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Note

Fluorescence of pesticides by treatment with heat, acid or base

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A method for the detection of naturally fluorescent pesticides on silica gel layers was described in a preceding paper[1]. Benomyl, Coumatetralyl, Diphacinone, Fuberidazole, Propyl isome and Quinomethionate were investigated, the fluorescence spectra were measured and visual limits of detection were estimated. In most cases, as little as a few nanograms were detected. In addition, the effects of heat treatment on the fluorescence were observed.

In this study, a few more pesticides were investigated in addition to those already reported. It was intended to show how selectivity may be increased through the use of acid or base as spray reagent along with heat treatment of the chromatogram.

EXPERIMENTAL

For a more detailed description, see the preceding paper[1].

The Farrand VIS-UV Chromatogram Analyzer requires filters in addition to the monochromators. The combination found to be most useful was a No. 7-54 (250–390 nm, effective band pass) filter in the exciter drawer and a No. 3-73 filter (430–800 nm, effective band pass) in the analyzer leg (system A). This filter combination was found to be excellent for excitation in the region from 250 to 390 nm while emission is in the region of 435–800 nm. The second filter combination (system B) was obtained by placing two No. 7-54 filters (320–385 nm, overall effective band pass) in the exciter drawer with a No. 3-75 filter (370–800 nm, effective band pass) in the analyzer leg. This has the advantage of decreasing spectra overlap. To avoid excessive reflection peak interference, the No. 3-75 filter may also be doubled. The doubling of filters, however, cuts down on the percentage of light transmitted and consequently, may affect the detection limits. All of the above mentioned spectral filters are from Corning Glass Works (Corning, N.Y., U.S.A.).

General procedure

Aqueous solutions of H_2SO_4, HCl, HNO_3, NH_4OH, NaOH and KOH were prepared at the following concentrations: 0.1, 1.0 and 2.5 *N*. The chromatograms were sprayed until moist with the acidic or basic solution and heated at an optimum temperature for a definite time period.

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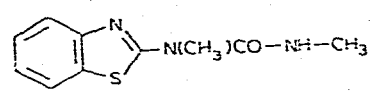
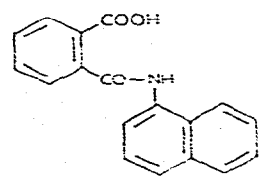
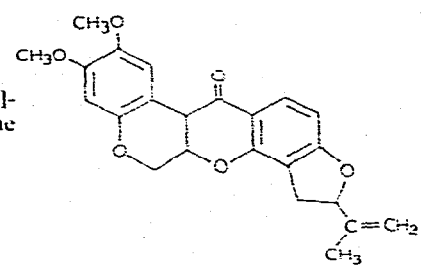
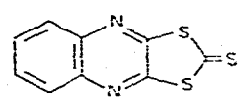
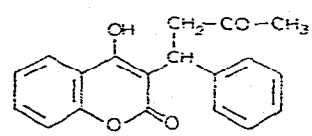
RESULTS AND DISCUSSION

The structures of the pesticides used in this study and not given earlier[1] are shown in Table I. To our knowledge, no reference is made in the literature on the *in situ* fluorimetric detection of the compounds Naptalam, Methabenzthiazuron, Rotenone, Thioquinox and Warfarin on silica gel chromatograms. However, Benomyl has been determined quantitatively by fluorescence measurements in solution[2]. Sensitivity was given as 0.1 ppm based on a 50 g sample. The other pesticides are analyzed either by UV-visible spectroscopy, *e.g.* Coumatetralyl[3], Fuberidazole[4], Rotenone[5], and Warfarin[6,7]; or by colorimetric methods, *e. g.* Naptalam[8–10], Methabenzthiazuron[11], Propyl isome[12], Quinomethionate[13,14], Thioquinox[13] and Rotenone[15]. Quinomethionate has been determined by polarography[16].

TABLE I

STRUCTURES OF SOME OF THE PESTICIDES USED

H = Herbicide; I = insecticide; F = fungicide; Ro = rodenticide.

