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THE FLUORIMETRIC DETECTION OF PESTICIDES ON ALUMINIUM OXIDE LAYERS

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SUMMARY

Several pesticides have been investigated for their fluorigenic properties on acidic and basic aluminium oxide layers. Fluorescence was obtained in several instances and the relative intensities were observed. Fluorescence spectra were recorded for the best fluorescence obtained before and after heat treatment of the chromatogram. The results are compared with those already reported for silica gel layers.

INTRODUCTION

During the last few years, a number of fluorimetric detection techniques for pesticides on thin-layer chromatograms have been reported. Many were based on the use of fluorigenic spray reagents with the spray reagent itself being primarily responsible for the fluorescence[1–3]. One technique was derived from the use of fluorigenic labelling compounds which were made to react with the pesticide in solution prior to separation on a thin-layer chromatogram. The work has been reviewed by Lawrence and Frei[4].

Another technique whereby the fluorescence originates from the pesticide has been developed by Brun *et al.*[5]. The chromatogram is heated and the pesticides appear as bright fluorescent spots. Sometimes spraying with an acid or base causes changes in the spectral characteristics[6,7]. Very recently, the detection of pesticides has been achieved by spraying the chromatoplate with inorganic reagents[8].

The development of such detection techniques has permitted the analysis of pesticide residues in a variety of substrates. For instance, Guthion has been analysed in blueberries[9] and Co-Ral and Bayrusil have been monitored in lake and sewage water[10]; a method has been reported for Maretin in milk and eggs[11] and Co-Ral has been determined in eggs[12].

Most of the above detection techniques, however, involve the use of silica gel thin-layer chromatograms; cellulose layers were used in a few instances[3]. It was intended in this study to evaluate the detection of pesticides on aluminium oxide layers with the expectation that some results could be applied eventually to residue analysis.

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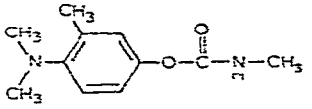
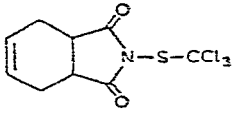
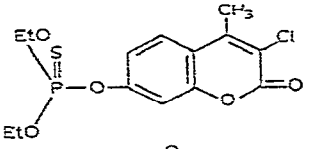
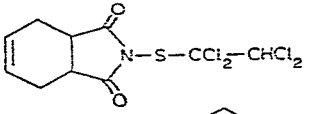
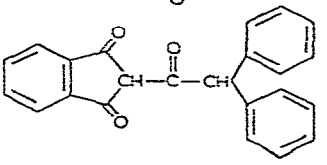
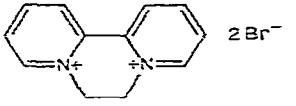
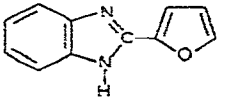
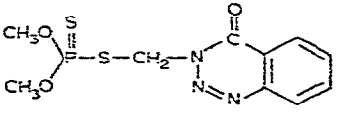
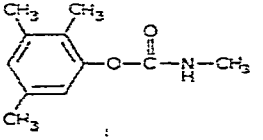
TABLE I

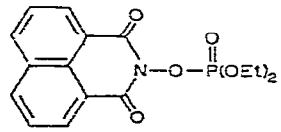
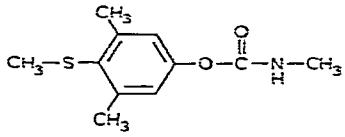
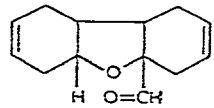
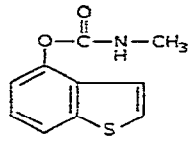
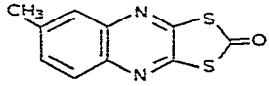
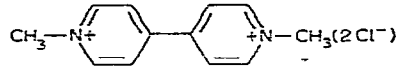
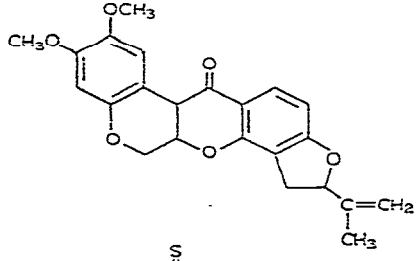
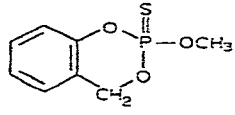
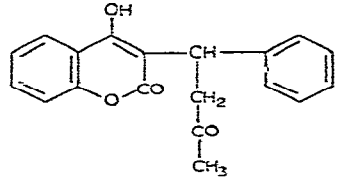
PESTICIDES THAT GIVE POSITIVE RESULTS

Abbreviations: Et = CH_3CH_2–; F = fungicide; H = herbicide; I = insecticide; M = miticide; P = parasiticide; R = repellent; Ro = rodenticide.

