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DETECTION OF NATURALLY FLUORESCENT PESTICIDES ON SILICA GEL LAYERS

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SUMMARY

A number of fluorescent pesticides, namely, benomyl, coumatetralyl, diphacinone, fuberidazole, propyl isome and quinomethionate, have been investigated on silica gel thin-layer chromatograms. Fluorescence spectra have been measured and visual detection limits have been estimated. In most cases, as little as a few nanograms can be detected. In addition, the effects of heat treatment on the fluorescence have been observed. The most pronounced effect is a large bathochromic shift of the fluorescence excitation and emission maxima.

INTRODUCTION

The combination of *in situ* fluorometry and thin-layer chromatography (TLC) is recognized as a valuable analytical technique for the evaluation of pollutants[1]. The method has found wide application to the analysis of various organic compounds such as steroids[2], air pollutants[3,4], nicotinic acid and derivatives[5], citrus oil[6], indoles[7], and many more too numerous to mention.

Application to pesticide residue analysis, however, is relatively new. RAGAB[8] was probably one of the first to use fluorogenic chelate spray reagents for the detection of organothiophosphorous pesticides on thin-layer chromatograms. This work led to the development of other chelate spray reagents[9], which were later applied to the detection of azinphosmethyl in water[10] and blueberries[11]. One of the latest developments in fluorogenic chelate spray reagents is that of BIDLEMAN *et al.*[12], who used a palladium – fluorescein combination for the quantitative evaluation of organothiophosphorous pesticides. Flavones[13–16] were also utilized as fluorogenic spray reagents. Other methods based on the principle of enzyme inhibition[17] and fluorogenic labelling[18] have been developed.

The tendency nowadays is to measure fluorescence derived from the pesticide to be determined whether it be through its natural fluorescence or by a process of conversion[19]. Either case is advantageous since each fluorescing species is characterized by its own absorption and emission spectra, which is not the case when fluorescence is produced indirectly such as with fluorogenic spray reagents.

In this study, the natural fluorescence of a number of organic pesticides has

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been measured directly from thin-layer chromatograms. In addition, the effects of heat treatment on the fluorescence have been investigated. The ultimate goal is to develop analytical methods suitable for the quantitative determination of pesticides in the environment and in our food supply, using a combination of TLC and fluorometry.

EXPERIMENTAL

Chemicals and apparatus

A list of the pesticides investigated has been provided in Table I. All were analytical standards and were utilized without further purification with the exception of propyl isome, which was purified by column chromatography over alumina by elution with a 1:50 mixture of acetone and *n*-hexane. All the solvents used were either spectranalysed or pesticide grade (Fisher Scientific Co., Montreal, Canada). Pre-coated (250 μ) TLC plates, Silica Gel 60 from Brinkman Instruments (Rexdale, Canada), were used.

Fluorescence spectra were recorded by means of a VIS-UV Chromatogram Analyzer (Farrand Optical Co., Inc., New York, U.S.A.) equipped with motorized monochromators. The source was provided by a 150-W high-pressure xenon arc. A No. 7-54 filter (230–420 nm) was used in the exciter drawer with a No. 3-73 filter (405–800 nm) in the analyzer leg for all pesticides investigated except quinomethionate (No. 7-60 and No. 3-75, respectively). An aperture reducer of 0.625 in. in diameter was used with both filters. Entrance and exit slits measuring 10 nm were also employed. The photomultiplier detector consisted of a 1-P21 tube.

Visual observation of the fluorescence was carried out in a Chromato-Vue Cabinet (Canlab Supplies Ltd., Montreal, Canada).

General procedure

Standard pesticide solutions of 1000 p.p.m. (w/v) were prepared in methylene chloride. A plate was spotted with 2 μg of each pesticide by means of 2-μl capillaries and eluted 10 cm in a 9:1 mixture of *n*-hexane and acetone. In order to estimate visual detection limits, dilution series of each pesticide were prepared in *n*-hexane from the original stock solutions such that 1-μl aliquots were spotted each time. The excitation and emission spectra were recorded in the usual manner[10]. When required, the chromatograms were heated at 200° for at least 45 min.

RESULTS AND DISCUSSION

Of all the pesticides listed in Table I, none to our knowledge have been examined by *in situ* fluorometry after separation on thin-layer chromatograms. However, benomyl has been analysed by direct fluorometric analysis in solution after conversion to 2-aminobenzimidazole[20]. Others, such as coumatetralyl and fuberidazole, have been determined by UV spectrometric analysis[21]. Product analysis for quinomethionate[21] was also carried out by UV spectroscopy but residues have also been determined by a colorimetric method and by GLC. Product analysis for propyl isome[21] is presently done by the reaction with gallic acid in sulfuric acid, the blue colour being measured at 600 nm.

TABLE I

LIST OF PESTICIDES

Abbreviations: F = fumigant; I = insecticide; M = miticide; Ro = rodenticide; S = synergist; s = saturated.

Pesticide and manufacturer	*Uses*	*Chemical name*	*Structure*
Benomyl (Du Pont)	F	Methyl N-[1-(butylcarbamoyl)-2-benzimidazole] carbamate	
Coumatetralyl (Chemagro)	Ro	4-Hydroxy-3-(1,2,3,4-tetrahydro-1-naphthyl)-coumarin	
Diphacinone (Velsicol)	Ro	2-Diphenylacetyl-1,3-indandione	
Fuberidazole (Chemagro)	F	2-(2[1]-Furyl)-benzimidazole	
Propyl isome (Penick)	S	Dipropyl 5,6,7,8-tetrahydro-7-methylnaphtho [2,3-*d*]-1,3-dioxole-5,6-dicarboxylate	
Quinomethionate (Chemagro)	I, M, F	6-Methyl-2,3-quinoxalinediyl cyclic S,S-dithiocarbonate	

All of the pesticides studied are naturally fluorescent and the spectral data are given in Table II. With the exception of diphacinone, most fluoresce in the blue region of the spectrum. The effect of heat on the fluorescence spectra is also given in Table II. The most noticeable change is a shift of the emission and excitation maxima to longer wavelengths. Another difference is an increase in the number of excitation and emission peaks. This is the case with diphacinone, for example, where the emission at 518 nm is decreased at the expense of another peak appearing at 529 nm (Fig. 1). The total emission upon heating is also much broader.

