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INORGANIC SALTS IN THE FLUOROMETRIC DETECTION OF PESTICIDES

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SUMMARY

A method is described for the detection and quantification of pesticides on silica gel chromatograms by *in situ* fluorometry. Simple inorganic salts are used to induce fluorescence in a number of pesticidal compounds. Intensified fluorescence is shown for one naturally fluorescent pesticide. Spectral data and instrumental limits of detection are given.

INTRODUCTION

Direct fluorometry of thin-layer chromatographic (TLC) spots has found wide application in the field of micro and trace organic analysis. *In situ* methods have been applied to the determination of long-chain aliphatic hydrocarbons[1], triglycerides[2], vitamins[3,4], amines[5], steroids[6], hormones[7], and pharmaceuticals[8-10], to name but a few.

Earlier work with pesticides involved the use of spray reagents[11,12] and labelling compounds[13]. In most instances the fluorescence came from the reagent and not from the pesticide. Recently, techniques whereby the fluorescence comes from the pesticide have been developed[14]. The effects of heat[15] and of strong acids or bases have been reported[16,17]. The obvious advantage of such techniques over those which use fluorogenic spray reagents is that each compound is characterized by its own excitation and emission wavelengths.

Numerous studies[18] have been carried out dealing with the fluorometric analysis of inorganic cations or anions. In most cases a fluorescing chelate is formed between the inorganic ion and a non-fluorescing organic compound. Since some of the pesticides studied are potential ligands, it was thought at first that they might become fluorescent if the proper cations or anions were present. The effects of a variety of inorganic salts upon a number of pesticides on silica gel thin layers were therefore investigated. At the same time the influence of heat treatment of the chromatogram was studied.

No similar work, as yet, has been reported for the determination of pesticides by *in situ* fluorometry, although some has been reported for triglycerides[2], cholesterol[6], and diaminopyrimidines[10] using either the sulfate or the bisulfate salt of ammonium.
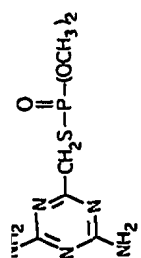

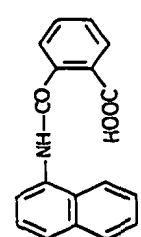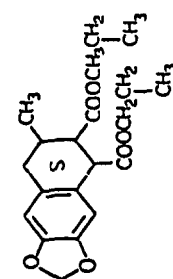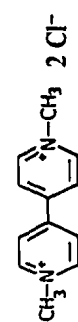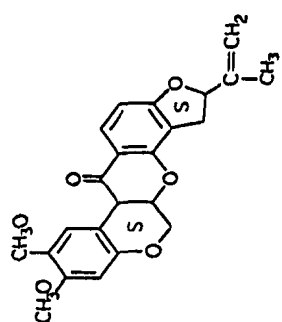* To whom correspondence is to be addressed.

TABLE I

LIST OF PESTICIDES GIVING POSITIVE RESULTS

Abbreviations: Et = CH_3CH_2-; S = "saturated"; F = fungicide; H = herbicide; I = insecticide; M = miticide; P = parasiticide; Sy = synergist.

Pesticide	Supplier	Type	Chemical name	Structure
Captan	Chevron (San Fransisco, Calif., U.S.A.)	F	N-Trichloromethylthio-4-cyclohexene 1,2-dicarboximide	
Devrinol	Stauffer (New York, N.Y., U.S.A.)	H	2-(α-Naphthoxy) N,N-diethylpropionamide	
Difolatan	Chevron	F	cis-N-[(1,1,2,2-Tetrachloroethyl)-thio]-4-cyclohexene-1,2-dicarboximide	
Diquat	Chevron	H	6,7-Dihydrodipyrido[1,2-a:2',1'-c]pyrazinedium dibromide	
Maretin	Chemagro (Kansas City, Mo., U.S.A.)	P	N-Hydroxynaphthalimide diethyl phosphate	

