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## Note

# Separation and detection of thiofanox and its metabolites by thin-layer chromatography

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Thiofanox (P), 3,3-dimethyl-1-(methylthio)-2-butanone O-[(methylamino)-carbonyl]oxime, is a systemic and contact carbamate insecticide effective in the control of cotton aphid, spider mites, serpentine leafminer, and many other leaf damage insects[1]. The pathway of degradation of thiofanox in plants, soil and water occurs by rapid oxidation to the sulfoxide derivative ($P_1$), {3,3-dimethyl-1-(methylsulfinyl)-2-butanone O-[(methylamino)carbonyl]oxime}, which is subsequently oxidized to the sulfone form ($P_2$), {3,3-dimethyl-1-(methylsulfonyl)-2-butanone O-[(methylamino)-carbonyl]oxime}[2–5]. In this paper an effective method for the separation and detection of P and its metabolites by thin-layer chromatography (TLC) is presented.

## EXPERIMENTAL

### *Materials*

Separate standards of P, $P_1$, $P_2$, O [3,3-dimethyl-1-(methylthio)-2-butanone oxime], $O_1$ [3,3-dimethyl-1-(methylsulfinyl)-2-butanone oxime], and $O_2$ [3,3-dimethyl-1-(methylsulfonyl)-2-butanone oxime] (Table I) were prepared as 1 $\mu$g/$\mu$l benzene solutions.

The TLC plates were obtained from Brinkman as silica gel 60/Kieselguhr pre-coated on aluminum. The plates were of the fast-running type with fluorescent indicators.

The three solvent systems effective in the separation of thiofanox and its degradation products were as follows: (A) hexane–acetone (7:3), (B) hexane–acetone (1:1), and (C) diethyl ether–ethyl acetate (1:1).

The chloroplatinic spray reagent was prepared by dissolving 0.06 g potassium iodide and 0.003 g chloroplatinic acid in 50 ml ethanol. A 0.5-ml portion of 2 *N* hydrochloric acid was added and the entire solution diluted to 100 ml with ethanol.

The basic permanganate spray reagent was prepared by combining 5 ml 20% sodium hydroxide, 5 ml 5% potassium permanganate, and 90 ml distilled water.

### *Procedure*

Separate standards of thiofanox and its degradation products corresponding to 30 $\mu$g each were applied as 3-mm spots to the bottom of three silica gel TLC plates.

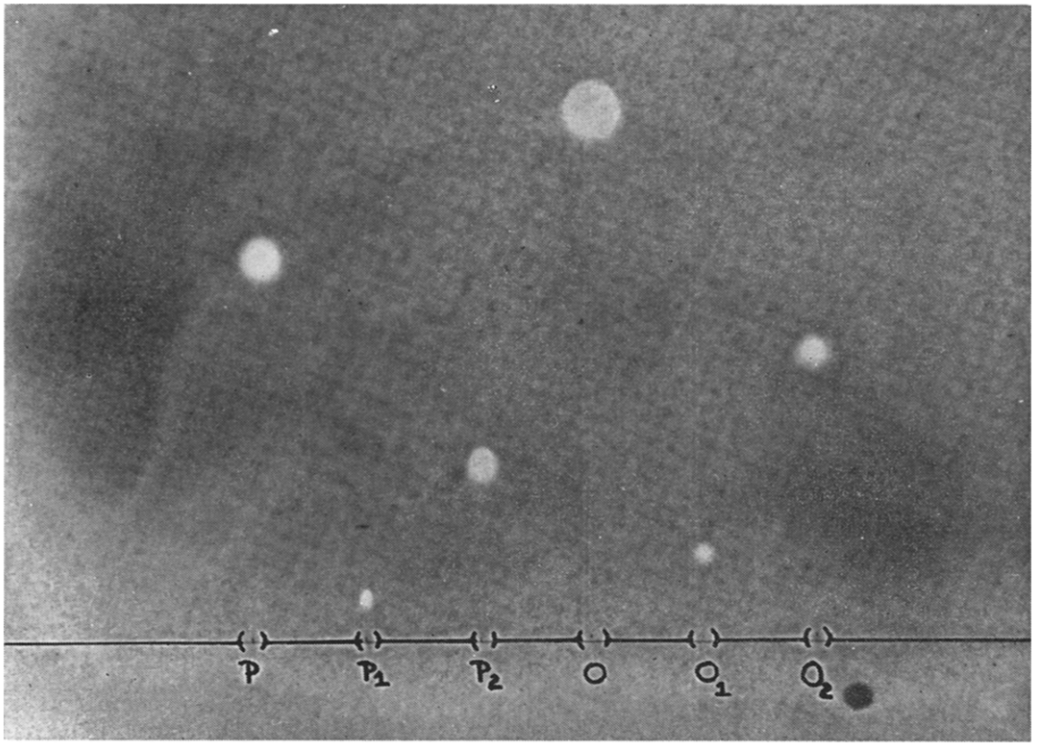



Fig. 1. Thin-layer chromatographic separation of thiofanox (P) and its metabolites on silica gel. Eluent, hexane–acetone (7:3); migration distance, 15 cm; visualization reagent, basic permanganate spray.

The plates were developed by ascending chromatography in solvent systems A, B, or C, respectively, to a distance of 15 cm from the origin.

After removal from the developing chamber and evaporation of solvent, the plates were lightly sprayed with the basic permanganate spray reagent. The standards were visualized as yellow spots on a pink-purple background. The $R_F$ values were determined for each standard in the respective solvent systems, as shown in Fig. 1.

