CHROM. 5801

FLUORIGENIC LABELLING OF CARBAMATES USING DANSYL CHLORIDE

II. FLUORESCENCE PHENOMENA OF THE DERIVATIVES

J. F. LAWRENCE AND R. W. FREI*
Department of Chemistry, Dalhousie University, Halifax, Nova Scotia (Canada)
(Received October 25th, 1971)

SUMMARY

The experimental parameters affecting the fluorescence of N-methyl carbamates as their dansyl (1-dimethylaminonaphthalene-5-sulfonyl) derivatives were investigated. Exposure to ultraviolet light causes a degradation of the dansyl derivatives, resulting in slight shifts in fluorescence excitation and emission maxima as well as in a decrease in fluorescence intensity. Spraying the derivatives with triethanolamine in isopropanol improved the *in situ* quantitative analysis of the carbamates. Further increase in fluorescence is observed with sprays consisting of aqueous buffers, but the derivatives are less stable with time. Stabilization is possible, however, by covering the moist chromatoplate with a thin glass sheet. Only 2–3 % loss in sensitivity is observed under these conditions. Long-range storage, however, is not possible with the aqueous buffers. At a pH of less than one, the fluorescence of the derivatives is quenched reversibly.

INTRODUCTION

The development of *in situ* fluorometric techniques by thin-layer chromatography (TLC) for carbamates[1–3] offers advantages over other methods of analysis. The fluorescence technique is extremely sensitive and a number of instruments are now available commercially for the quantitative evaluation of thin-layer chromatograms. TLC has proven useful in residue analysis, although it is not as widely used as gas chromatography because of the lack of suitable quantitative procedures. With the utilization of dansyl chloride as a fluorigenic labelling reagent for carbamates[2,3], analysis of these compounds in nanogram amounts is possible. The choice of this reagent is favoured since highly fluorescent derivatives are formed with amines and phenols, both of which are hydrolysis products of carbamates and therefore can be analysed by this method. The reaction was studied in detail and can be carried out in less than 60 min for most N-methylcarbamates, with detection limits of less than 1 ng per spot for each derivative[3].

In this work, the fluorescence phenomena of the dansyl derivatives were investigated under various experimental conditions in order to obtain optimum results.

* To whom all correspondence should be addressed.

EXPERIMENTAL

Reagents

Analytical-grade dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride) obtained from Mann Research Laboratories (136 Liberty St., New York N.Y. 10006, U.S.A.) was dissolved in redistilled acetone to form a 0.2 % solution The carbamates used were recrystallized analytical-grade materials, which were checked by nuclear magnetic resonance (NMR) and IR spectroscopy (see Table I for chemical names). Solutions of the pesticides were prepared at a concentration of 0.01 mg/ml in methylene chloride. The spray solution consisted of a 20 % solution of triethanolamine in isopropanol, which was compared to spray solutions of aqueous buffers from less than pH 1 to pH 14. Reagent-grade solvents were used throughout

TABLE I

CARBAMATES STUDIED

Carbamate	*Chemical name*
Sevin	1-Naphthyl N-methylcarbamate
Mesurol	4-Methylthio-3,5-xylyl N-methylcarbamate
Baygon	2-Isopropoxyphenyl N-methylcarbamate
Landrin	3,4,5-Trimethylphenyl N-methylcarbamate
Bux	*m*-(1-Ethylpropyl)phenyl N-methylcarbamate and *m*-(1-methylbutyl)phenyl N-methylcarbamate
Mobam	Benzo(*b*)thien-4-yl N-methylcarbamate
Carbofuran	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate
Matacil	4-Dimethylamino-m-tolyl N-methylcarbamate

The thin-layer plates were prepared with a slurry consisting of 30 g of Silica Gel G (Macherey, Nagel and Co., Duren, G.F.R.) and 60 ml of distilled water. This was applied to the plates using the Desaga thin-layer applicator at 250 μm thickness.

