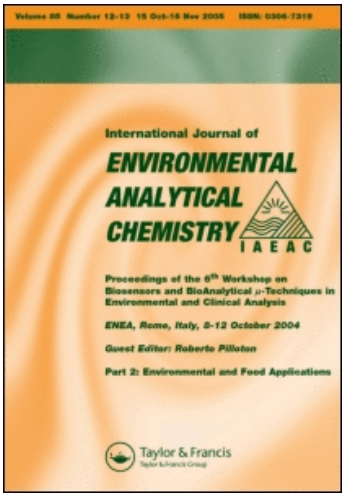

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Analysis of Carbamates as Fluorescent 1-Dimethylamino-naphthalene-5-sulfonate Esters

J. F. Lawrence[a]; R. W. Frei[a]
[a] Department of Chemistry, Dalhousie University, hali, Nova Scotia, Canada

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Analysis of Carbamates as Fluorescent 1-Dimethylamino-naphthalene-5-sulfonate Esters

J. F. LAWRENCE and R. W. FREI

Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada

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The fluorigenic labelling of several N-methyl carbamates is carried out using 1-dimethylaminonaphthalene-5-sulfonyl chloride (dansyl chloride). Prior to chromatography the carbamates are reacted in aqueous solution with the dansyl chloride. The derivative is extracted from the solution with *n*-hexane and subsequently spotted on a thin-layer plate for chromatography. For each N-methyl carbamate, two highly fluorescent spots are obtained. One is common to all N-methyl carbamates studied, whereas the other is characteristic of each individual carbamate. N.m.r. and infrared spectroscopic data indicate that the common spot is the methylamine derivative, and the second spot is the derivative from the phenyl moiety of each carbamate. The sensitivity of the method is less than one nanogram and has been successfully applied to the analysis of natural water samples.

INTRODUCTION

Much work has been reported on the thin-layer (TLC) and gas (GC) chromatographic analysis of carbamates. Direct GC analysis of carbamates is not feasible and methods utilizing derivatization procedures are not entirely satisfactory. Besides being tedious and suffering from poor sensitivity, elaborate clean-up is needed for the majority of samples and materials. The TLC methods available at present are mostly of a semi-quantitative nature and detection limits are fairly high (0.1 to 1 mcg/spot). Recently an attempt

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has been made to remedy this situation by developing an *in situ* fluorimetric technique for Sevin (carbaryl) and 1-naphthol[1] which made use of the native fluorescence of these species after hydrolysis on the plate. The method proved that it is possible to carry out a sensitive and accurate analysis by TLC. However, it is restricted more or less to compounds such as Sevin which exhibit strong native fluorescence in basic media.

In the search for a more generally applicable method which combines the selectivity of TLC and the high sensitivity of fluorimetric techniques, it was decided to investigate fluorigenic labelling procedures. Such an approach has been used in 1956 for amino acids[2] with 1-dimethylaminophthalene-5-sulfonyl chloride (DNS chloride, dansyl chloride). The properties of dansyl derivatives have since been studied by several workers[3–5] in connection with studies carried out on biological systems such as peptides, proteins, alkaloids, antibiotics, enzymes, amines, and phenols. Most of this work was done in connection with TLC, although high-voltage electrophoresis methods have also been reported.[6]

In this study, the possibility of using dansyl chloride as a fluorigenic labelling reagent for N-methyl carbamates was investigated, for the purpose of developing improved analytical methods.

EXPERIMENTAL

Reagents

Analytical-grade dansyl chloride, obtained from Mann Research Laboratories (136 Liberty St., New York. N.Y. 10006), was dissolved in redistilled acetone to form an 0.2% solution. The carbamates tested were Sevin (1-naphthyl-N-methyl carbamate), Matacil (4-dimethylamino-m-tolyl-N-methyl carbamate), Mesurol (4-methylthio-3,5-xylyl-N-methyl carbamate), Baygon (2-isopropoxyphenyl-N-methyl carbamate), Landrin (3,4,5-trimethylphenyl-N-methyl carbamate), Zectran (4-dimethylamino-3,5-xylyl-N-methyl carbamate), IPC (isopropyl-N-phenyl carbamate), and CIPC (isopropyl-N-3-chlorophenyl carbamate). One organophosphate, Proban (O,O-dimethyl-O-p-sulfamoylphenyl phosphorothioate), was also tested. Solutions of these were prepared in methylene chloride at concentrations of 0.001–0.1 mg per ml. The buffer consisted of an 0.1M solution of sodium bicarbonate in distilled water. The spray solution was triethanolamine and isopropanol (20:80, v/v). Reagent-grade chromatographic solvents were used throughout.

