentiated from the above phenolic compounds and carbamet insecticides by a mixed spotting technique, in which monocrotophos with mixtures of the above interfering components were spotted on TLC plate along with individual standards. The plate

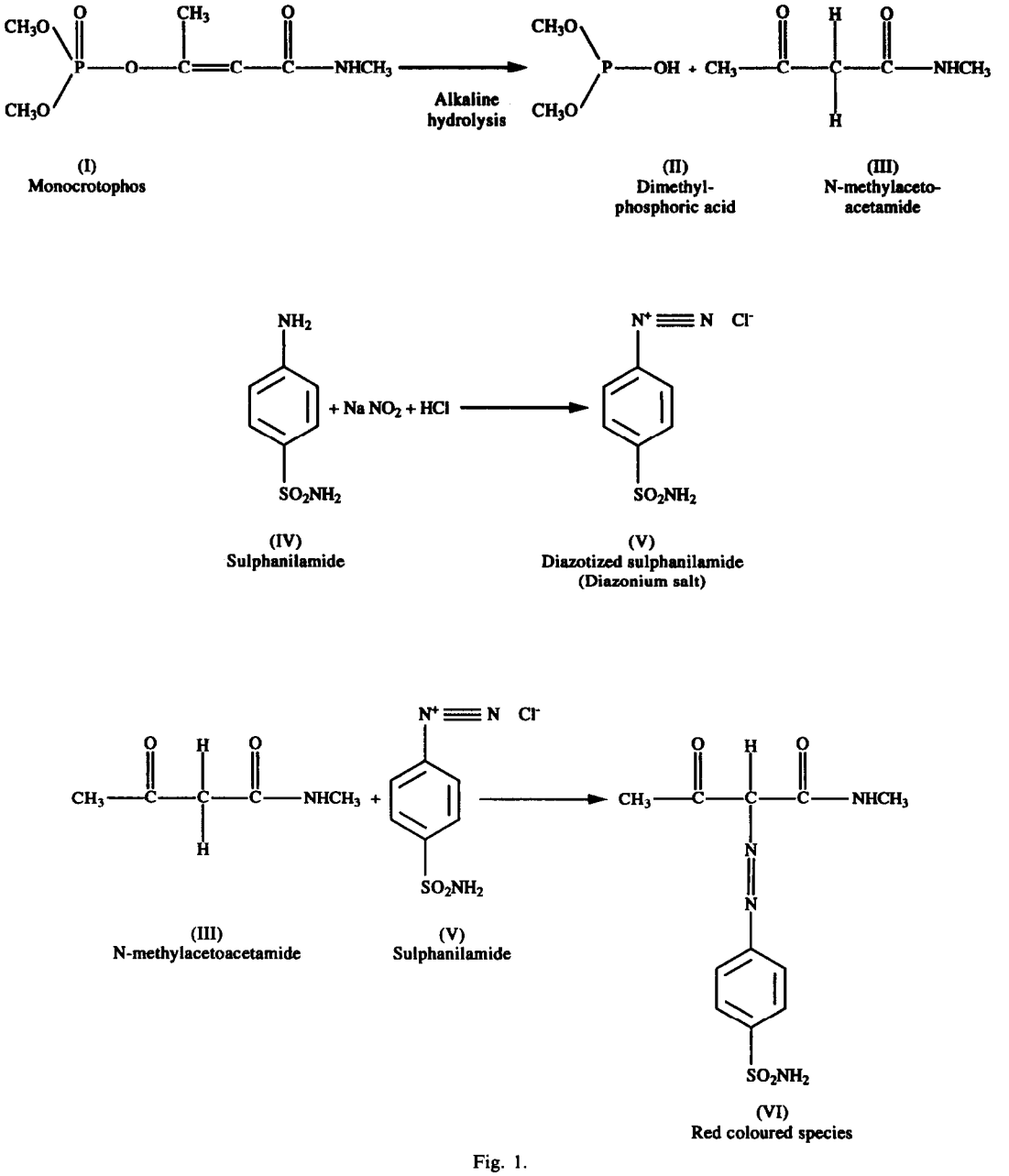

Fig. 1.

was then developed in a previously saturated TLC chamber using benzene: acetone (9:1) as solvent up to a height of 10 cm. The air-dried plate was then sprayed with the reagents as described under 'Procedure'. From the R_F values and colour of the spot developed for monocrotophos and other interfering components: (Table 1) monocrotophos can be detected and differentiated from other interfering components.

The reported reagent was selective for monocrotophos, within the organophosphorus group of insecticides. Other organophosphorus insecticides such as malathion, parathion-ethyl, parathion-methyl quinalphos, phorate, fenthion, fenitrothion, thiometon, phosphamidon, dichlorvos, and trichlorfon; organochlorine insecticides such as endrin, aldrin, dialdrin, endosulfan, DDT and BHC, and pyrethroid insecticides such as fenvalerate, cypermethrin and deltamethrin did not give coloured spots.

Since the various chromogenic reagents described for the screening of organophosphorus insecticides in biological materials by TLC failed to give a coloured reaction with monocrotophos, and since there is a need to screen

Table 1. The colour of the spot before and after alkaline hydrolysis and R_F values of monocrotophos, some phenolic compounds and carbamate insecticides in two solvent systems

Compound	Colour of the spot		R_F value in solvent system	
	Before hydrolysis	After hydrolysis	Benzene:acetone (9:1)	Chloroform:acetone (7:3)
Monocrotophos	—	Red	0.10	0.45
Propoxur	—	Orange	0.57	0.82
Carbaryl	—	Violet	0.55	0.82
Carbofuran	—	Orange	0.52	0.82
Phenol	Yellow	Dark yellow	0.57	0.91
o-Cresol	Yellow	Red	0.71	0.94
m-Cresol	Red	Orange	0.73	0.94
p-Cresol	Yellow	Red	0.71	0.94
1-Naphthol	Pink-violet	Violet	0.66	0.82
2-Naphthol	Orange	Reddish-brown	0.50	0.82

biological samples for the presence of monocrotophos in forensic toxicology, the reagent described here is very sensitive and can be routinely used for this purpose.

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