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FLUOROGENIC LABELING OF ORGANOPHOSPHATE PESTICIDES WITH DANSYL CHLORIDE

APPLICATION TO RESIDUE ANALYSIS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY AND THIN-LAYER CHROMATOGRAPHY*

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SUMMARY

The analysis of some organophosphorus pesticides by fluorogenic labeling with dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride) was investigated. The pesticides were hydrolysed in sodium hydroxide to the corresponding phenols. The reaction of dansyl chloride with the phenols was accomplished in a two-phase system. The resulting fluorescent derivatives were separated and analysed quantitatively by *in situ* thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC). As little as 10–25 ng/spot of pesticide was detected by both TLC and HPLC.

INTRODUCTION

The use of fluorogenic labeling for pesticide residue analysis has recently been reviewed[1]. Reagents such as dansyl chloride (5-dimethylaminonaphthalene-1-sulfonyl chloride) and NBD-chloride (4-chloro-7-nitrobenz[2,1,3]oxadiazole) have been used for the analysis of N-methyl- and N,N-dimethyl-carbamate insecticides[2–4], phenylcarbamate, and phenylurea herbicides[5,6], as well as triazine herbicides[7]. In most cases quantities as low as 1–10 ng/spot could be detected by thin-layer chromatography,

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(TLC) after minimal sample cleanup. Dansyl chloride reacts with primary and secondary amines, imidazoles and phenols to produce the corresponding fluorescent sulfonamides and phenolic esters[8].

The analysis of organophosphate insecticides by TLC and fluorescence has been attempted with the use of fluorogenic spray reactions. Several of these depend on the reaction of bromine with easily oxidizable sulfur atoms in the pesticide to produce hydrobromic acid on the TLC plate. The hydrobromic acid may react with a pH sensitive fluorescent indicator[9] or may liberate a fluorescent ligand from a non-fluorescent metal chelate[10]. Ligand-exchange reactions have been applied to the analysis of organothiophosphates with detection limits in the low nanogram range[11]. The disadvantages of these methods are that they cannot detect organophosphates which do not contain sulfur and they cannot be easily applied to high-pressure liquid chromatography (HPLC). The method described herein is capable of detecting organophosphates which yield phenols upon hydrolysis and is also directly applicable to HPLC with fluorescence detection.

EXPERIMENTAL

Reagents

Analytical-grade dansyl chloride (Aldrich, Milwaukee, Wisc., U.S.A.) was dissolved in reagent-grade methyl isobutyl ketone (MIBK) at a concentration of 1 mg/ml. The organophosphates used are listed in Table I. These compounds were characterized by NMR spectroscopy. Solutions of the pesticides were prepared in redistilled reagent-grade methylene chloride. All pesticide and reagent solutions were stored at 10°.

The triethanolamine spray consisted of a 10% (v/v) solution of triethanolamine in isopropanol.

All other solvents were reagent-grade materials.

TABLE I

ORGANOPHOSPHATES INVESTIGATED

Common name	*Chemical name*
Fenthion	Dimethyl 3-methyl-4-methylthiophenyl phosphorothionate
Crufomate	4-*tert.*-Butyl-2-chlorophenyl methyl N-methylphosphoroamidate
Fenchlorphos (Ronnel)	Dimethyl 2,4,5-trichlorophenyl phosphorothionate
Methylparathion	Dimethyl 4-nitrophenyl phosphorothionate
Fenitrothion	Dimethyl 3-methyl-4-nitrophenyl phosphorothionate

Reaction procedure

An aliquot of the pesticide extract was placed in a 3-ml test tube and the methylene chloride was evaporated in a stream of nitrogen at room temperature. A 0.2-ml volume of 0.5 *M* sodium hydroxide was added and the tube heated for 45 min at 80°. Following this, 0.2 ml of dansyl chloride solution was added and the test tube was heated for 90 min at 80°. The remaining MIBK was evaporated from the test tube with a stream of nitrogen. The contents were cooled to room temperature

and 0.2 ml of benzene was added. The test tube was gently shaken followed by the removal of the benzene layer by Pasteur pipette. This was dried with a few granules of anhydrous sodium sulfate before spotting on the TLC plate.