Reaction procedure

Ten microlitres of an 0.1 mg per ml solution of each pesticide and one sample blank were placed into individual "Concentratubes" (Laboratory Research

Co., P.O. Box 36509, Los Angeles, Calif. 90036) with a 10-mcl Hamilton syringe. The methylene chloride was evaporated to dryness in a warm water bath for about 5 min. Five microlitres of the dansyl chloride solution were then added, followed by 2 mcl of the buffer. The sides of the tubes were rinsed with 5 mcl of acetone. They were then sealed and allowed to stand at room temperature for at least 2 hr. After this time the content of each tube was spotted on a thin-layer plate for separation of the products.

For the spectroscopic studies, about 30 mg of the dansyl derivative from the phenyl moiety of Sevin was prepared. The reaction conditions were the same as for the residue-size samples, with the exception of the excess of the dansyl chloride.

Chromatography

Silica gel G (Macherey-Nagel, Düren, G.F.R.) was used for most separations. Silica gel N and Alumina G (Merck, Darmstadt, G.F.R.) were used for comparison only. Preparative plates used to isolate the phenyl derivative of Sevin (30 mg) were prepared in the same manner but with a 1 mm adsorbent thickness.

The solvent systems used were benzene-chloroform (2:1) for the derivatives from the carbamates and acetic acid:ethanol:chloroform (5:20:75) for the dansyl derivative of Proban. The chromatography was performed by the ascending technique at room temperature.

After separation, the chromatograms were sprayed with the triethanolamine solution and visualized under a u.v. lamp. The fluorescence spectra were recorded *in situ* with the Aminco-Bowman Spectrofluorometer equipped with a thin-layer scanning attachment.

Spectroscopic studies

The n.m.r. spectrum of the dansyl-phenyl derivative of Sevin was recorded in deuterochloroform with the Varian T-60 n.m.r. Spectrometer. The infrared spectrum was recorded on the dry sample, using sodium chloride discs with the Perkin-Elmer 237B Spectrophotometer.

RESULTS AND DISCUSSION

Chromatography

Chromatographic results indicated that for each N-methyl carbamate, two derivatives were formed which were not present in the blank (Figure 1). One of these spots (R_f 0.09) was constant for all N-methyl carbamates studied. The second spot was found to be characteristic for each individual N-methyl carbamate. No derivatives were observed for the N-phenyl car-

bamates IPC and CIPC, due to absence of hydrolysis under present conditions. The organophosphate Proban (containing an $-NH_2$ group) formed a single derivative which remained at the origin with the same solvent system. Separation of this derivative from the dansyl-OH was possible by using acetic acid:ethanol:chloroform (5:20:75).

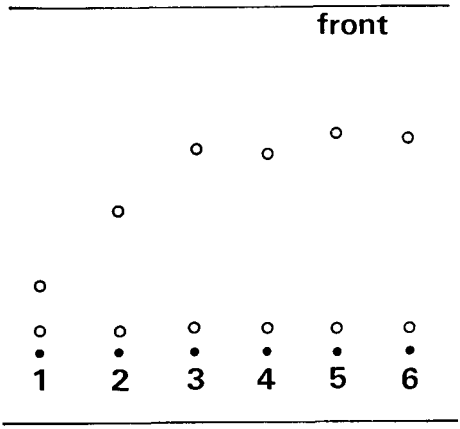


FIGURE 1 A developed chromatogram of the derivatives studied. The adsorbent is silica gel G and the eluting solvent is chloroform:cyclohexane (85:15). The spots R_f 0.09 are the spots common to all N-methyl carbamates, whereas the spots near R_f 0.60 and at R_f 0.21 and R_f 0.44 are the derivatives characteristic for each N-methyl carbamate. 1. Matacil; 2. Baygon; 3. Mesurol; 4. Landrin; 5. Sevin; 6. Zectran. The spots at the origin are dansyl-OH.

