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FLUORIGENIC LABELLING OF CARBAMATES USING DANSYL CHLORIDE

III. THIN-LAYER CHROMATOGRAPHIC PROPERTIES OF THE DERIVATIVES

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SUMMARY

The chromatographic separation of the dansyl derivatives of several carbamate insecticides has been investigated. Three solvent systems were utilized for the development of the derivatives on Silica Gel G layers. Silver oxide was incorporated into the adsorbent for the separation of the Mesurol phenol derivative from that of Landrin. The silver oxide plates caused a decrease in the fluorescence of the derivatives with time when compared with silver-free plates. Ultraviolet light was found to have a hydrolytic effect on the derivatives and a number of fluorescent products were obtained. Light at 254 nm wavelength caused a greater degradation of the adsorbed compounds than at 350 nm. The solvent systems are relatively non-polar and fast-running. One-dimensional chromatography over a distance of 13 cm can be accomplished in 30–40 min. All chromatography must be carried out in the dark to avoid decomposition of the spots.

INTRODUCTION

Recent investigations[1,2] have shown fluorigenic labelling to be a promising new approach to residue analysis of carbamate and urea pesticides, comparing well to gas chromatographic techniques. The dansyl (1-dimethylaminonaphthalene-5-sulfonyl) derivatives of the phenyl or anilino moiety of the pesticides permit their identification and evaluation at nanogram levels. An earlier study of the fluorescence characteristics of these derivatives[3] suggested a good potential for the quantitative analysis as well. One condition for the successful adaptation of this method to residue analysis is the availability of good chromatographic separation procedures to eliminate interferences from similarly labelled compounds, other hydrolysis products of the pesticides, and excess reagent. For this reason some of the chromatographic conditions necessary for the separation of the dansyl derivatives of a number of carbamate pesticides are investigated.

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EXPERIMENTAL

Reagents

The carbamates investigated were listed earlier[2,3]. In addition, Pirimicarb (2-dimethylamino-4,5-dimethyl-1,3-pirimidin-6-yl N,N-dimethylcarbamate) has been studied. All reagents and solutions were prepared as described in earlier papers[2,3].

Reaction procedure

The dansyl derivatives were prepared by placing 10 μl of a stock solution of a carbamate into a 5-ml centrifuge tube which was then heated in a warm water bath to evaporate the methylene chloride. 0.5 ml of 0.1 *M* $NaHCO_3$ was added to the residue and the tube was heated for 60 min at 45°. It was cooled and 0.5 ml of acetone was added along with 0.2 ml of dansyl chloride solution. The contents were heated for another 20 min at 45°. After this, 0.5 ml of cyclohexane was added and the tube was shaken. The cyclohexane layer containing the derivatives was transferred by pipette to a clean test tube and stored at 2° until further use. 10-μl aliquots of these solutions were used for chromatography.