Thin-layer chromatography

The TLC plates were prepared at a thickness of 0.25 mm from a slurry of silica gel G (Brinkmann, Rexdale, Ontario, Canada) consisting of 30 g silica gel and 60 ml of distilled water. The plates were allowed to air dry, then stored in the dark.

Ten microlitres of the benzene solution from the reaction procedure were spotted on silica gel G plates with a 10-μl Hamilton syringe. The plates were developed with solvents such as benzene, benzene–hexane (20:5) or benzene–acetone (100:0.4). After chromatography the plates were dried and sprayed with the triethanolamine solution until visibly moist. The plates were then dried under a stream of air for analysis.

An Aminco-Bowman (Silver Springs, Md., U.S.A.) spectrophotofluorometer equipped with a thin-layer scanning accessory was used to record the fluorescence spectra of the derivatives directly from the TLC plates. All quantitative TLC results were obtained with a Zeiss (New York, N.Y., U.S.A.) chromatogram spectrophotometer. A mercury lamp was used to excite the derivatives (Zeiss 356-nm filter). Emission was monitored through a monochromator set at the appropriate wavelength.

High-pressure liquid chromatography

The chromatographic system consisted of a Haskel air-driven piston pump (Model No. 17082-3; Haskel Engineering, Burbank, Calif., U.S.A.); a fluorescence detector (Model 1209; Laboratory Data Control, Riviera Beach, Fla., U.S.A.); and a sample injection port described earlier[12]. All connective tubing was 1/8-in.-O.D. stainless steel joined by Swagelok fittings.

The analytical column consisted of 3/32-in.-I.D., seamless stainless steel 40 cm in length. This was packed with small particle silica gel (10 μm) (Brinkmann) by a balanced-density slurry-packing technique[13]. The eluting solvent was 10% chloroform in hexane at a flow-rate of 1.0 ml/min (1500 p.s.i.).

Extraction of water samples

A 500-ml sample of water was extracted with two 50-ml volumes of methylene chloride. The combined organic extracts were dried with anhydrous sodium sulfate and reduced to about 1 ml by rotary vacuum evaporation. This was transferred to a 3-ml test tube and evaporated to dryness at room temperature under a stream of nitrogen. The residue was treated as described in the reaction procedure for formation of the fluorescent products.

RESULTS AND DISCUSSION

Reaction

Several reaction procedures were attempted for the labeling of the phenols originating from the organophosphate pesticides. The original method of Frei and Lawrence[2] was not suitable. The hydrolysis of the organophosphates required

stronger conditions (0.5 *M* NaOH) than that for the methylcarbamates (0.1 *M* Na_2CO_3). Also the dansylation of the phenols does not proceed as well in the stronger base as it does in sodium carbonate. Thus attempts were made to hydrolyse the organophosphate separately, then extract the phenol from the mixture for dansylation. This proved tedious since the pH of the hydrolysis mixture had to be carefully adjusted for reproducible extractions. Even under optimum pH conditions recoveries of the phenols were poor. The final reaction procedure attempted avoided the extraction problem and made use of the two-phase reaction system described for NBD-chloride labeling[3]. This reaction scheme was satisfactory and thus was chosen for the present work.