Most of the excess dansyl chloride was hydrolyzed to the sulfonic acid, dansyl-OH. It was present in all chromatograms as a bright blue fluorescent spot which remained at the origin with the solvent systems studied and thus does not interfere with the evaluation of the carbamate derivatives. The unreacted dansyl chloride travelled with the solvent front.

Identification of the derivatives

An investigation into the chemical nature of the derivatives was carried out. Since most carbamates are unstable in basic media,[7] it was assumed that they were first hydrolyzed to methylamine and a phenol and each in turn would react with the dansyl chloride. A suggested reaction is shown in Figure 2. The spots common to all N-methyl carbamates (Figure 1, R_f 0.09) would be the dansyl derivative of the methylamine moiety. The second spots would be the dansyl derivative of the phenyl portions which are different for each carbamate and therefore have different R_f values.

Support for the above assumptions is found in the results of the n.m.r. and infrared analyses of the phenyl derivative of Sevin (carbaryl). The n.m.r. spectrum of the derivative (Figure 3) was compared to that of the parent

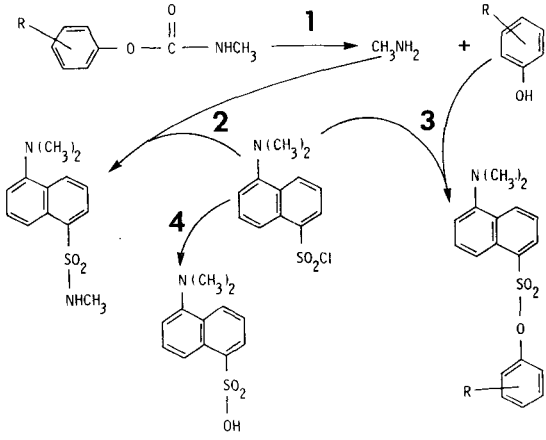


FIGURE 2 A suggested reaction mechanism for the labelling procedure.

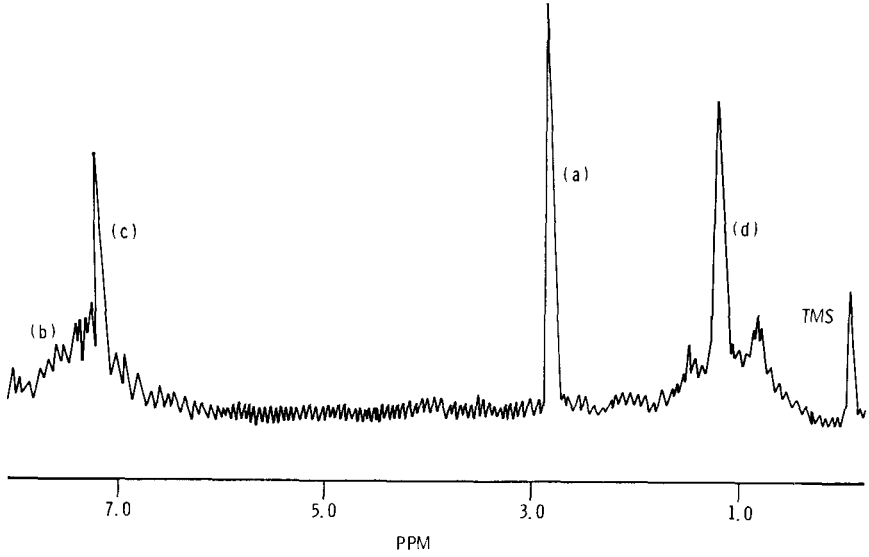
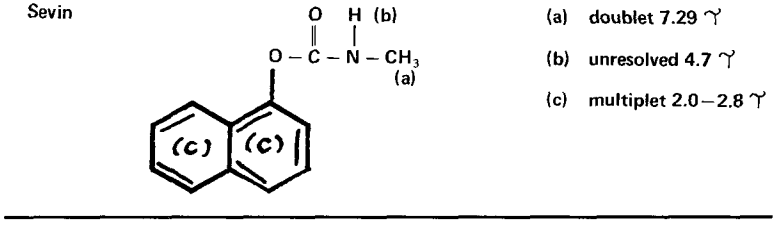