Chromatography

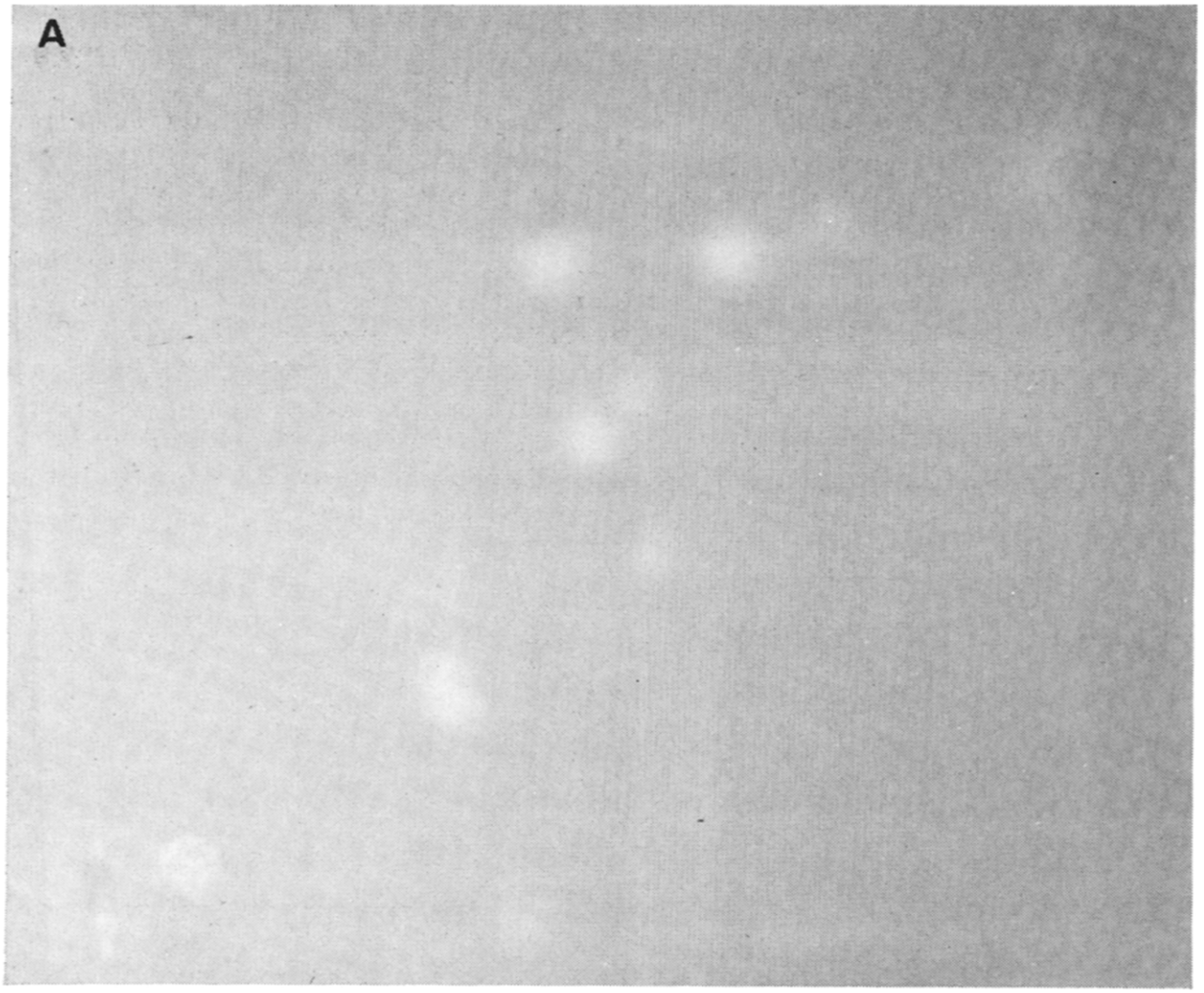
The thin-layer plates (20 × 20 cm) were prepared from aqueous slurries of the adsorbents at a thickness of 250 μ with a thin-layer applicator (Desaga, Heidelberg, G.F.R.). The Silica Gel G (calcium sulfate binder) (Macherey, Nagel & Co., Düren, G.F.R.) slurry was made by mixing 30 g of the adsorbent with 60 ml of distilled water. Silica Gel N (no binder) (Macherey, Nagel & Co.) plates were coated with a slurry consisting of 30 g of adsorbent and 70 ml of distilled water. Aluminium oxide plates (Camag, Muttenz, Switzerland) were made from 55 g aluminum oxide without binder and 40 ml of distilled water. Silica Gel G–silver oxide plates were prepared by mixing 30 g of Silica Gel G with 0.15 g of silver nitrate dissolved in 50 ml of distilled water, followed by the addition of 10 ml of 0.05 *N* NaOH. The plates were air-dried and stored at room temperature in the dark. All plates were activated by heating at 75° for 15 min before use.

