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Note

Separation and detection of Propamocarb by thin-layer chromatography*

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Propamocarb, propyl-(3-dimethylaminopropyl) carbamate monohydrochloride, $[(CH_3)_2N–CH_2–CH_2–CH_2–NH–CO–O–C_3H_7 \cdot HCl]$, is a synthetic organic product belonging to the dialkylcarbamate class, recently introduced by Schering (Berlin/Bergkamen, G.F.R.) under code SN 66752. Its action characteristics make it particularly interesting for the control of horticultural and ornamental plant diseases caused by *Oomycetes*.

As with other carbamate substances, Propamocarb suffers from some analytical problems associated with molecular instability. Generally, the analytical procedures applicable are limited and spectrophotometry, gas chromatography and other techniques are not sufficiently responsive and/or specific in all instances. High-performance liquid chromatography (HPLC) seems to be more promising but, in absence of such sophisticated facilities, we have attempted to devise a thin-layer chromatographic (TLC) method that is simple, rapid and sufficiently sensitive for application to low Propamocarb levels. It is our intention to use this technique for the detection and determination of Propamocarb in plant tissues.

EXPERIMENTAL

Silica gel 60G plates (Merck), 200 μm thick, were used after heating in an oven at 110°C for 20 min. Six 3-cm wide tracks were marked on each plate and 100-μl volumes of different concentrations of the active ingredient (94% standard for analysis, supplied by courtesy of Schering) were deposited on the starting line at the centre of each track.

The plates were developed in a saturated chamber using as mobile phase the series of solvent systems listed in Table I. After development, the chromatograms were dried in air.

The active ingredient was located using the detection reagents and methods cited in Table II.

In detection method No. 6 a fluorescent TLC plate, obtained by adding 0.04% sodium fluoresceinate solution to the silica gel 60G, loses its fluorescence in the presence of the carbamate following exposure to bromine vapour. Because during

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development of the plate in the chosen solvent the sodium fluoresceinate is carried along by the solvent, thus making any further treatment pointless, we modified the method as follows: the sodium fluoresceinate solution (1 ml, 0.04%) was added to the mobile phase and, after development, the plates were dried in air, sprayed with 20% aluminium sulphate solution and exposed to bromine vapour. When viewed under ultraviolet light in these conditions, the compound appears as a blue spot against a yellow-green background.

The quantitation of Propamocarb on the chromatograms was accomplished in two ways:

(1) by measuring the absorbance of the coloured spots with a Quick Scan R & D densitometer (Helena Laboratories) at 420 nm;

(2) by using the linear relationship between the logarithm of the weight of compound in the spot and the square root of the spot area and then calculating the amount of product present by reference to a calibration graph (Fig. 1).

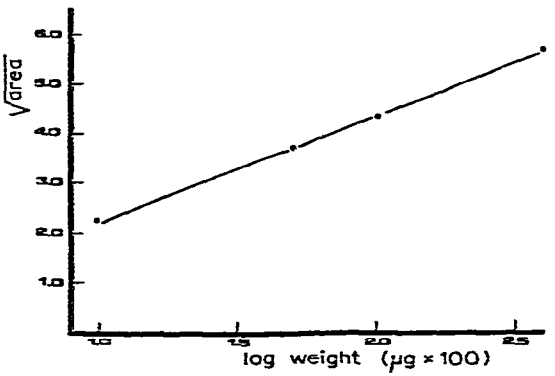

Fig. 1. Spot area *versus* weight relationship for Propamocarb.

Recovery procedure for vegetable samples

Pepper plant samples (20 g), to which 0.5–2.5 ml of 0.1% Propamocarb monohydrochloride solution and 40 ml of 0.1 *N* hydrochloric acid were added, were ground in a Sorvall Omnimixer (J. Norwack, U.S.A.) for about 2 min. The slurry thus obtained was immediately filtered through cheese-cloth and the sample tissue was re-extracted in the same way. The combined filtrates were centrifuged at 14,000 *g* for 10 min and the acidic supernatant was transferred into a separating funnel and made alkaline with 10 *N* sodium hydroxide solution. Finally, the Propamocarb was extracted twice from this basic solution using 20-ml volumes of chloroform. The organic phase, concentrated to 10 ml in a rotary vacuum evaporator at 40°C, was used for analysis.