Name	Type	Chemical name	Manufacturer	Structure
Menazon	I, M	S-[(4,6-Diamino-s-triazin-2-yl)-methyl] O,O-dimethyl phosphorodithioate	Imperial (London, Great Britain)	
Naptalam	H	N-1-Naphthylphthalamic acid	Uniroyal (Naugatuck, Conn., U.S.A.)	
Propyl isome	Sy	Dipropyl 5,6,7,8-tetrahydro-7-methylnaphtho[2,3-d]-1,3-dioxole-5,6-dicarboxylate	Penick (New York, N.Y., U.S.A.)	
Paraquat	H	1,1'-Dimethyl-4,4'-dipyridinium dichloride	Chevron	
Rotenone	I	1,2,12,12a-Tetrahydro-2-isopropanyl-8,9-dimethoxy-benzo[1]pyrano[3,4-b]furo[2,3-b]benzo[1]pyran-6(6aH)-one	Penick	

EXPERIMENTAL

Chemicals and apparatus

More detailed descriptions of reagents, solvents and apparatus used are to be found elsewhere[14-17].

The majority of the pesticides were supplied as analytical-grade standards (Table I) and were utilized as such. Propyl isome was purified by a method described earlier[16] while rotenone and warfarin were purified by multiple recrystallisation from ethanol–water and acetone–benzene systems, respectively.

The inorganic salts investigated were the chlorides of aluminium, copper and calcium and the sulfates of beryllium, magnesium, manganese, nickel, and zinc. Sodium borate, potassium chromium sulfate and selenious acid were also investigated. All were analytical grade reagents (Fisher Scientific, Pittsburgh, Pa., U.S.A.). Solutions were prepared in de-ionized distilled water.

The fluorescence spectra were recorded on a VIS-UV Chromatogram Analyzer (Farrand Optical Co. Inc.) equipped with appropriate filters. A list is provided in Table II. A Turner Model 111 fluorometer (G. K. Turner) fitted with a Camag TLC scanner was utilized for the evaluation of instrumental detection limits. A No. 7-60 primary filter was used for excitation and a No. 2-A barrier filter was used for emission. The fluorescence intensity of the spots was recorded on a Brinkmann Servogor S Model 2543. A minimum deflection of 1 cm was taken as being the least detectable peak.

General procedure

Silica gel H thin-layer chromatograms with a layer thickness of 250 mμ were prepared in the usual way. In some cases, the salt under investigation was incorporated directly into the sorbent layer. This was accomplished by using a solution of the desired salt instead of distilled water.

In order to proceed in the rapid screening of all the pesticides and inorganic reagents, it was found suitable to scribe the layer into 64 small squares. Two-microlitre

TABLE II

PESTICIDES AND FILTER COMBINATIONS

Compound	Filter	
	Ex	Em
Captan*	7-54	3-73
Devrinol**	7-60	3-75
Difolatan*	7-54	3-73
Diquat*	7-54	3-73
Maretin*	7-60	3-75
Menazon*	7-54	3-73
Naptalam*	7-54	3-73
Paraquat*	7-59	3-72
Propyl isome*	7-54	3-73
Rotenone**	7-60	3-75

* With UV-visible excitation lens.
** With UV excitation lens.

Table III. The instrumental limits of detection (ILD) are also given. Maretin and propyl isome are the only two compounds which fluoresce naturally to any great extent on silica gel thin layers. The natural fluorescence of maretin has been reported earlier[16] but measurements were made with different filters. The presence of aluminium chloride results in an increase in IDL. Maretin is unstable on silica gel layers and the initial fluorescence fades by some 50% after only 1 h 30 min. The stability is much greater on $AlCl_3$-treated layers, a decrease of only 5% in initial fluorescence intensity being noted after 2 h. After 72 h, the fluorescence of 1-μg spots is decreased by 45% while on untreated layers the fluorescence has disappeared. Thus, $AlCl_3$-treated layers should be preferred for developing an analytical method for maretin. The fluorescence of propyl isome is decreased in the presence of aluminum chloride as compared to its natural fluorescence[14]. Irradiation with UV light, however, returns the fluorescence. This is accompanied by a bathochromic shift of the emission maximum. When untreated propyl isome spots are irradiated in the same manner, no change is observed (Fig. 1). The shift in the emission maximum can be attributed to additional fluorescent species produced by irradiation in the presence of $AlCl_3$.

The interaction of menazon with selenious acid to produce fluorescence is of interest since determination of this compound by GLC is difficult due to its thermal instability. It was reported earlier[16] that menazon becomes fluorescent when heated on a chromatoplate at 225° for 30 min. Since the fluorescence spectra are different for both techniques, there is an added degree of selectivity for identification purposes.