The chromatography was carried out in the dark at room temperature, by the ascending technique. All liquid systems were freshly prepared from reagent-grade solvents in volume/volume ratios. They were allowed to equilibrate in the chromatography chambers for at least 15 min before performing any separations.

RESULTS AND DISCUSSION

The carbamates, with the exception of Pirimicarb, form two dansyl derivatives. One is the derivative of the phenyl moiety and the other the derivative of the methylamine moiety. Both derivatives are fluorescent and can be separated by thin-layer chromatography[1]. The phenol derivative is of major interest, since it is characteristic of each individual carbamate. Pirimicarb (a dimethylcarbamate) forms only the dimethylamine derivative. No significant reaction was observed with the pirimidinyl hydrolysis product. This can be explained by the fact that the basicity of the hydroxyl oxygen is reduced due to resonance of the electron pair with the heterocyclic ring.

Silica Gel G was found to be the best support for the separation of the dansyl



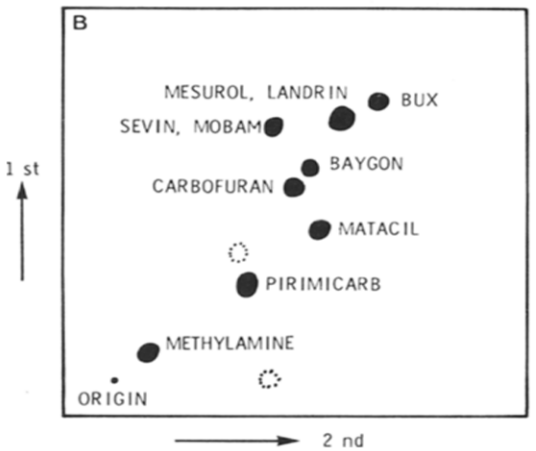


Fig. 1. Thin-layer chromatogram of the dansyl derivatives of the carbamates by two-dimensional chromatography on Silica Gel G using benzene–acetone (98:2) in the first dimension and petroleum ether–triethylamine (3:1) in the second. (a) photograph under UV light; (b) labelled diagram.

derivatives. The spots remained round and homogeneous after two-dimensional chromatography. This is important if quantitative results are required. Separation on aluminum oxide resulted in considerable diffusion of the spots and hence its use was discontinued. Silica Gel N showed little difference from the Silica Gel G, but the latter was preferred because of the higher physical stability of the layers.

Two solvent systems, benzene–acetone (98:2) and petroleum ether–triethyl amine (3:1) were found to be most suitable for the separation of the majority of the carbamate derivatives on Silica Gel G. A typical separation of some of the derivatives is shown in Fig. 1. For two-dimensional separation, the benzene–acetone system was run first and the plate was air-dried before developing in the second dimension. The phenolic derivatives of Mobam and Sevin (carbaryl) appear as a single spot. These can be separated from one another in one dimension with carbon tetrachloride–methanol (99:1). Those of Landrin and Mesurol could not be separated from one another with any of the solvent systems studied, without the incorporation of silver oxide in the adsorbent. Fig. 2 shows the separation of these two derivatives along with several