Pesticide and manufacturer	*Chemical name*	*Structure*
Methabenzthiazuron (H) (Bayer)	1-(2′-Benzothiazolyl)-1,3-dimethylurea	
Naptalam (H) (Uniroyal)	N-1-Naphthylphthalamic acid	
Rotenone (I) (Niagara)	1,2,12,12a-Tetrahydro-2-isopropenyl-8,9-dimethoxy[1]benzopyrano[3,4-*b*]-furo[2,3-*b*][1]benzopyran-6(6a*H*)-one	
Thioquinox (F) (Chemagro)	2-Thio-1,3-dithiolo[4,5-*b*]quinoxaline	
Warfarin (Ro) (Penick)	3-(α-Acetonylbenzyl)-4-hydroxycoumarin	

Some of the results obtained in this study are given in Table II. The pesticides that were not previously studied, namely Methabenzthiazuron, Naptalam, Rotenone, Thioquinox and Warfarin, all give fluorescence upon heat treatment. With the exception of Rotenone, all are not (or very weakly) fluorescent naturally. An exceptionally good limit of detection is obtained with Methabenzthiazuron under such conditions.

TABLE II

FLUORIMETRIC RESPONSE OF SOME PESTICIDES

Pesticide	*Filter combination*	*Wavelength (nm)*		*Optimum conditions* *	*I.L.D.* **
		Excitation	*Emission*		
Coumatetralyl	B	358	450	a,c	0.01
Diphacinone	A	330	514	a,b	2.0
Fuberidazole	B	333	410	a,d	0.006
Methabenzthiazuron	B	353	439	a	0.002
Naptalam	A	361	455	a	0.08
	A	298	455	b,f	0.04
Propyl isome	A	352	472	a,d	0.008
Quinomethionate	B	357	458	a,e	0.04
	B	335	455	a,c	0.01
	B	362	417	a,d	0.06
Rotenone	A	362	453	F	0.8
	A	370	440	a	0.6
Thioquinox	B	329	441	a	0.04
	B	329	435	a,c	0.08
Warfarin	A	363	456	a	0.06

* F = Fluorescent naturally; a = heated at 200° for 45 min; b = sprayed with 2.5 *N* KOH; c = sprayed with 0.1 *N* NH_4OH; d = sprayed with 0.1 *N* HCl; e = sprayed with 0.1 *N* H_2SO_4; f = heated at 220° for 30 min.

** I.L.D. = Instrumental limit of detection.

In most cases, the spraying with a strong electrolyte, such as an acid or base, prior to the heat treatment does not increase the limit of detection markedly as compared to the heat treatment alone. However, there is sometimes a change in the spectra as shown with Naptalam and Thioquinox. The change is more drastic in other cases already mentioned[1]. For instance, Coumatetralyl has excitation and emission maxima at 330 and 415 nm naturally; Fuberidazole at 328 and 402 nm; and Propyl isome at 343 and 460 nm. It should be noticed that most of the compounds studied do not give the same combination of excitation and emission maxima.

Heat treatment has definitely an effect on organic compounds spotted on silica gel thin layers. The actual mechanisms involved are not as yet fully understood, although work in this area is presently being undertaken. It may be stipulated, however, that in some cases degradation of the initial substance takes place. It is known that Naptalam is unstable at elevated temperatures (200°), tending to form the imide[4]. Also, Propyl isome, Quinomethionate and Naptalam are hydrolysed by strong alkali[3]. Thioquinox, on the other hand, is resistant to hydrolysis[4] but susceptible to oxidation to sulfur oxides. Quinomethionate is closely related

to Thioquinox in chemical properties, but is more stable to oxidation[4]. Chemical or physical bonding of the compounds to the silica gel layers is still another factor which may very well affect fluorescence intensities. Thus, as solvent polarity and pH affect quantum efficiency in solution, the polarity, as well as the water content of the layer, are also important factors.

CONCLUSION

The ease with which TLC can be applied to pesticide residue analysis is by far the greatest asset of the technique. Coupled with the sensitivity of fluorimetric methods, a relatively inexpensive and time saving analytical method is possible. With detection limits well into the sub-microgram level, sensitivity is guaranteed. Work already done with Quinomethionate[17] gives limits of detection at the ppb level.

Alkali spray reagents were found to be most useful as fluorescence intensifiers. However, no marked difference was noted between NaOH or KOH as spray reagents. Acid causes shifts in the spectra, but, unfortunately, it is almost always followed by a decrease in fluorescence intensity. The most useful of the acidic sprays used was HCl. Nitric acid is useless since it tends to act as a quencher.

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