Reaction procedure

Ten microlitres of a pesticide solution were placed in a "concentratube" (Laboratory Research Co., P. O. Box 36509, Los Angeles, Calif. 90036, U.S.A.) with a 10-μl Hamilton syringe. The methylene chloride was evaporated by heating in a warm water bath for about 5 min. Five microlitres of a sodium bicarbonate solution of pH 9 were added and the tube was heated at 45° for 30 min. After this time, 3 μl of the dansyl chloride solution were added and the mixture was stirred with the tip of the syringe. The tube was then heated for a further 15 min at 45°. The total content of the tube was then spotted on a Silica Gel G thin-layer plate and eluted with chloroform by the ascending technique. The separated derivatives were sprayed until the plate was barely moist and they were instrumentally evaluated using the Zeiss PMQII Chromatogram–Spectrophotometer and the Aminco-Bowman Spectrophotofluorometer equipped with the thin-layer scanning attachment. When using the triethanolamine spray, the plates were subjected to a cool air stream in the dark for 2 min to remove the isopropanol present before instrumental analysis.

RESULTS AND DISCUSSION

Effect of spray reagents

No significant variations in excitation or emission maxima were observed for any of the derivatives when aqueous buffers above pH 1 or the triethanolamine solution were used as sprays. The emission maxima measured with the Aminco-Bowman instrument varied from 526 to 528 nm with the aqueous sprays. With dioxane as a spray, a large bathochromic shift to about 544 nm was noted for most of the derivatives. This same shift was also observed for amino acids in solution[4], where it was found that this shift varied with the dielectric constant of the solvent used. The excitation maxima remained between 356 and 367 nm for the carbamate derivatives. Although the dioxane spray increased the fluorescence of the spots relative to the triethanolamine, it was unsuitable for quantitative work because of its volatility. The aqueous buffer sprays increased the fluorescence almost two-fold compared with the triethanolamine, but were also found to be less stable with time. This is due to the evaporation of water, as the fluorescence of the derivatives must be measured while the plates are moist. Significant improvement is achieved if the chromatoplate is covered with a 1-mm thick glass sheet immediately after spraying and before instrumental analysis. This results in only a 2–3 % loss in fluorescence intensity. It is not possible to store the plates for any length of time after such treatment since evaporation continues to take place with a corresponding irreversible loss in fluorescence.

Another interesting feature of the aqueous sprays is that whereas the fluorescence of the phenol derivative of the carbamates increases, a decrease in the fluorescence of the methylamine derivative and hydrolyzed dansyl chloride (dansyl-OH) is observed. This could result in the elimination of some interferences in actual sample analyses of the phenol derivatives.

The fluorescence intensity of the derivatives was found to decrease sharply with spray solutions of pH 1 or less (Fig. 1) and is quenched completely when sprayed with a 5 % solution of sulphuric acid. A possible explanation is that the dimethyl-

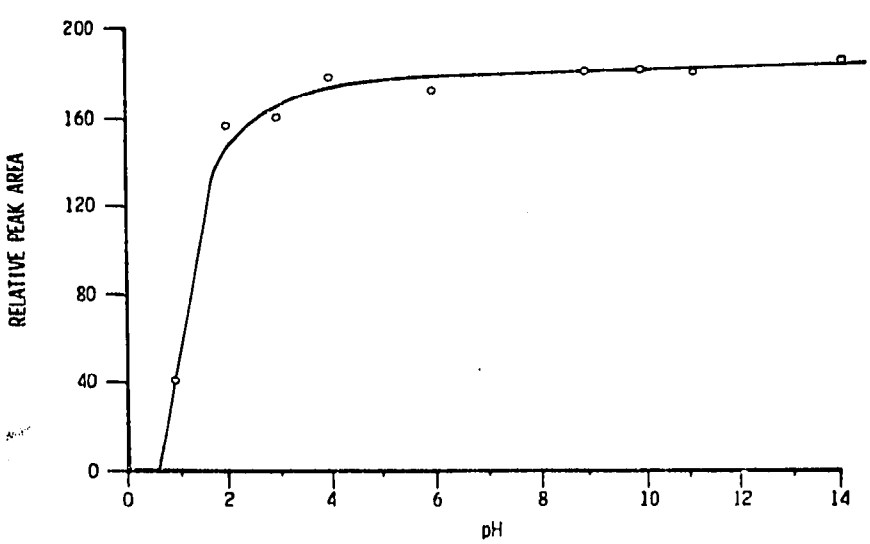

Fig. 1. Fluorescence intensity of the dansyl derivative of the phenolic hydrolysis product of Mesurol (0.1 μg) as a function of pH.

amino group of the naphthyl ring becomes protonated, preventing resonance of the nitrogen lone-pair electrons with the ring (Fig. 2). A similar view was suggested for the change in the fluorescence of 1-naphthol in going from an acidic to a basic medium[5]. In basic solution, 1-naphthol forms the naphtholate anion, which is capable of forming structures similar to those shown in Fig. 2. This results in a visible fluorescence when the molecules return to the ground state from the lowest excited singlet state. In neutral or acidic media this fluorescence disappears due to protonation.