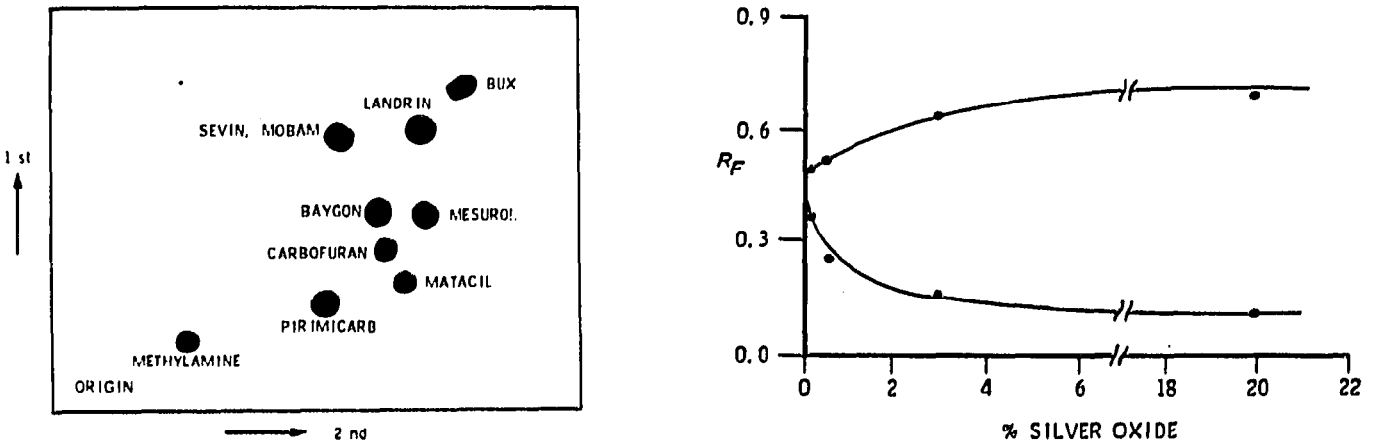


Fig. 2. Two-dimensional separation of the dansyl derivatives of several carbamates using Silica Gel G–0.34% silver oxide as adsorbent. The solvent systems are those used in Fig. 1.

Fig. 3. Effect of silver oxide content of the Silica Gel G on the R_F values of the dansyl phenol derivatives of Mesurol and Landrin. The eluting solvent consists of benzene–acetone (98:2).

others on a silver oxide plate. It was found that the content of silver oxide in the plates had a direct influence on the R_F values of Mesurol, although it did not greatly affect the other derivatives (see Fig. 3). It is believed that complexation of silver with the sulphur atom of the Mesurol causes the molecule to be held more strongly by the adsorbent, resulting in lower R_F values. The degree of complexation would be a function of concentration of silver, as shown in Fig. 3. The optimum amount of silver oxide is between 0.2 and 0.5%. Larger amounts did not improve the separation and caused a significant decrease in the stability of the derivatives after chromatography. This degradation effect could be due to catalytic and photo-oxidative processes. The stability of the derivatives on Silica Gel G and Silica Gel G–0.34% silver oxide as a function of time is shown in Fig. 4.

The influence of UV light on the stability of the derivatives was discussed in a previous paper[3], but no detailed investigations were carried out on the irradiated spots. In order to study this further, the dansyl derivatives of Mesurol and Sevin were

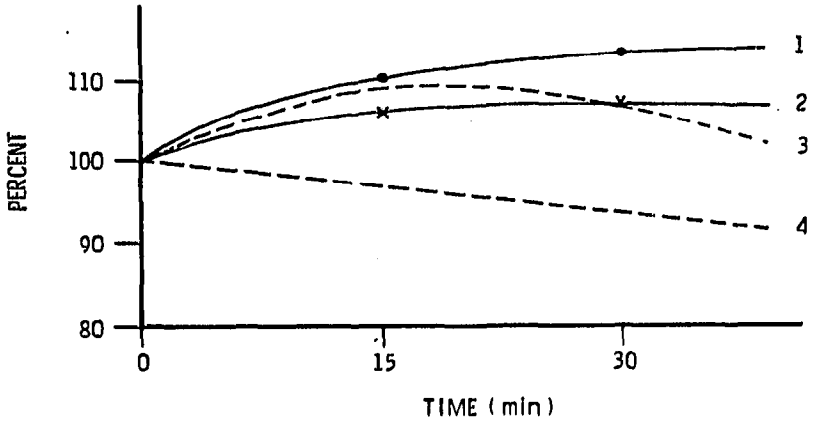
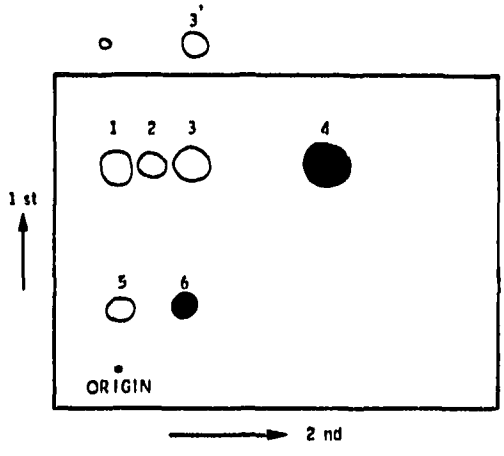


