New Spray Reagents for the Detection of Thiophosphate Insecticides on Paper Chromatograms

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The use of Metanil Yellow, Yellow RFS, and methyl orange as reagents for the instantaneous location of some thiophosphate insecticides on paper chromatograms is described.

THE CONVERSION of organophosphates to inorganic phosphates by oxidation and then to molybdenum blue (*4, 6*) often leaves the paper fragile, and the procedure is cumbersome. Spraying the paper with *N*-bromosuccinimide and fluorescein for the detection of sulphur-containing phosphate esters (*1, 7*) requires careful adjustment of reagent concentration, two successive sprays, and viewing under ultraviolet light. In the method using 2, 6-dibromo-*N*-chloro-*p*-quinoneimine in cyclohexane as the spray reagent (*7*), only with subsequent heating of the paper are the spots revealed. Iodine azide has been used in the detection of thiophosphoric esters (*3*), and a method exists for the visual location of in vitro inhibitors in situ on the chromatogram (*2*). Recently, MacRae and McKinley (*5*) reported a method in which the paper chromatogram was first exposed to bromine vapor, and then sprayed successively with ferric chloride and salicylsulphonic acid solutions. In this method, two sprays are required.

In a search for a single spray reagent that did not involve any subsequent heating, several dyes were investigated. It was found that if, after exposure to bromine vapor, the paper was sprayed with Metanil Yellow [sodium salt of 4-(3-sulphophenylazo) diphenylamine], Yellow RFS [disodium salt of 4-sulpho-4′-(sulphomethylamino) azobenzene], or methyl orange, colored spots appeared immediately.

Procedure

After the chromatogram is developed, the paper is thoroughly dried by letting it stand at room temperature (about 30° C.). The paper is then exposed to bromine vapor for about 30 seconds. Exposure to bromine vapor emanating from carbon tetrachloride saturated with bromine in a dessicator (*5*) was found to be satisfactory. The paper is then left standing in the air for a few minutes to remove excess bromine in the paper. This step is important for excessive residual bromine in the paper will result, when subsequently sprayed, in a heavy background color obscuring the spots (Table I). The paper is then sprayed with any one of the three sprays containing 0.5% of the substance in 50% ethyl alcohol.

Results

If, after spraying and drying the chromatograms, they are kept between sheets of filter paper, away from light, the spots persist for a few days; otherwise, they tend to fade away overnight.

Discussion

All chromatograms were eluted by the aqueous solvent system of Mitchell (*8*)—immobile phase, 10% heavy mineral oil; mobile phase, 50% aqueous solution of dimethylformamide. The Metanil Yellow spray is the most superior of the three sprays. It gives good clarity of spots, and the contrast in color between spot and background is the best. DDVP (dimethyl 2,2-dichlorovinyl phosphate), a phosphate insecticide, could not be detected by the above technique. All locally available thiophosphate insecticides—parathion, malathion, diazinon, and Rogor [*O,O*-dimethyl *S*(*N*-methylcarbamoylmethyl)phosphorodithioate]—were found to give a positive reaction. The limit of detection is about 1 to 2 μg.

Table I. Colors Resulting from Spray Reagents

Spray Reagent	Color of Spot	Background Color
Metanil Yellow	Purple	Orange-yellow
Yellow RFS	Red	Yellow
Methyl orange	Pink	Yellow

The above method has the advantage over previous methods in that only one spray is needed and spots appear immediately after spraying.

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