FIGURE 3 The n.m.r. spectrum of the dansyl derivative of the phenyl portion of Sevin. Peak *A* is the N-dimethyl signal of the dansyl molecule. Peaks at *B* are the naphthalene ring protons signals. Peaks *C* and *D* are due to impurities.

carbamate (Table I) as well as to that of the pure dansyl chloride (Figure 4). The spectrum of the derivative displayed signals which corresponded to the various substituents on the benzene ring of the Sevin. The N-methyl doublet of the parent Sevin however is not present in the spectrum of the derivative. This indicates that the derivative does not contain the methylamine portion of the Sevin, hence suggesting that the Sevin molecule is hydrolyzed either before or during the dansyl labelling.

TABLE I



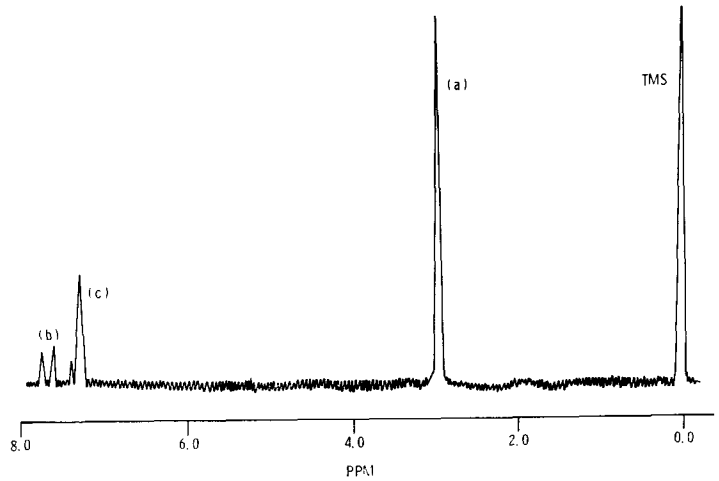


FIGURE 4 The n.m.r. spectrum of the dansyl chloride used in the formation of the derivatives. Peak *A* is the N-dimethyl signal. Peaks *B* are the naphthalene ring protons signals. Peak *C* is due to solvent impurity.

Infrared analysis (Figure 5) also indicates that a hydrolysis of the Sevin occurs, since the strong carbonyl stretching band of the Sevin is absent in the derivative.

For a chemical verification of these assumptions Sevin was hydrolyzed with 2.5N sodium hydroxide.[7] The methylamine was extracted with methylene chloride, and then reacted with dansyl chloride (without evaporating the extract to dryness before dansyl chloride was added, since the methylamine would evaporate as well) as outlined in the experimental procedure. The remaining aqueous solution was acidified and extracted to remove the 1-naphthol which was then reacted with dansyl chloride in the same manner

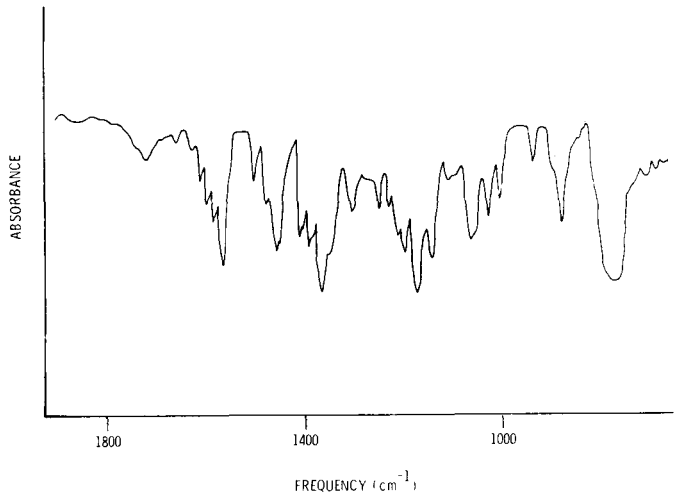


FIGURE 5 The infrared spectrum of the dansyl derivative from the phenyl moiety of Sevin.

as the methylamine. The chromatographic results showed that the methylamine spot was identical to the spot (Figure 1, R_f 0.09) which was common to all the N-methyl carbamates studied. The 1-naphthol extract gave a spot which corresponded to the characteristic Sevin derivative (Figure 1, R_f 0.62). The same reaction was also carried out on methylamine and 1-naphthol obtained from commercial suppliers. The chromatographic results indicated that they formed the same derivatives as those obtained through the hydrolysis of Sevin.