Pesticide	*Supplier*	*Type*	*Chemical name*	*Structure*
Aminocarb	Chemagro (Kansas City, Mo., U.S.A.)	I, M	4-(Dimethylamino)-*m*-tolyl methylcarbamate	
Captan	Chevron (San Francisco, Calif., U.S.A.)	F	N-Trichloromethylthio-4-cyclohexene 1,2-dicarboximide	
Coumaphos	Chemagro	I	O,O-Diethyl O-3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl-phosphorothioate	
Difolatan	Chevron	F	*cis*-N-[(1,1,2,2-Tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide	
Diphacinone	Velsicol Chemical Corp. (Chicago, Ill., U.S.A.)	Ro	2-Diphenylacetyl-1,3-indanedione	
Diquat	Chevron	H	6,7-Dihydrodipyrido-[1,2*a*:2′,1′-*c*]pyrazidinium dibromide	
Fuberidazole	Chemagro	F	2-(2′-Furyl)benzimidazole	
Guthion	Chemagro	I	O,O-Dimethyl S-(4-oxo-1,2,3-benzotriazin-3-[4*H*]-ylmethyl)phosphorodithioate	
Landrin	Shell Chem. Co. (New York, N.Y., U.S.A.)	I	2,3,5-Trimethylphenyl methylcarbamate	

(Continued on p. 131)

TABLE I *(continued)*

Pesticide	*Supplier*	*Type*	*Chemical name*	*Structure*
Maretin	Chemagro	P	N-Hydroxynaphthalimide diethyl phosphate	
Mesurol	Bayer (Leverkusen, G.F.R.)	I, M	4-(Methylthio)-3,5-xylylmethylcarbamate	
MGK Repellent II	MGK (McLaughlin, Gormley, King Co.) (Minneapolis, Minn., U.S.A.)	R	2,3:4,5-bis-(2-Butylene)-tetrahydro-2-furaldehyde	
Mobam	Mobil Chem. Co. (Metuchen, N.J., U.S.A.)	I	4-Benzothienyl-N--methylcarbamate	
Morestan	Chemagro	I,M,F	6-Methyl-2,3-quinoxalinediyl cyclic S,S-dithiocarbonate	
Paraquat	Chevron	H	1,1′-Dimethyl-4,4′-dipyridinium dichloride	
Rotenone	Penick (New York, N.Y., U.S.A.)	I	1,2,12,12*a*-Tetrahydro-2-isopropanyl-8,9-dimethoxybenzo[1]pyrano-[3,4-*b*]furo[2,3-*b*]benzo-[1]pyran-6-(6*aH*)-one	
Salithion	Sumitomo Chem. Co. (Osaka, Japan)	I	2-Methoxy-4H-1,3,2-benzodioxaphosphorine-2-sulphide	
Warfarin	Penick	Ro	3-(α-Acetonylbenzyl)-4-hydroxycoumarin	

EXPERIMENTAL

Chemicals and apparatus

The pesticides were supplied as analytical standards and were utilized as such (Table I). Solutions were prepared in chloroform, except those for diquat and paraquat, which were prepared in 50% aqueous acetone. All of the adsorbents were obtained from Brinkmann Instruments (Rexdale, Canada).

The fluorescence was observed in a Chromato-Vue Cabinet (Canlab Supplies, Montreal, Canada) under long wavelength UV light. The spectra were measured using a VIS-UV Chromatogram Analyser (Farrand Optical Co., New York, N.Y., U.S.A.) equipped with motorized monochromators and appropriate filters.

General procedure

The thin layers were prepared as recommended by the supplier to a thickness of 250 μm. A 2-μl volume of a 1000-ppm solution of each pesticide was applied on a chromatogram by means of a micro-pipette. After observation under UV light, the chromatogram was heated in an oven at a specific temperature for a definite period. The chromatogram was again examined under UV light for fluorescence.

Prior to measurement of the fluorescence spectra, the chromatogram was spotted with a particular pesticide and eluted 5 cm in an appropriate solvent system.

RESULTS AND DISCUSSION

In this study, 61 pesticides were examined for fluorigenic properties, but only those for which positive results were obtained are discussed (see Table I).

Acidic aluminium oxide layers

All of the compounds listed in Table II, except aminocarb, show a detectable natural fluorescence on acidic aluminium oxide layers. The fluorescence is most intense, however, for coumaphos, diphacinone, Maretin and Morestan. Heat treatment of the chromatogram yields intensified fluorescence for most pesticides but the effect is much more noticeable for those which are originally barely or non-fluorescent. The fluorescence of Morestan is decreased slightly while Maretin appears as a red-orange UV-absorbing spot. Coroxon (not shown in this paper), which is the oxygen analogue of coumaphos, behaves in the same manner as coumaphos.

