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DETECTION OF ORGANOPHOSPHOROUS PESTICIDES BY *IN SITU* FLUOROMETRY ON THIN-LAYER CHROMATOGRAMS

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SUMMARY

A method for the detection of organophosphorous pesticides directly on thin-layer chromatograms is described. One approach involves heating the chromatogram for a definite period of time at a specific temperature. The other requires spraying the chromatoplate with a strong acid or base prior to the heat-treatment. From a total of thirty-five organophosphorous pesticides investigated, twelve gave positive results. Instrumental limits of detection at the nanogram level are possible.

INTRODUCTION

A new technique for rendering certain organophosphorous pesticides fluorescent on thin-layer chromatograms was described in a previous paper[1]. The pesticide was spotted on Silica Gel H layers and after development the chromatogram was simply heated at an optimum temperature for a definite period of time. The effect of a strong base and heat was also investigated (see ref. 1). The results were that fluorescence was improved in a few cases and produced with pesticides that were non-fluorescent. Because fluorescence was produced without the use of fluorogenic spray reagents, background fluorescence was practically non-existant. Another advantage was added selectivity introduced from the different excitation and emission wavelengths obtained by varying the experimental conditions. The technique was found especially important with certain compounds such as menazon and coumaphos which are difficult to detect by other means[2]. It afforded an excellent sensitivity and limits of detection in the nanogram range were observed.

Acids and bases have been used before on thin-layer chromatograms, but not quite for the same purpose or under similar experimental conditions. For instance, Askew *et al.*[3] used hydroiodic acid for the detection of pesticides after thin-layer chromatography (TLC). The reaction involved hydrolysis on the thin layer, with formation of specific or semi-specific colours by the breakdown products which were used as an aid in the characterization of the parent pesticide. Carbamate, thiocarbamate, substituted ureas and some nitrogen-containing organophosphorous

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pesticides were detected after hydrolysis to amines, with a detectability at the microgram level.

Frei *et al.*[4] also described an *in situ* fluorometric method for the determination of the insecticidal carbamate Sevin and its hydrolysis product, α-naphthol. Sevin was converted to α-naphthol on the chromatogram by hydrolysis with aqueous NaOH. Visual limits of detection of 0.006 μg per spot were reported.

In this study, it was intended to produce fluorescence on thin-layer chromatograms for a larger number of organophosphorous pesticides than had been done previously[1]. This was accomplished by the heat-treatment alone and also by first spraying the plate with an acid or a base prior to heating at an optimum temperature for a definite period of time.

EXPERIMENTAL

Chemicals and apparatus

The pesticides used in this study were analytical standards except for azinphosmethyl and Maretin which were purified by multiple recrystallizations from ethanol. Structures and chemical names were obtained from Kenaga and Allison[5].

All the solvents used were either spectranalysed or pesticide grade (Fisher Scientific Co., Montreal, Canada). Binder-free Silica Gel H and Silica Gel 60 precoated TLC plates were obtained from Brinkmann Instruments (Rexdale, Ontario, Canada).

The fluorescence spectra were recorded with a Farrand VIS-UV Chromatogram Analyzer (Farrand Optical Co., Inc., New York, U.S.A.) equipped with motorized monochromators. For quantitative studies a 1-P28 photomultiplier detector tube was used. The exciter drawer was fitted with a No. 7–54 filter and an attenuator of 0.625 in. while a No. 3–73 filter and a 0.500-in. attenuator were put in the analyzer leg. For visual observation of the fluorescence a Chromato-Vue Cabinet (Canlab Supplies Ltd., Montreal, Canada) with long wavelength (366 nm) ultraviolet light was utilized.

General procedure

TLC plates (20 × 20 cm) were coated 250 μ thick with a Desaga applicator from a suspension of 30 g of Silica Gel H in 80 ml of distilled water. Aqueous solutions (1.0 *N*) of NaOH, KOH, and H_2SO_4 were prepared in the usual way. Stock solutions of 1000 p.p.m. of each pesticide except menazon which was dissolved in ethanol, were prepared in methylene chloride. Dilution series were made with *n*-hexane.