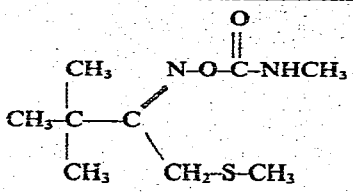
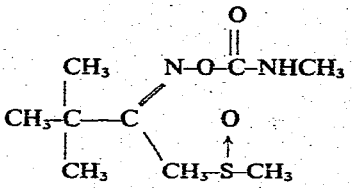
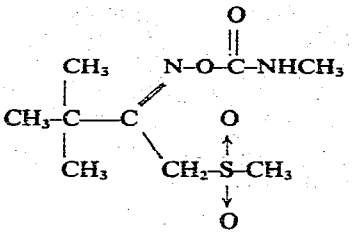
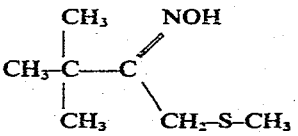
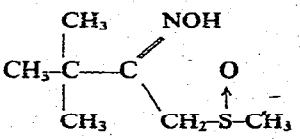
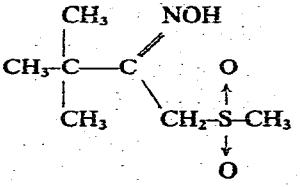
The sensitivity of the spray reagent was determined by applying each of the standards in quantities ranging from 1 to 30 $\mu$g to three thin layer chromatographic plates. Development of all plates was accomplished in solvent system A. One plate was exposed to iodine vapors which resulted in the detection of the standards as brown spots on a light yellow background. The second plate was sprayed with the chloroplatinic acid reagent to visualize the standard as white spots on a tan background. The third plate was treated with the basic permanganate spray reagent.

## RESULTS

From the $R_F$ data presented in Table I, it is evident that the best overall separation of thiofanox and its metabolites was obtained using solvent system A. Development in system B resulted in greater separation near the origin and may be used for

TABLE I

TLC $R_F$ VALUES OF THIOFANOX (P) AND ITS MAJOR METABOLITES ON SILICA GEL IN THREE SOLVENT SYSTEMS

| *Symbol* | *Chemical name* | *Structure* | *Solvent system* | | |
|---|---|---|---|---|---|
| | | | *A* | *B* | *C* |
| P | 3,3-Dimethyl-1-(methylthio)-2-butanone-O-[(methylamino)-carbonyl]oxime |  | 0.56 | 0.86 | 0.76 |
| $P_1$ | 3,3-Dimethyl-1-(methylsulfinyl)-2-butanone-O-[(methylamino)-carbonyl]oxime |  | 0.07 | 0.24 | 0.07 |
| $P_2$ | 3,3-Dimethyl-1-(methylsulfonyl)-2-butanone-O-[(methylamino)carbonyl]oxime |  | 0.29 | 0.66 | 0.54 |
| O | 3,3-Dimethyl-1-(methylthio)-2-butanone oxime |  | 0.75 | 0.91 | 0.88 |
| $O_1$ | 3,3-Dimethyl-1-(methylsulfinyl)-2-butanone oxime |  | 0.17 | 0.41 | 0.24 |
| $O_2$ | 3,3-Dimethyl-1-(methylsulfonyl)-2-butanone oxime |  | 0.42 | 0.80 | 0.78 |

purification of the more polar metabolites of thiofanox. Solvent system C gave poor separation of thiofanox and its $O_2$ metabolite.

The basic permanganate spray reagent was found to be the most sensitive of the three visualization reagents investigated (Table II). As little as 1 $\mu$g each of thiofanox and its $P_1$, $P_2$, and $O_2$ metabolites was detected.

TABLE II

SENSITIVITY OF THIOFANOX AND ITS METABOLITES TO DETECTION BY THREE VISUALIZATION REAGENTS

Abbreviations: ND = no detection; W = weak; G = good; S = strong.

| *Compound* | *Quantity (μg)* | *Visualization reagent* | | |
|---|---|---|---|---|
| | | *Basic permanganate reagent* | *Chloroplatinic acid reagent* | *Iodine vapors* |
| P | 1 | W | — | — |
| | 3 | G | — | — |
| | 5 | G | — | — |
| | 10 | S | G | S |
| | 20 | S | G | S |
| | 30 | S | G | S |
| $P_1$ | 1 | G | — | — |
| | 3 | S | — | — |
| | 5 | S | — | — |
| | 10 | G | G | ND |
| | 20 | G | G | ND |
| | 30 | G | G | ND |
| | 50 | — | — | G |
| $P_2$ | 1 | W | — | — |
| | 3 | W | — | — |
| | 5 | G | — | — |
| | 10 | G | ND | ND |
| | 20 | G | ND | ND |
| | 30 | G | ND | ND |
| | 50 | — | — | G |
| O | 10 | S | S | S |
| | 20 | S | S | S |
| | 30 | S | S | S |
| $O_1$ | 10 | G | G | W |
| | 20 | G | G | W |
| | 30 | G | G | W |
| $O_2$ | 1 | W | — | — |
| | 3 | G | — | — |
| | 5 | S | — | — |
| | 10 | S | ND | ND |
| | 20 | S | ND | ND |
| | 30 | S | ND | ND |
| | 50 | — | — | G |

Iodine was much less sensitive, especially in detecting $P_1$, $P_2$, or $O_2$. A minimum of 50 μg of each of these compounds was required for visualization.

The $P_2$ and $O_2$ metabolites were not detected at less than 30 μg by the chloroplatinic acid reagent. However, good visualization of the other standard compounds tested was observed at the 10-μg level.

It may be concluded, therefore, that rapid separation and sensitive detection of thiofanox and its metabolites by TLC may be achieved by using solvent system A and the basic permanganate spray.

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