Generally, the triethanolamine–isopropanol spray was preferred for further work because it stabilized the fluorescence. Consequently, repeated scanning of the spots can be performed without any significant loss in fluorescence intensity. Typical fluorescence spectra using triethanolamine are shown in Fig. 3 for the dansyl derivatives of Baygon.

Effect of ultraviolet irradiation

Irradiation of the derivatives adsorbed on Silica Gel G with UV light caused hypsochromic shifts in the emission maxima (Table II). Studies were performed on both the Zeiss and the Aminco-Bowman instruments. The effect of UV irradiation varied for the different carbamate derivatives. Most exhibited shifts in emission maxima of only 5–10 nm when using the triethanolamine spray and some not at all. Sevin and Mesurol were affected most by the UV light. When the derivatives were irradiated with the Aminco-Bowman instrument, they remained more stable than with the Zeiss instrument. This could be due to the fact that the Zeiss instrument uses an excitation filter which gives a band-pass of UV light extending from 320 to

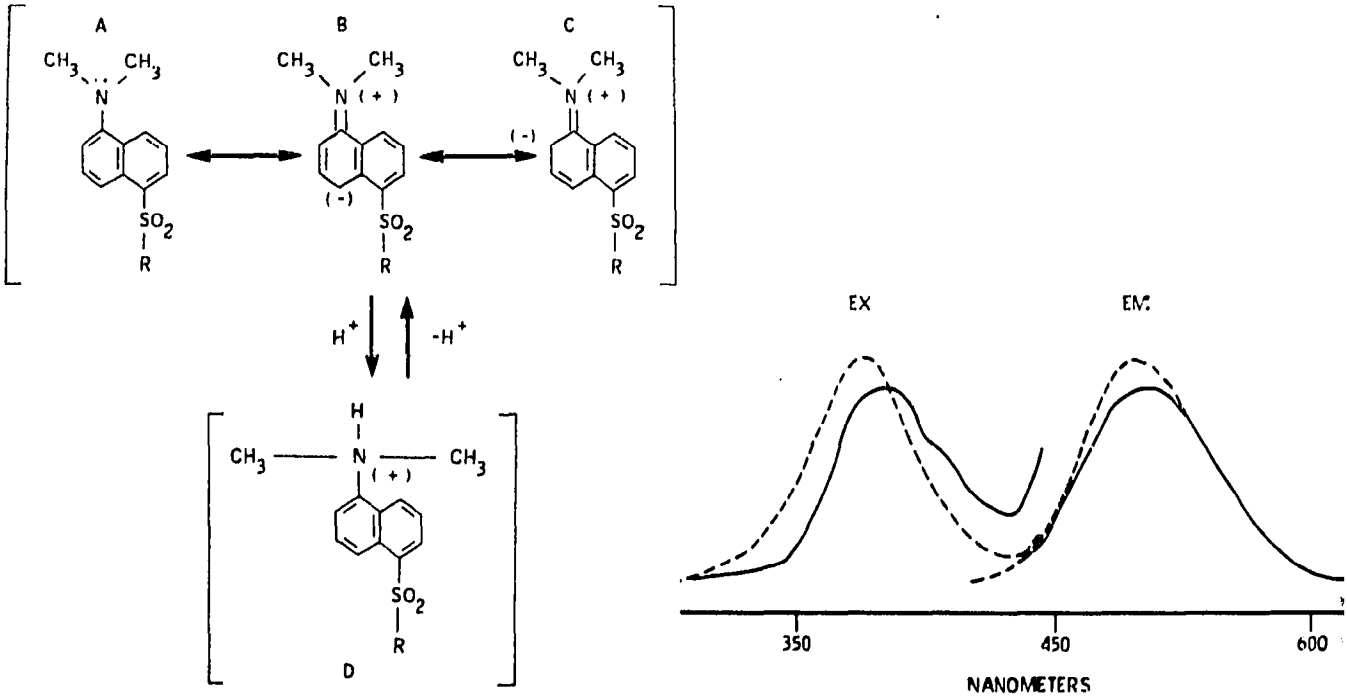

Fig. 2. Resonance forms of the dansyl derivatives. R = $NHCH_3$ or O-aryl.