For chromatographic separation the pesticide was spotted 2 cm from the bottom of the plate by means of a 1-μl micropipet. Elution of the plate was carried out at least 10 cm with a solvent system consisting of *n*-hexane–acetone (7:2) or (5:1). The plate was dried over a cold air-stream and when necessary, sprayed with the appropriate reagent by means of a chromatography spray gun (Brinkmann Inst.). The plates were then heated at various temperatures: 50, 75, 100, 125, 150, 175, 200, 225, and 250° for varying periods of time; 10, 20, 30, 45, 60, and 120 min in order to establish the optimum conditions for maximum fluorescence.

Once the optimum conditions were known the fluorescence spectra were recorded in the usual manner[1].

Instrumental detection limits

For a particular fluorescent compound the excitation and emission monochromators of the VIS-UV Chromatogram Analyzer are set at the wavelengths of maximum excitation and emission, respectively. The xenon lamp must be properly adjusted until a maximum deflection on the recorder is obtained. The instrument fluorescence gain is set at maximum sensitivity and the plate is scanned at a speed of 1.0 in./min. The recorder speed is kept at 4 in./min. Since the baseline of the recorder is always very stable even at the highest gain of the fluorometer, the lowest limit of detection was taken as a deflection of a least 1 cm. Silica Gel 60 chromatoplates were used for quantitative purposes.

RESULTS AND DISCUSSION

A list of the organophosphorous pesticides studied, which gave positive results either by the heating or acid–base and heating techniques, is given in Table I. They are generally classified as either aryl or heterocyclic organophosphorous derivatives. Other pesticides studied which, however, gave negative results included: ABATE, Chi 4133, Cyanox, CYOLANE, CYTROLANE, Delnav, Diazinon, dicapthon, Dyfonate, fenthion, Folithion, methyl parathion, methyl trithion, parathion, R-2596, ronnel, Ruelene, Salithion, Surecide, Torak, Trithion, WARBEX, and Zytron (see ref. 5).

Some organophosphorous pesticides are naturally fluorescent on thin-layer chromatograms. Such an example is MARETIN[1] whose structure is derived from a highly conjugated naphthalimide moiety. Other non-fluorescent compounds can be rendered fluorescent simply by heating[1], which produces fluorescence probably through decomposition or rearrangement of the original molecule into a more stable or more conjugated species. A few additional examples are given in Table II. It seems odd at first glance that the spectral data are very similar for Fospirate and Noltran. A close look at the structures (see Table I), however, indicates that both compounds are almost identical.

As was mentioned earlier, fluorescence of the pesticide can sometimes be obtained by spraying the chromatogram with a strong acid[3] or base[4]. For example, Bayrusil becomes fluorescent upon spraying with aqueous NaOH or KOH (Table III) followed by heating. The same spectral data are obtained with both bases except that the temperature is 100° less with aqueous KOH. These data are very similar to those obtained by the heating technique alone (see Table II). By spraying the pesticide with aqueous H_2SO_4 and heating, a tremendous shift in fluorescence emission is produced from a blue spot at 440 nm (with aqueous base) to a yellowish green spot at 510 nm.

Certain difficulties were encountered with coumaphos because of the presence to the extent of 3–5% of a secondary product named Potasan in the technical-grade material. The only difference between the two compounds is the absence of the 3-chloro substituent in Potasan. For that reason, purification is difficult to achieve and even the analytical-grade material contains a substantial quantity of Potasan. Both compounds are fluorescent on thin layers but coumaphos has an R_F value somewhat greater than that of Potasan. However, because Potasan has insecticidal properties and could be one of the degradation products of coumaphos, it is important not to confuse the two on the chromatographic plate.