Fig. 4. Comparison of the stabilities of the dansyl phenol derivatives of Mesurol and Baygon on chromatoplates with and without silver. Curves 1 and 3: Mesurol; curves 2 and 4: Baygon. — — —, Silver-Silica Gel G; ———, Silica Gel G.

spotted in the corner of separate 20 × 20 cm Silica Gel G plates and eluted to at least 10 cm in the darkness with the benzene–acetone solvent system. The plates were dried and placed 1 in. from the surface of a Camag Universal UV lamp at 350 nm for 10 min. After this treatment the plates were developed in the second dimension with the same solvent system. This procedure was carried out with two other similarly spotted plates using the 254 nm light for irradiation. All plates were sprayed with triethanolamine–isopropanol (1:4) before visualization[3]. Figs. 5 and 6 show the combined results for each pesticide at the two wavelengths. The methylamine derivatives of the carbamates did not decompose under the 350 nm light but formed a blue fluorescent product as a result of the 254 nm irradiation. This compound (spot No. 5, Fig. 5; No. 7, Fig. 6) did not move during the development in the second direction. The phenol derivatives of the carbamates exhibited a blue spot (Nos. 1 in Figs. 5 and 6)



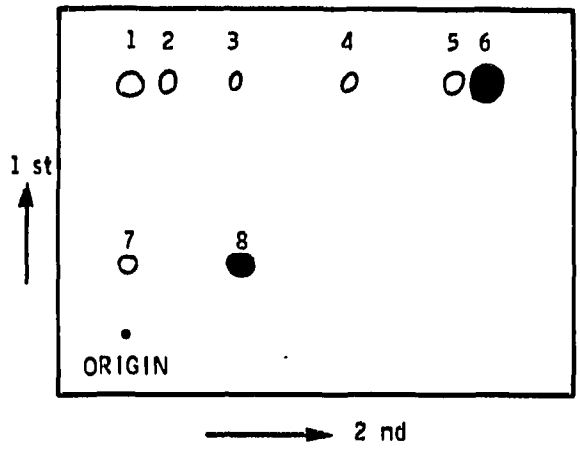


Fig. 5. Chromatogram of the dansyl derivatives of Sevin. The first dimension was run in the dark, the second after 10 min of UV irradiation. Spot Nos. 1–3, decomposition from 254 and 350 nm light; spot No. 3 corresponds to 1-naphthol; spot No. 5 results from 254 nm irradiation. Spot Nos. 4 and 6 are the undecomposed dansyl naphthyl and dansyl methylamine derivatives, respectively.

Fig. 6. Chromatogram of the dansyl derivatives of Mesurol. The first dimension was run in the dark, the second after 10 min of UV irradiation. Spot Nos. 1 and 5 result from 350 nm irradiation. UV light at 254 nm caused the formation of all the decomposition products. Spot Nos. 6 and 8 are the undecomposed dansyl phenol and dansyl methylamine derivatives, respectively.

from the influence of both 350 nm and 254 nm light, similar to that obtained with the methylamine derivatives. In all cases the blue spot was considered to be dansyl-OH (the sulfonic acid). To confirm this, a TLC plate was spotted with separate 1-μg amounts of the dansyl derivatives of the phenyl moiety of Mesurol, the naphthyl moiety of Sevin, and methylamine, along with one spot each of dansyl chloride and dansyl-OH. All spots were irradiated with 254 nm light for 15 min and developed with acetic acid–ethanol–chloroform (5:20:75). The plate was then sprayed with triethanolamine–isopropanol (1:4)[3] and dried in a cool air stream. The result is shown in Fig. 7. It can be seen that all compounds form a blue fluorescent spot identical in