Fluorescence spectra were recorded on acidic aluminium oxide layers. The results are summarized in Table III and include, for the species that show the best fluorescence under optimum conditions, the filter combination as well as the eluting solvent used in each instance. No spectra were recorded for Fuberidazole because upon elution many fluorescent spots appear, owing to impurities in the original formulation. Maretin is fluorescent only for a short time and the spot becomes red-orange UV-absorbing on standing. Upon heating, rotenone becomes fluorescent but the fluorescence is masked by the appearance of a brown colour beneath the spot, which makes it very difficult to record spectra.

It is interesting (see Table III) that the combination of excitation and emission wavelength maxima is characteristic for each pesticide, which make the detection very

TABLE II

ACIDIC ALUMINIUM OXIDE THIN LAYERS

Specification: type T, pH = 4.0. N.F. = Non-fluorescent at the 2.0 μg level; B.F. = barely fluorescent at the 2.0 μg level; + + = equivalent to a visual limit of detection of 2.0 μg per spot; + + + + = equivalent to a visual limit of detection of 0.2 μg per spot; ± + + + = equivalent to a visual limit of detection of 0.02 μg per spot or less.

Pesticide	*Natural fluorescence at room temperature*	*Relative fluorescence intensity at 200°/45 min*
Aminocarb	N.F.	± ∓ ∓
Captan	B.F.	+ + ∓
Coumaphos	± + +	± + + +
Difolatan	B.F.	± + +
Diphacinone	+ + +	+ + +
Fuberidazole	∓ +	+ ∓ ∓
Guthion	B.F.	∓ ± ∓ +
Landrin	B.F.	+ + +
Maretin	+ + ±	Red-orange spot
MGK Rep. II	B.F.	+ + +
Morestan	+ + +	∓ +
Rotenone	B.F.	+ + +
Salithion	B.F.	± + + +
Warfarin	B.F.	− + + +

TABLE III

FLUORESCENCE SPECTRAL DATA ON ACIDIC ALUMINIUM OXIDE LAYERS

Specification: type T, pH = 4.0. H = heat treated at 200° for 45 min; R.T. = room temperature (not heat treated). Eluting systems: a = hexane–acetone (8:1); b = hexane–acetone (13:1); c = acetone–toluene (1:9); d = hexane; e = hexane–acetone (16:3); f = hexane–acetone (14:3). Filter combinations (Ex, Em): A = (3-75, 7-60); B = (3-74, 7-54); C = (3-73, 7-54); D = (3-75, 7-54).

Pesticide	*Eluting system*	*Filter combination*	*Wavelength (nm)*	
			Excitation	*Emission*
Aminocarb (H)	b	A	372	473
Captan (H)	c	B	372	460
Coumaphos (R.T.)	f	A	340	412
(H)	f	A	360	435
Difolatan (H)	c	C	372	467
Diphacinone (R.T.)	a	A	368	490
(H)	a	A	370	493
Guthion (H)	a	A	347	421
Landrin (H)	a	A	373	483
MGK Rep. II (H)	h	C	375	457
Morestan (R.T.)	d	A	366	413
(H)	d	A	345	423
Salithion (H)	b	A	371	474
Warfarin (H)	c	D	375	462

selective. Also, coumaphos and Morestan give different spectra before and after the heat treatment.

Basic aluminium oxide layers

Results obtained on basic aluminium oxide layers are given in Table IV. As on acidic layers, most of the pesticides show detectable fluorescence prior to heat treatment, with the exception of paraquat, which does not fluoresce. Heat treatment improves the fluorescence substantially in most instances.

TABLE IV

BASIC ALUMINIUM OXIDE LAYERS

Specification: type T, pH = 9.0. Abbreviations as in Table II.

Pesticide	*Natural fluorescence at room temperature*	*Relative fluorescence intensity at 200°/45 min*
Captan	B.F.	+ + +
Coumaphos	+ + +	+ + + +
Difolatan	B.F.	+ + +
Diquat	B.F.	+ + +
Diphacinone	+ + +	+ + +
Guthion	B.F.	+ + +
Landrin	B.F.	+ + +
Mesurol	B.F.	+ + +
Mobam	B.F.	+ + +
Paraquat	N.F.	+ + +
Rotenone	B.F.	+ + +
Salithion	B.F.	+ − + +
Warfarin	+ +	+ + +

It is important that although some pesticides fluoresce equally well on both acidic and basic aluminium oxide layers, others fluoresce only on one type of layer. For instance, aminocarb, fuberidazole and MGK Rep. II fluoresce only on acidic layers, while diquat, Mesurol, Mobam and paraquat fluoresce only on basic layers. There does not seem to be a strong correlation between the chemical structure of a pesticide and its behaviour on either acidic or basic layers.