RESULTS AND DISCUSSION

The best solvent system among those tried for the separation of Propamocarb was methanol–25% ammonia solution (30:1); the development time was about 45 min and the R_F value was 0.2 (Table I).

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF PROPAMOCARB USING VARIOUS SOLVENT SYSTEMS

Solvent system	*Development time (min)*	$R_F \times 100$
Methanol	59	7
Methanol–water (1:1)	125	14
Methanol–water (7:3)	105	13
Methanol–water (9:1)	75	7
Water–formic acid–methanol (4:1:5)	126	1
Water–acetone (6:4)	122	35
Methanol–25% ammonia solution (30:1)	45	16
Acetone	26	1
Methanol–diisopropyl ether (1:1)	68	4
Methanol–diisopropyl ether (1:4)	65	3
Hexane–acetone (9:1)	33	0
Hexane–acetone (7:3)	30	0
Chloroform	57	0
Chloroform–acetone (7:3)	47	0
Chloroform–acetone (9:1)	48	0
Diethyl ether	35	0

With the other solvents, either the development time was too long, the spots had tails and poor shapes or the R_F values were either too high or too low. The solvent system chosen was excellent even for the separation of the active ingredient from vegetable tissues as it avoids interferences resulting from co-extracted pigments, which have very different R_F values.

Regarding the detection of Propamocarb on the chromatograms, many of the reagents used gave negative results or showed poor sensitivity (Table II) for the product sought. Reagents 8 and 9 are highly sensitive, although not specific, with a

TABLE II

DETECTION OF PROPAMOCARB WITH VARIOUS REAGENTS

No.	*Method of detection*	*Minimal determinable amount (μg)*	*Colour*	*Colour stability (min)*
1	Rhodamine B–UV[1]	0.0	–	–
2	*p*-Dimethylaminobenzaldehyde[2]	0.0	–	–
3	Potassium permanganate[3]	0.0	–	–
4	Silver nitrate + bromophenol blue[4]	100	Blue against yellow background	>60
5	Pinacriptol yellow–UV[1]	10	Blue against fluorescent background	>60
6	Sodium fluoresceinate–UV[5]	10	Black against blue background	>60
7	Sodium fluoresceinate–UV (modified method)	5	Blue against yellow-green background	>60
8	Iodine vapour	0.01	Brown	<5
9	Iodine spray	0.01	Brown	<5

minimal determinable amount of 0.01 μg, but their action is reversible and as their coloration disappears in a few minutes the subsequent quantitative determination is difficult. Reagent 7, although not very sensitive (minimal determinable amount 5 μg), results in persistent coloration and hence allows subsequent determination without difficulty.

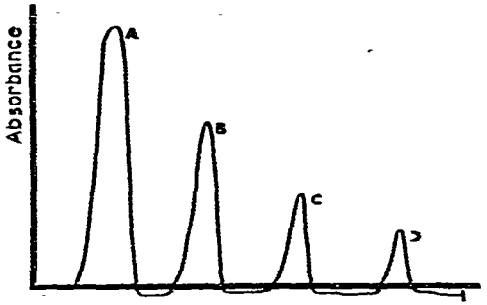

Fig. 2. Densitometric measurements after detection with iodine vapour (λ = 420 nm). Each peak represents a spot of propamocarb: A = 50 μg; B = 25 μg; C = 10 μg; D = 5 μg.

The densitometric analysis (Fig. 2) provided results that are comparable to those obtained by applying the linear relationship between the square root of the spot area and the weight of compound present in the spot.

By applying the procedure described above, the separation, detection and determination of the active ingredient in the vegetable tissue were achieved. The mean recovery (24 determinations) was 95.75% with a standard deviation of 2.25%.

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