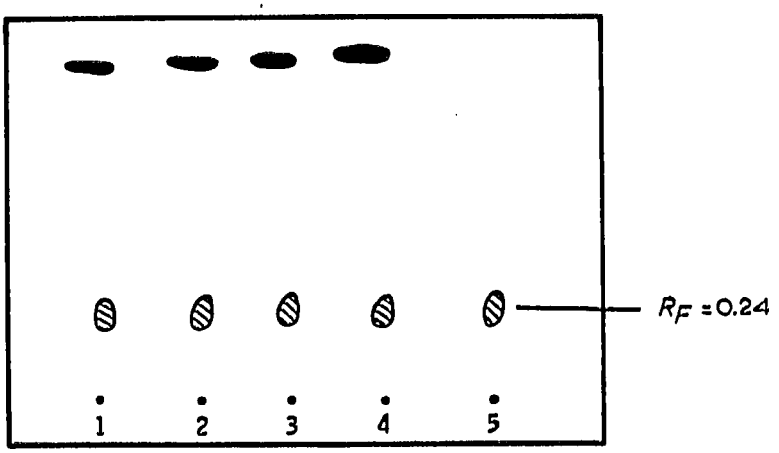


Fig. 7. Chromatogram after 15-min exposure to 254 nm UV light. Developing solvent is acetic acid–ethanol–chloroform (5:20:75). Spot No. 1, dansyl naphthyl derivative of Sevin; No. 2 dansyl phenol derivative of Mesurol; No. 3, dansyl methylamine; No. 4, dansyl chloride; No. 5 dansyl-OH.

R_F to dansyl-OH (R_F 0.24). The fluorescence spectra of these compounds are exactly the same as those of dansyl-OH (ex. 365 nm, em. 485 nm). A control plate was run after being spotted and stored in the dark for 15 min. No blue spots were observed for the derivatives after chromatography. Thus, from these data, it is concluded that one of the products from UV irradiation of the derivatives is dansyl-OH. This indicates that a hydrolysis of the derivatives has taken place. This view is supported by the presence of 1-naphthol as one of the UV decomposition products of the naphthyl derivative of Sevin. To verify this a known 1-naphthol standard was developed parallel to the second dimension (No. 3′, Fig. 5). 1-Naphthol is non-fluorescent when sprayed with triethanolamine–isopropanol, but fluoresces in the blue region of the visible spectrum when sprayed with 1 *N* NaOH[4]. Further identification of the other decomposition products was not carried out, but it can be seen that the effect of UV light must be considered in order to perform meaningful analytical work.

CONCLUSIONS

A TLC system has been devised for the separation of the dansyl derivatives of a number of carbamate pesticides. The possibility of separating carbamate derivatives with low polarity solvents permits a more rapid separation than with non-labelled carbamates. After two-dimensional chromatography, the spots are approximately

twice the size in area as compared to one-dimensional chromatography, which is a disadvantage. Therefore, when spotting, the spots should be kept as small as possible for better resolution and easier *in situ* quantitation. In much of the practical work with carbamates, in water, soil, and formulations analyses for example, two-dimensional chromatography need not be used, since one or the other of the previously mentioned solvent systems will separate the carbamates and interferences sufficiently in one dimension. On the other hand, two-dimensional chromatography is advantageous when analysing complex biological samples where many interferences may be encountered. The incorporation of 0.34% silver oxide in the adsorbent enables a good separation of the phenolic derivatives of Mesurol and Landrin. The silver content must be kept as small as possible in order to prevent significant degradation, which would make reliable quantitative analyses difficult. It was found that UV light causes a hydrolysis of the carbamate derivatives. For practical work, reactions and chromatography should, therefore, be carried out in the dark. The quantitative aspects of this method are currently under investigation.

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