Stability studies

It was found that u.v. light caused degradation of the dansyl-phenyl derivatives,[8, 9] Three or more products were isolated by TLC as a result of u.v. irradiation. Thus, for analytical purposes the formation and chromatography of the dansyl derivatives had to be done in the dark.

The effect of drying of the chromatoplate after development was studied also. It was found that the fluorescence decreased considerably upon drying. The most intense fluorescence was observed while the plate was kept moist with some organic solvent of low volatility, such as triethanolamine, which for this reason was used to spray the plates before instrumental analysis.[10]

Fluorescence spectra

The fluorescence excitation and emission spectra for both dansyl derivatives of Sevin are shown in Figure 6. Both derivatives have approximately the same emission wavelength maxima (530 nm). Similar results were found for all the other N-methyl carbamates studied. The excitation maxima varied from 365–390 nm. The above similarity is not surprising, since the major contributor to the fluorescence of the derivatives is the naphthyl ring of the dansyl

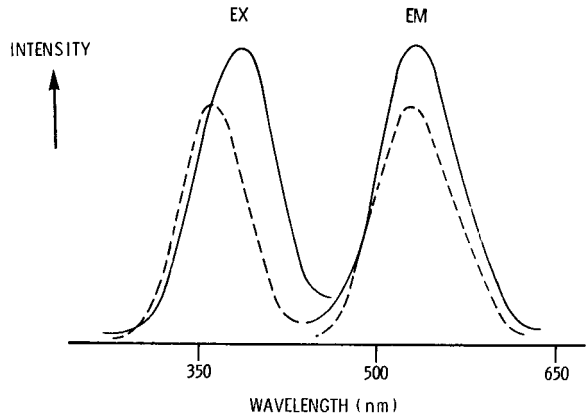


FIGURE 6 The fluorescence spectra of the dansyl derivatives of Sevin. ———dansyl-phenyl derivative. .....dansyl-methylamine derivative.

molecule. In order to greatly influence this fluorescence, the amine or phenyl substituent must be conjugated to the naphthyl ring.[11] This is not the case with the dansyl derivatives, since the presence of the sulfone group makes resonance with the naphthyl ring impossible. The only influence the substituents could have is a weak inductive effect which alters the fluorescence only slightly.

Detection limits

The visual detection limits for this methoapproached about 51 ng of pesticide, whereas instrumental detection limits taken at a 2:1 signal to background

ratio were often less than 1 ng per spot. It was found that detection limits varied from one carbamate to the other. This is believed to be due to the different rates of hydrolysis and reaction with the dansyl chloride.[8]

CONCLUSIONS

Fluorigenic labelling of N-methyl carbamates with dansyl chloride has been shown to have considerable potential as an analytical technique. The reagent is available from commercial sources. The range of applications will depend upon the efficiency of the chromatographic separation techniques available. As with amino acid derivatives, two-dimensional TLC could be used for complex mixtures. The relatively low polarity of the carbamate derivatives permits the use of non-polar, fast-flowing solvents for rapid separations, leaving interfering polar derivatives such as those of amino acids and others at the origin, or permitting reverse-phase high-speed liquid-liquid column chromatography. Studies on the chromatographic properties of these derivatives are currently under way.

The fluorigenic labelling process can serve as a clean-up step, eliminating many interferences from complex samples. Preliminary investigations have shown that dansyl derivatives can also be formed with other pesticides, for example of the organophosphorus, urea, and triazine groups. Other fluorigenic labelling reagents which may form more stable derivatives are also being studied.

Acknowledgements

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