Fig. 3. Fluorescence spectra of the dansyl derivatives of Baygon. ——, Phenyl moiety; - - - - methylamine moiety.

TABLE II

INFLUENCE OF ULTRAVIOLET LIGHT ON THE FLUORESCENCE SPECTRA OF THE PHENOLIC DANSYL DERIVATIVES OF THE CARBAMATES

A = Aminco-Bowman instrument, excitation and emission, respectively; B = Zeiss instrument, emission using 365-nm ex filter.

Compound	*Wavelength maxima (nm)*							
	Before irradiation		*After irradiation*					
			5 min		*10 min*		*20 min*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Sevin	400;530	530	400;530	500	400;530	495	400;530	485
Mesurol	367;528	525	367;515	510	367;515	500	367;515	500
Matacil	365;540	540	365;540	540	365;535	535	365;535	535
Baygon	365;530	530	365;530	530	360;530	530	360;530	530
Landrin	366;530	530	365;530	530	365;530	530	365;530	530
Bux	365;530	530	365;530	525	360;530	520	360;530	520
Carbofuran	375;530	510	375;530	510	370;522	510	367;522	510
Mobam	395;528	505	395;528	505	395;528	505	395;528	505
CH_3NH_2	356;530	530	365;530	530	365;530	530	365;530	530

400 nm. This shorter wavelength light could be responsible for the greater degree of decomposition than the monochromatic light from the Aminco-Bowman instrument. Another reason could be that each instrument uses a different light source, possibly emitting different intensities of light.

The effect of UV light on the fluorescence intensities of the derivatives was also studied (Figs. 4 and 5). Unlike the wavelength shifts, which were generally small, there was a significant change in fluorescence intensities. All intensities were found to decrease under constant irradiation from the Zeiss lamp. The pattern was different when the derivatives were exposed to light from the Aminco-Bowman lamp. Although some decreased with time, many actually increased in intensity. This increase is due to the dissolution of the adsorbed dansyl derivatives into the triethanolamine after spraying. Once the derivatives are in this liquid environment, they fluoresce more intensely, causing the observed increase with time. For those compounds which exhibited a decrease with time, the UV light most likely has an overriding effect. Thus, when performing practical work, it is necessary to keep this degradation to a minimum. All reactions and chromatographic procedures should be carried out in darkness. On the other hand, the time any one spot spends under the beam of the excitation light is only about 15–30 sec per scan. In this short time very little degradation occurs. The shift in excitation and emission maxima will be negligible under these conditions.

Effect of heat

The dansyl derivatives of the carbamates were found to be stable when heated at 100° for 20 min after chromatography and before spraying. For some carbamates there was a slight increase in fluorescence intensity when compared to an unheated sample. The fluorescence spectra were not affected by the heat treatment.