The results obtained for captan and difolatan are interesting. Both compounds differ only in the acyclic part of the molecule. Since the data are identical, it seems that the fluorescence originates from the same species. Thus, it should be possible to analyse for both compounds in a sample on the same chromatogram.
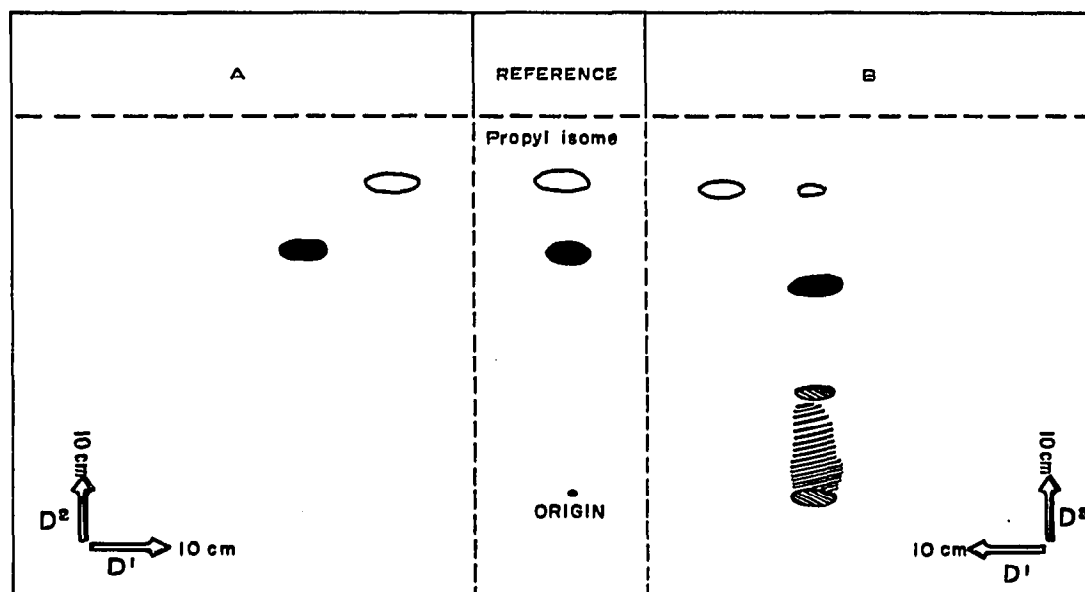

Fig. 1. Behavior of propyl isome upon irradiation with UV light. (A) Untreated layer; (B) treated layer.

Diquat and paraquat are similar in that they are both quaternary salts. The results obtained, however, are significantly different. Good limits of detection are obtained with devrinol, naptalam and rotenone. Naptalam and rotenone can also be rendered fluorescent by the heat treatment alone[17]. In the case of naptalam, treatment with $AlCl_3$ increases the ILD and a bathochromic shift in spectra is observed. For rotenone, the ILD is not increased and the fluorescence spectrum is similar to that obtained from its natural fluorescence. Devrinol and naptalam give better results on pre-coated silica gel than otherwise.

The mechanisms involved in all of these reactions are presently being studied. Each compound must be looked at separately since the reactions involved are characteristic and the products are numerous. Propyl isome is presently known to degrade into at least four products after UV irradiation on $AlCl_3$-treated layers (cf. Fig. 1). Some preliminary work[27] indicates that naptalam is split in half, viz. phthalic acid and 1-naphthylamine. Further breakdown occurs with 1-naphthylamine, which yields over thirty fluorescent species. It was thought initially that the fluorescence could result from the formation of chelates and although this possibility has not been ruled out it is becoming increasingly clear that inorganic reagents act as catalysts for the degradation of a pesticide into many fluorescent species.

CONCLUSION

One of the most important advantages of this technique is its inherent selectivity. The fluorescence is very characteristic since it is derived directly from the pesticide. Sensitivity is no detriment since submicrogram amounts can be detected for all the pesticides reported. Background fluorescence which plagues many fluorogenic spraying techniques is absent, thus assuring more reproducible results. In some cases the salt reagent can be either incorporated into the sorbent layer or sprayed onto the chromatogram without affecting the limits of detection. Spot spreading associated with spray techniques is eliminated in the first instance and this also has a bearing on reproducibility.

It should be possible to work out analytical procedures for most of the pesticides listed. Obviously the use of inorganic salt as a detection technique should be applicable to other pesticides and other organic pollutants as well.

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