This technique of heating the chromatogram at a specific temperature for a definite period of time to obtain the desired fluorescence has already been applied successfully to organophosphorous pesticides[10]. The actual mechanism is not yet

TABLE II

FLUORESCENCE SPECTRAL DATA

Pesticide	*Wavelength (nm)*			
	As is		*After heat treatment*	
	Ex	*Em*	*Ex*	*Em*
Benomyl	298	422	362	464
Coumatetralyl	330	415	365 (348)	463
Diphacinone	330 (365)	518	330 (365)	529 (518)
Fuberidazole	328	402	323 (373)	447
Propyl isome	343	460	347	453
Quinomethionate	363	418	335 (360)	465 (478)

fully understood and it is not known what the fluorescing species are. Nevertheless, it is certain that heat causes degradation of the original compound to one or more fluorescing species (*cf.* Fig. 1).

This was demonstrated further with quinomethionate. A thin-layer chromatogram was spotted with 20 μg of the technical product and eluted in the usual way. The plate was heated and eluted again at 90° to the first elution. Results are illustrated in Fig. 2. Spots Nos. 1,2,3,4, and 6 are impurities in the original material. Upon heating, the major component (spot No. 5) gives a number of degradation products (spots Nos. 7 to 10) of which spot No. 9 is the major constituent. The degradation products have not yet been identified although they are presently being investigated.

The visual detection limits both prior to and after the heat treatment are given

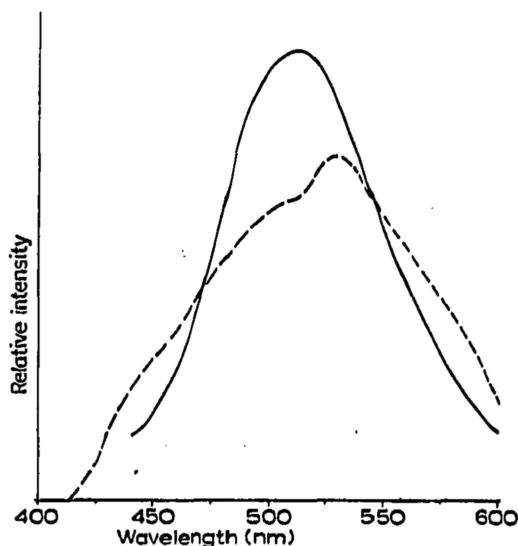

Fig. 1. Fluorescence spectra of diphacinone. ——, Natural fluorescence; ----, heated at 200° for 45 min.

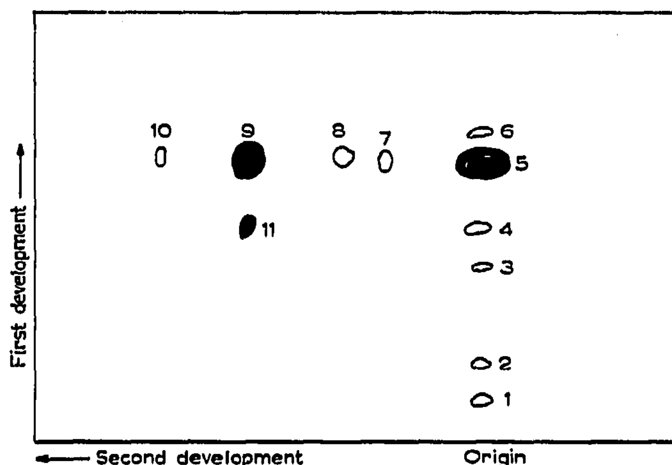

Fig. 2. Chromatogram showing quinomethionate before and after the heat treatment. Dark shaded = strong fluorescence; light shaded = weak fluorescence.

TABLE III

VISUAL LIMITS OF DETECTION

Pesticide	*Limit of detection* (μg)	
	Prior to heat treatment	*After heat treatment*
Benomyl	0.06	0.02
Coumatetralyl	0.001	0.01
Diphacinone	0.005	0.1
Fuberidazole	1.00	0.005
Propyl isome	0.005	0.06
Quinomethionate	0.004	0.04

in Table III. There does not seem to be any correlation, *i.e.*, some spots become more fluorescent upon heating probably because the degradation species are more fluorescent than the original material, while others decrease in fluorescence intensity. The decrease in fluorescence can probably be attributed either to a destruction of the initially fluorescent species or the formation of less fluorescent degradation products. It is interesting to note that the fluorescence intensity of fuberidazole is markedly increased.

CONCLUSION

The fact that the fluorescence characteristics of a species may be altered upon physical treatment is most interesting both in terms of qualitative and quantitative analysis. In practice, the fluorescence could be measured both prior to and after the heat treatment.

Thus, dual information about the nature of the species present would be obtained through very selective absorption and emission wavelengths and, with added

selectivity from R_F values normally associated with the technique of TLC, compounds could be identified positively without the help of additional information.

The other advantage stems from the fact that in certain cases fluorescence intensity is markedly increased, thus greatly facilitating the detectability.

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