Fospirate does not show a large shift in fluorescence maxima upon treatment

TABLE I

ORGANOPHOSPHOROUS PESTICIDES GIVING POSITIVE RESULTS

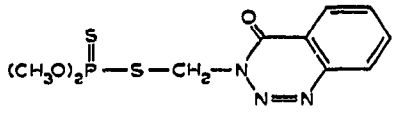
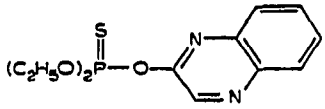
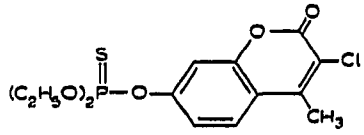
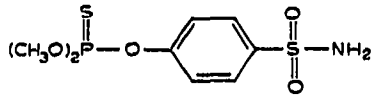
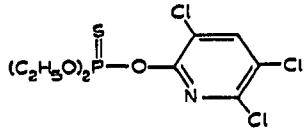
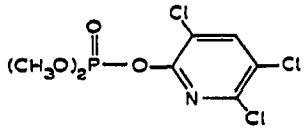
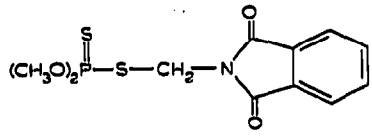
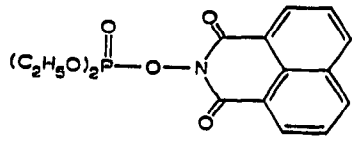
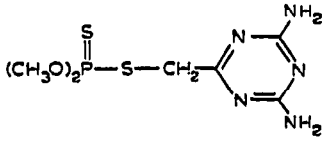
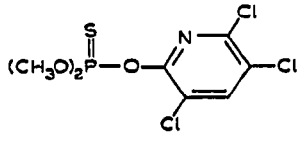
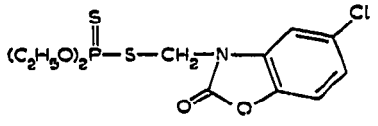
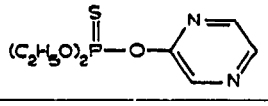
Common or trade name (manufacturer)	*Chemical name*	*Structure*
azinphosmethyl (Chemagro Corp.)	O,O-Dimethyl S-(4-oxo-1,2,3-benzotriazin-3 (4H)-ylmethyl) phosphorodithioate	
Bayrusil (Bayer)	O,O-Diethyl O-(2-quinoxalyl) phosphorothioate	
coumaphos (Chemagro Corp.)	O-(3-Chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl) O,O-diethyl phosphorothioate	
cythioate (American Cyan)	O,O-Dimethyl O-*p*-sulfamoylphenyl phosphorothioate	
Dursban (Dow Company)	O,O-Diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	
Fospirate (Dow Company)	Dimethyl 3,5,6-trichloro-2-pyridyl phosphate	
Imidan (Stauffer)	O,O-Dimethyl S-phthalimidomethyl phosphorodithioate	
MARETIN (Chemagro Corp.)	N-Hydroxynaphthalimide diethyl phosphate	

TABLE I *(continued)*

Common or trade name (manufacturer)	*Chemical name*	*Structure*
menazon (Imperial)	S-[(4,6-Diamino-*s*-triazin-2-yl) methyl] O,O-dimethyl phosphorodithioate	
Noltran (Dow Company)	dimethyl 3,5,6-trichloro-2-pyridyl thiophosphate	
phosalone (Rhodia)	S-[(6-Chloro-2-oxo-3-benzoxazolinyl) methyl] O,O-diethyl phosphorodithioate	
ZINOPHOS (American Cyan)	O,O-Diethyl O-2-pyrazinyl phosphorothioate	

with aqueous base but Noltran does (compare Tables II and III). In general, there is only a slight change in fluorescence excitation and emission wavelengths when aqueous KOH is used instead of aqueous NaOH (see Table III) and therefore any one of the two could be chosen.

The spectral data obtained for coumaphos, Dursban, MARETIN and menazon (see Table III) upon treatment with aqueous acid or base show a bathochromic shift when compared to results obtained by the heating technique[1].