An overall reaction scheme for hydrolysis and dansylation is shown in Fig. 1. The pesticide was hydrolysed in the aqueous phase to the phenol. The phenol then

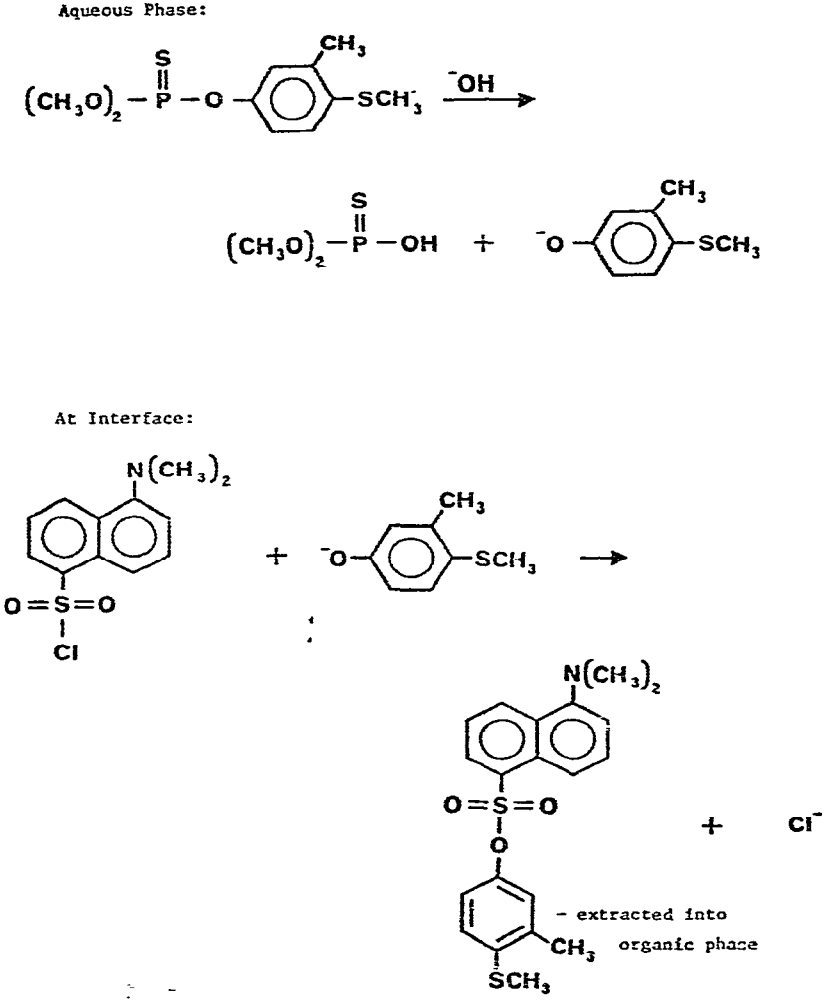

Fig. 1. Overall reaction scheme for the hydrolysis and dansylation of fenthion. Aqueous phase: hydrolysis. Interface: dansylation.

reacted with the dansyl chloride at the interface to form the fluorescent product which remained in the organic phase. Any hydrolysed dansyl chloride was extracted into the aqueous base and was removed from the product.

Hydrolysis of the organophosphates was complete in about 30–40 min. Dansylation of the phenols required about 90 min. Lower reaction temperatures increased these times.

Fig. 2 shows the effect of dansyl chloride concentration on the yield of dansyl derivative with 10 μg of the phenol from fenthion. The plot levels off above 0.05% dansyl chloride in MIBK. The 0.1% concentration was chosen for further work to ensure high yields of products.