The fluorescence spectral data on basic aluminium oxide layers are given in Table V. Comments made on the spectral data listed in Table III are also applicable in this instance. In addition, for pesticides that are fluorescent on both acidic and basic layers, *e.g.*, coumaphos, there is sometimes a noticeable difference in the spectral data.

Comparison with silica gel layers

The natural fluorescence of coumaphos, Maretin, fuberidazole, diphacinone and Morestan on silica gel layers has been reported[5,13] and the effects of heat treatment on the fluorescence of these compounds, and also that of Guthion and warfarin, have been assessed in another study[5,7]. Data on the fluorescence of captan, difolatan, diquat and paraquat after treatment of the chromatogram with an inorganic reagent and, for captan and difolatan, after subsequent heating of the chromatogram, have also been published[8]. These data are compared in Table VI.

It can be observed that the spectral data vary from one layer to another. A

TABLE V

FLUORESCENCE SPECTRAL DATA ON BASIC ALUMINIUM OXIDE LAYERS

Specification: type T, pH = 9.0. Abbreviations as in Table III. Eluting systems: a–f as in Table III; g = acetone–toluene (2:9); h = acetone–toluene (1:10); i = benzene–*n*-butanol–methanol–1 *N* HCl (1:1:2:1); j = hexane–acetone (8:2). Filter combinations: A–D as in Table III; E = (3-74, 7-60).

Pesticide	*Eluting system*	*Filter combination*	*Wavelength (nm)*	
			Excitation	*Emission*
Captan (H)	g	B	372	465
Coumaphos (H)	e	A	363	447
(R.T.)	e	A	340	418
Difolatan (H)	g	B	375	462
Diphacinone (R.T.)	a	A	370	495
Diquat (H)	i	B	372	452
Guthion (H)	a	E	350	431
Landrin (H)	b	A	368	454
Mesurol (H)	b	A	368	455
Mobam (H)	b	A	371	480
Paraquat (H)	i	C	367	460
Rotenone (H)	j	A	370	470
Salithion (H)	b	A	374	470
(R.T.)	g	A	375	470
Warfarin (H)	g	A	374	456

TABLE VI

COMPARISON BETWEEN SILICA GEL AND ALUMINA

Abbreviations as in Table III. I.R. = inorganic reagent.

Pesticide and conditions	*Silica gel*		*Aluminium oxide*			
			Acidic		*Basic*	
	Excitation	*Emission*	*Excitation*	*Emission*	*Excitation*	*Emission*
Coumaphos (R.T.)	325	434	340	412	340	418
(H)	344	440	360	435	363	447
Diphacinone (R.T.)	330	518	368	490	370	495
Guthion (H)	342	442	347	421	350	431
Warfarin (H)	363	456	375	462	374	456
Captan (I.R.)	360	465				
(H)			372	460	372	465
Difolatan (I.R.)	360	465				
(H)			372	467	375	462
Diquat (I.R.)	375	472				
(H)					372	452
Paraquat (I.R.)	420	510				
(H)					367	460

typical example is coumaphos, for which the spectral data are different before and after the heat treatment. The behaviour with coumaphos is well documented and it is known[14] that the species that fluoresces after heat treatment is chlorferone, the hydrolysis product.

Other layers

Neutral aluminium oxide layers (type T, pH 7.5) were also tested but the results obtained were very similar to those obtained on basic aluminium layers.

The same was true for aluminium oxide containing a binder (aluminium oxide G, type E) and aluminium oxide 25 pre-coated sheets.

CONCLUSION

The results offer an alternative to the use of silica gel layers for the determination of pesticides. More important, however, is the fact that different spectral data are obtained when acidic and basic aluminium oxide layers are used. In addition, the spectral data change with the experimental conditions preceding measurement of spectra. Evidently, these data can be useful when it becomes necessary to characterize a compound or for confirmation purposes.

Another important aspect is that some pesticides are fluorescent on aluminium oxide layers and not on silica gel layers under ordinary conditions. These pesticides are captan, difolatan, diquat and paraquat, which have to be detected on silica gel layers after treatment with an inorganic reagent[8]. Most important, however, is the fact that aminocarb, landrin, Mesurol, MGK Rep. II, Mobam and salithion have not previously been detected on silica gel layers.

As a result of this study, it is planned to investigate further the behaviour of the fluorescent pesticides on aluminium oxide layers and eventually to develop analytical procedures for their determination in environmental samples.

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