In Table IV the instrumental limits of detection obtained either by the heating technique alone or by the action of acid or base prior to heating are compared. The

TABLE II

FLUORESCENCE SPECTRAL DATA OF ORGANOPHOSPHOROUS PESTICIDES AFTER HEATING

Pesticide	*Optimum heating conditions*		*Wavelength of maximum (nm)*	
	Temp. (°C)	*Time (min)*	*Ex**	*Em***
Bayrusil	100	30	353	441
Fospirate	200	45	351	440
Noltran	200	45	351	440

* Ex = excitation.
** Em = emission.

TABLE III

FLUORESCENCE SPECTRAL DATA OF ORGANOPHOSPHOROUS PESTICIDES AFTER SPRAYING WITH AN ACID OR BASE

Pesticide	*Spray reagent*	*Optimum heating conditions*		*Spectral data (nm)*	
		Temp. (°C)	*Time (min)*	*Ex*	*Em*
Bayrusil	NaOH	200	30	356	440
Bayrusil	KOH	100	30	356	440
Bayrusil	H_2SO_4	100	30	373	510
coumaphos	NaOH	200	30	372	474
coumaphos	KOH	200	30	332	455
cythioate	NaOH	200	45	350	458
cythioate	KOH	200	45	345	450
Dursban	KOH	200	30	370	510
Fospirate	NaOH	150	45	353	439
Fospirate	KOH	150	45	357	436
MARETIN*	NaOH	200	20	370	500
MARETIN*	KOH	200	20	365	492
MARETIN*	H_2SO_4	75	20	355	440
menazon	KOH	200	30	350	495
Noltran	KOH	200	45	377	505

* The chromatoplate is covered with a glass plate while heating.

TABLE IV

INSTRUMENTAL LIMITS OF DETECTION (μg)

Pesticide	*Heating*	*Acid–base technique*		
		NaOH	*KOH*	*H_2SO_4*
azinphosmethyl	0.04	N.F.*	N.F.	N.F.
Bayrusil	0.02	0.004	0.006	0.008
coumaphos	0.001	B.F.**	B.F.	B.F.
cythioate	>1.0	0.01	0.6	N.F.
Dursban	0.06	N.F.	0.04	N.F.
Fospirate	0.2	1.0	1.0	N.F.
Imidan	>1.0	N.F.	N.F.	N.F.
MARETIN	0.004	0.002	0.006	0.001
menazon	0.009	B.F.	0.08	B.F.
Noltran	0.08	N.F.	0.1	N.F.
Phosalone***	0.02	–	–	–
ZINOPHOS***	0.08	N.F.	N.F.	N.F.

* N. F. —non-fluorescent at the 1.0-μg level.
** B.F. —barely fluorescent at the 1.0-μg level.
*** See ref. 1 for spectral data and experimental conditions.

favourable action of the acid–base technique is quite noticeable in a few cases. The intensity of the fluorescence is increased almost ten-fold in the case of Bayrusil and is 10 to 100 times better with cythioate. In other cases such as azinphosmethyl, coumaphos, Imidan, and ZINOPHOS, the action of acid or base is almost negligible.

CONCLUSION

The technique just described constitutes a more extensive study than the one previously reported[1]. From a total of thirty-five organophosphorous pesticides investigated only twelve gave positive results under various experimental conditions. The use of acid or base improved the limits of detection markedly in the case of Bayrusil, but in other cases there was either little improvement or a slight decrease in fluorescence. The importance of the acid- or base-treatment, however, was reflected more by the changes in the fluorescence excitation and emission maxima. In combining the spectral data of both techniques, a great deal more selectivity should be introduced in practice. Another advantage is that the use of acid or base does not have any effect whatsoever on the background of the plate.

It is now intended to develop quantitative analytical methods for those pesticides that gave the best fluorescence. Those are Bayrusil, coumaphos, MARETIN and menazon.

ACKNOWLEDGEMENTS

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