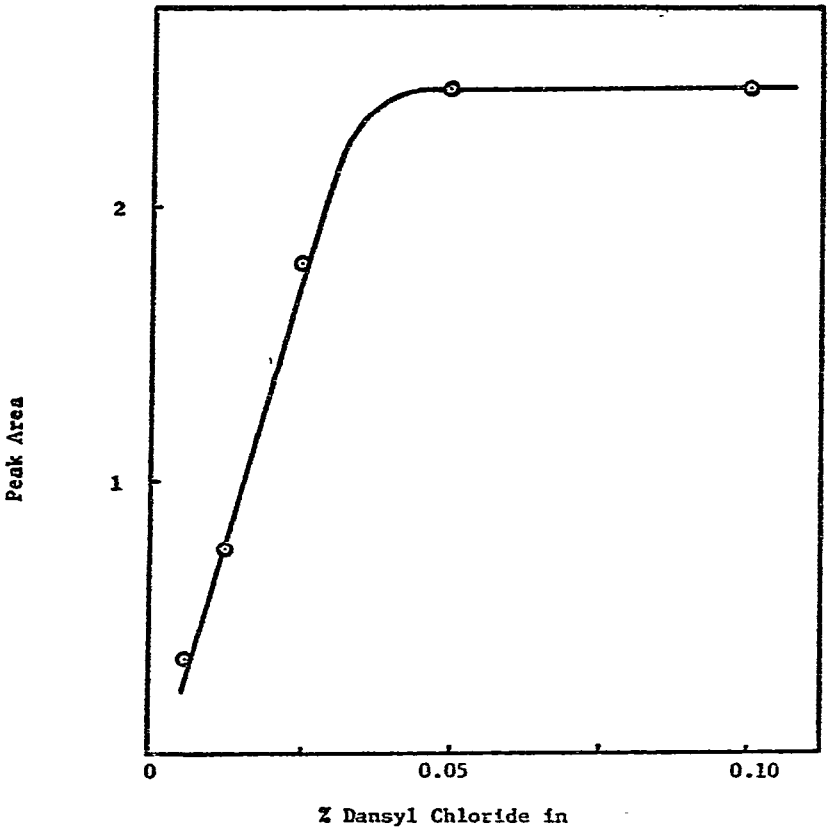

Fig. 2. Effect of dansyl chloride concentration on the yield of fluorescent product from the phenol derived from fenthion.

TLC fluorescence measurements

The excitation (ex) and emission (em) maxima of the dansyl derivative of fenthion phenol were found to be 378 nm ex and 538 nm em. The spectra of the other phenol derivatives were very similar. This is not surprising, since the fluorescence is generated by the dansyl portion of the molecule.

It was found that upon exposure to UV light, the fluorescent spots increased in intensity by two-fold and changed from yellow to blue. The fluorescence intensity of the dansylated phenols stabilized after 1 h of exposure to 365-nm irradiation. No increase was observed after this time. Fluorescence spectra of the spots after the irradiation were identical for all the dansyl derivatives (350 nm ex, 495 nm em). This seemed to indicate hydrolysis of the dansyl derivatives since the spectra correspond to the fluorescence spectrum of dansyl-OH. It was shown earlier that dansyl derivatives can hydrolyse under the influence of UV light to form dansyl-OH[14]. For quantitative purposes the plates were exposed to the UV light for 1 h before scanning at 350 nm excitation and 495 nm emission.

The degradation effect of UV light on the derivatives in solution was negligible. A solution of the dansyl derivative of fenthion was exposed to the same UV light for 1 h. This treatment had no effect on the fluorescence intensity or the wavelength maxima. This indicates that the adsorbent and/or spray solution may play some part in the degradation on the plates. The TLC degradation was found useful for the analysis of organophosphates such as parathion, methylparathion, and fenitrothion. Their corresponding phenols readily yield dansyl derivatives but since they contain a nitro group, no fluorescence was observed. However, the UV exposure of these compounds on the plates resulted in a blue fluorescence which was as sensitive as those of dansyl derivatives containing no NO_2 groups[8]. No response was observed for the dansyl derivatives of these pesticides by HPLC. Attempts to reduce the NO_2 group of methylparathion to a primary amine resulted in three dansyl derivatives being formed under the reaction conditions described above. This was probably due to incomplete labeling at both the $-NH_2$ and $-OH$ sites of the phenol. However, different reaction conditions might provide a single product.

Quantitation

The visual detection limits by TLC for this method approached 10–25 ng/spot of equivalent pesticide. However, for best reproducibility during scanning at least 50 ng/spot was necessary. The linear range of fluorescence occurs from 50–500 ng/spot.