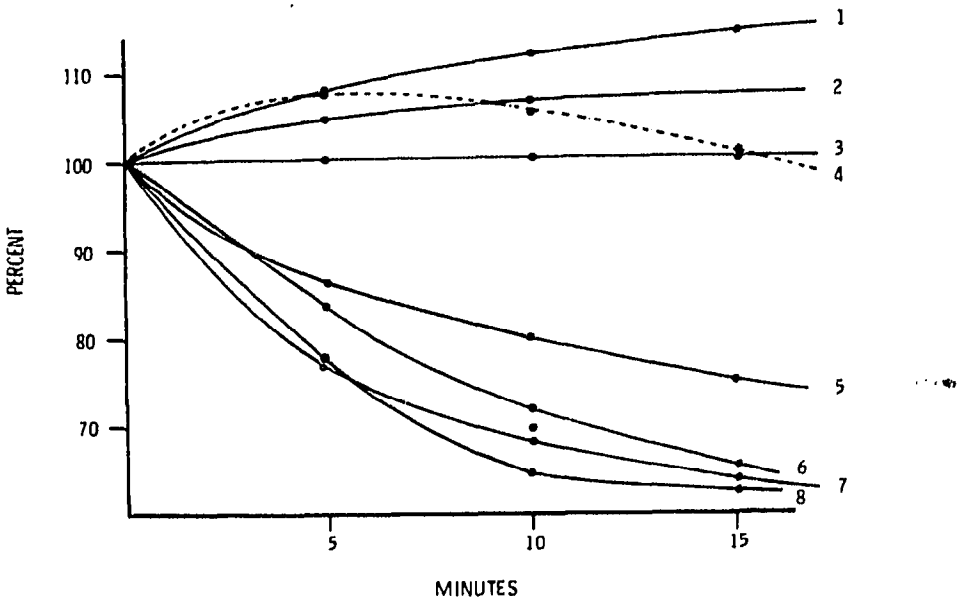

Fig. 4. Influence of initial excitation UV light from the Aminco-Bowman Spectrophotofluorometer on the fluorescence intensities of the phenolic dansyl derivatives of: 1, Mesurol; 2, Baygon; 3, Landrin and methylamine; 4, Sevin; 5, Bux; 6, Mobam; 7, Matacil; and 8, Carbofuran.

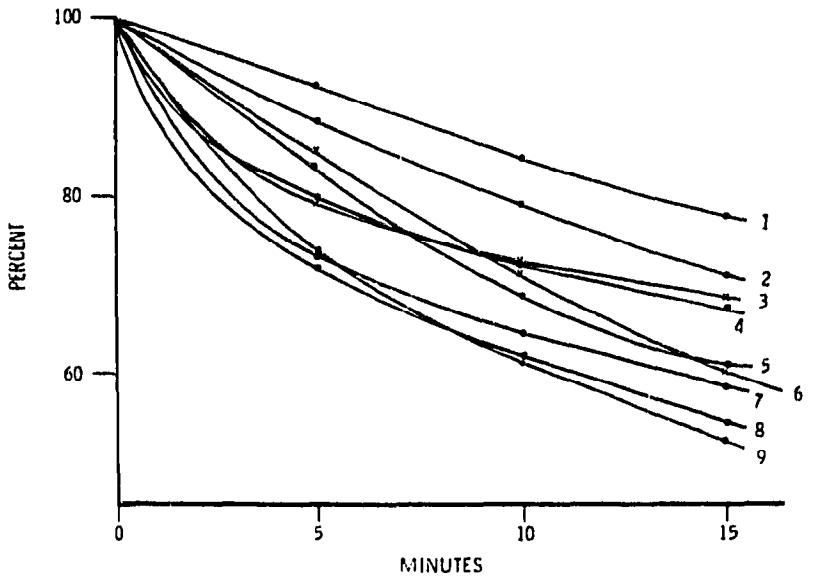

Fig. 5. Influence of UV light from the Zeiss Chromatogram-Spectrophotometer (365-nm filter) on the fluorescence intensities of the phenolic dansyl derivatives of: 1, Sevin; 2, Matacil; 3, Bux; 4, Mesurol; 5, Carbofuran; 6, Landrin; 7, Baygon; 8, Mobam; and 9, methylamine.

CONCLUSIONS

It was found that the dansyl derivatives of some of the carbamates decompose upon prolonged exposure to UV light. With certain precautions, however, the effect in actual sample analysis is minimal. The fluorescence of the derivatives was quenched when sprayed with aqueous solutions below pH 1. Above this value aqueous solutions increased the fluorescence of the derivatives two-fold compared to the triethanolamine spray. These sprays have some use in removing possible interferences in practical work. If no significant interferences are encountered, the triethanolamine spray

should be used because of its ability to stabilize as well as enhance the fluorescence of the derivatives. The method shows good potential for the quantitative analysis of carbamates, which is currently being investigated.

ACKNOWLEDGEMENTS

This work was supported by grants from the Canada Department of Agriculture, the Department of National Health and Welfare, and the National Research Council of Canada.

REFERENCES

1 R. W. FREI, J. F. LAWRENCE AND P. E. BELLIVEAU, *Z. Anal. Chem.*, 254 (1971) 271.
2 R. W. FREI AND J. F. LAWRENCE, *Proceedings of IUPAC Pesticide Congress, Tel Aviv, Israel, February 1971*, Gordon and Breach, New York and London, 1971.
3 R. W. FREI AND J. F. LAWRENCE, *J. Chromatogr.*, 61 (1971) 174.
4 R. F. CHEN, *Arch. Biochem. Biophys.*, 120 (1967) 609.
5 R. T. WILLIAMS, *J. Roy. Inst. Chem.*, 83 (1959) 611.