Fig. 3 shows the HPLC separation of the dansyl-phenols resulting from the three pesticides. Complete separation is achieved in less than 8 min. The separation by TLC, based on retention times and R_F values, was not as good and much worse when chromatographic efficiences were considered. Both methods, however, separated the derivatives from interfering side products such as dansyl-amide or the sulfonic acid.

The sensitivity of HPLC was found to be superior to *in situ* TLC. The lower limit of the linear concentration range was about ten-fold better by HPLC. Table II compares the two systems. The linearity of the HPLC analysis extends to zero concentration for all three compounds. Values given are the actual detection limits at a signal-to-noise ratio of 2:1. The visual detection limits of the derivatives by TLC is about 20–50 ng/spot but calibration curve linearity is distorted below the values stated in Table II and quantitation in this range is impractical.

Reproducibility studies were carried out by HPLC for eight dansylation reactions of fenthion (5 μg each). An aliquot equivalent to 80 ng of pesticide was injected into the chromatograph. The relative standard deviation obtained was 8.5%. This level of injection could not be carried out by TLC since it is below the linear

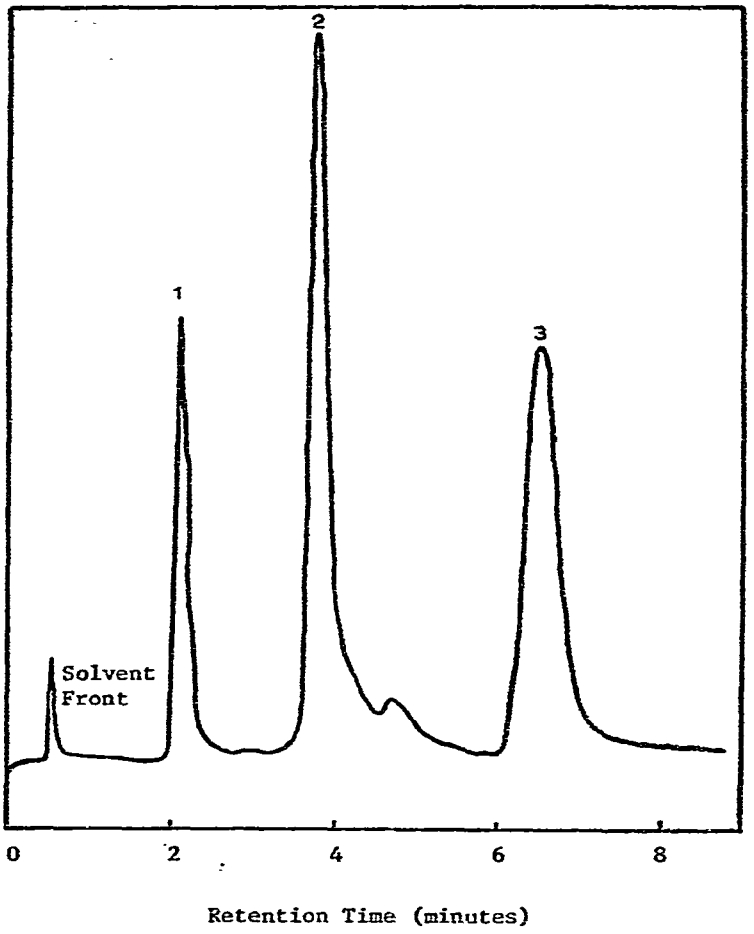

Fig. 3. HPLC separation of the dansyl–1-phenol derivative of fenchlorphos (1), crufomate (2) and fenthion (3). Conditions, as described in the text, 1 ml/min flow rate, 1500 p.s.i.

TABLE II

INSTRUMENTAL DETECTION LIMITS (LOWER END OF LINEAR RANGE)

Compound	*Detection limit (ng)*	
	TLC	*HPLC*
Fenchlorphos	150–200	10
Crufomate	120	5
Fenthion	150–200	5

range for fenthion. The reproducibility of injections was found to be 5% relative standard deviation for eight injections of a single solution.

The reproducibility for the TLC analysis above 100 ng/spot was of the order of 10–15%. This error was probably due to the error in spotting and of non-uniform spot degradation during the UV exposure period. However, it has been reported that

TABLE III

COMPARISON OF TOTAL ANALYSIS TIME FOR EIGHT SAMPLES OF FENTHION

Step	*Time (min)*	
	TLC	*HPLC*
Spotting (TLC)	20	—
Chromatography	60 (including drying and spraying)	64 (8 × 8 min)
Analysis	60 (UV exposure)	0 (done simultaneously with chromatography)
	30 (scanning)	
Total	170	64

reproducibilities for spotting dansyl derivatives of other compounds were of the order of 4–6% relative standard deviation[9]. No UV exposure was performed in this case and the dansylation reaction was carried out in a different manner.

A comparison of time of analysis was made for the two chromatographic techniques and is presented in Table III. It is clearly seen that HPLC was more rapid for 8 analyses of fenthion. If no separation of the fenthion derivative from other fluorescent compounds is necessary then chromatography time by HPLC can be reduced. An example of this is the analysis of a water sample spiked with fenthion. Fig. 4 shows the resulting chromatogram run at a flow-rate of 1.3 ml/min. The peak

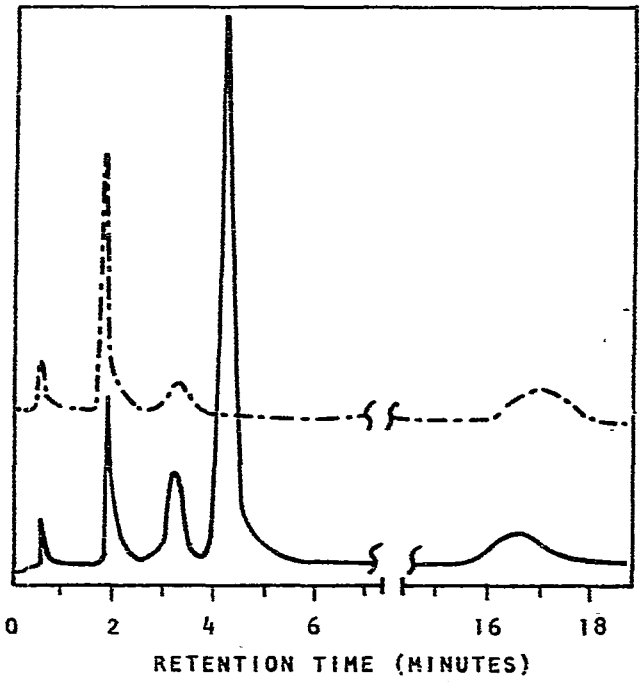

Fig. 4. Water sample analysis of fenthion spiked at 20 ppb. Conditions, as described in the text for Fig. 3, except that the flow-rate was 1.3 ml/min and the pressure 2000 p.s.i. ———, Fenthion spiked; —·—·—, unspiked water.

corresponding to fenthion appears just after 4.3 min and is well separated from interfering substances.

No detection of organophosphates which hydrolyse to thiols was observed with this reaction procedure. Seiler and Wiechmann[8] report that thiols react in the presence of dansyl chloride to form disulfides with no dansyl coupling.

CONCLUSIONS

The use of dansyl chloride for the analysis of phenol-generating organophosphorus pesticides in samples was examined by TLC and HPLC. The method can be useful for confirming residue data obtained by other techniques such as gas–liquid chromatography (GLC). The combination of TLC, which is an inexpensive technique, and the sensitivity of fluorescence can provide suitable means of analysis of pesticide residues in laboratories where more expensive instrumentation for methods such as GLC or HPLC is not available.

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