CHROM. 5528

Fluorigenic labelling of carbamates with dansyl chloride

I. Study of reaction conditions

Carbamate pesticides have frequently been analysed by thin-layer chromatography[1–3]. Though the solvent systems used provide adequate separation, the detection methods leave much to be desired in the terms of sensitivity and reproducibility. *In situ* fluorimetric techniques, though not widely used in residue analysis, show great potential in this respect. The analysis of Sevin (carbaryl) and 1-naphthol by *in situ* fluorimetry[4] has indeed shown that this method of residue determination can be both sensitive and quantitative. For some compounds which are not fluorescent, the possibility of fluorigenic labelling appears promising. This procedure has found wide use in the field of amino acid and peptide chemistry[5]. The reagent most often used in such labelling techniques is dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride). Much information on the chemistry of dansyl chloride reactions is contained in a recent review by SEILER AND WIECHMANN[6]. It reacts with phenols and amines to form highly fluorescent derivatives. The suitability of dansyl chloride in the analysis of carbamates has been confirmed by recent preliminary work[7]. In this paper further investigations of this method are discussed.

Experimental

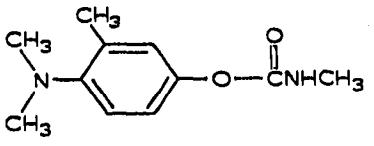
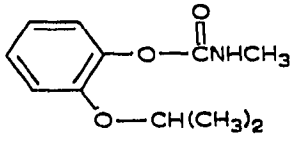
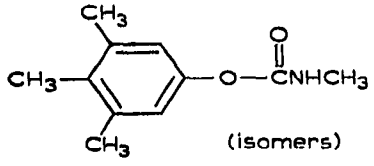
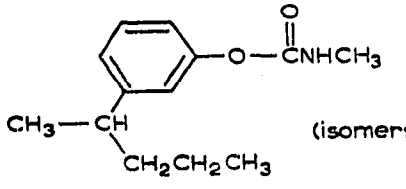
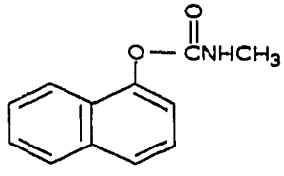
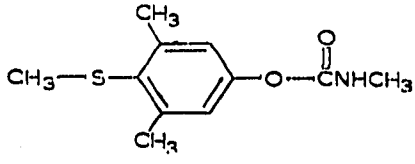
Reagents. Analytical grade dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride) obtained from Mann Research Laboratories was dissolved in redistilled acetone to form a 0.2 % solution. The carbamates used were analytical grade materials recrystallized and verified by NMR and IR spectroscopy (see Table I for the structures and suppliers). Solutions of the pesticides were prepared at a concentration of 0.01 mg/ml in methylene chloride. The spray solution consisted of 20% (by volume) triethanolamine dissolved in isopropanol. All solvents were redistilled reagent grade materials.

Reaction procedure. 10 μl of a pesticide solution were placed in a "concentratube" (Laboratory Research Co.) with a Hamilton 10 μl syringe. The methylene chloride was completely evaporated by heating in a warm water bath for a few minutes. 5 μl of a 0.1 *M* sodium bicarbonate solution were then added to the dry residue and the tube heated in a water bath at 45° for 30 min. After this 3 μl of the dansyl chloride solution were added and the content of the tube stirred with the tip of the syringe. The reaction mixture was then heated for a further 20 min at 45°. At this point the content of the concentratube was spotted on a Silica Gel G (Macherey-Nagel) thin-layer plate and eluted with chloroform–benzene (1:1). The separated derivatives were then sprayed and instrumentally evaluated using the Zeiss PMQII Chromatogram spectrophotofluorometer. The 365 nm excitation filter was utilized for the analysis of all the derivatives with the emitted light being monitored with a monochromater (most of the derivatives exhibited emission maxima near 530 nm).

The above procedure was performed at a variety of reaction times, temperatures for each N-methyl carbamate.

TABLE I

THE STRUCTURES AND SUPPLIERS OF THE CARBAMATES USED IN THIS STUDY

Trade name	*Formula*	*Produced by:*
Matacil		Chemagro corp.
Baygon		Chemagro corp.
Landrin	 (isomers)	Shell
Bux	 (isomers : ortho, 8.8 %; meta, 66.2 %; para, 22.8 %)	Chevron chemical Co.
Sevin		Union carbide
Mesurol		Chemagro corp.

Results and discussion

From preliminary work[7] it was found that the labelling reaction involved a hydrolysis of the carbamates with subsequent coupling of each hydrolysis product with the dansyl chloride. The coupling reaction proceeds faster than either the carbamate hydrolysis or the reagent hydrolysis (the dansyl chloride is converted to the sulfonic acid). Since the hydrolysis of the dansyl chloride is constant at a constant pH or temperature, only the rate of hydrolysis of the different carbamates will govern the yield of dansyl derivatives (Fig. 1). For carbamates such as Mesurol the

hydrolysis rates are fast enought to produce high yields of products without the need of prehydrolysing the compounds. The majority of the carbamates, however, have slow hydrolysis rates compared to that of the dansyl chloride. Therefore, they must be hydrolysed before the labelling reagent may be introduced for coupling. A comparison of the yields of the phenolic dansyl derivatives of Baygon under conditions of prehydrolysis and no prehydrolysis is shown in Fig. 2. The prehydrolysis reactions increase the yield of product almost ten-fold over the reactions without prehydrolysis. Also there is essentially no difference between the results of the prehydrolysis reactions even though different dansyl coupling temperatures were used. This

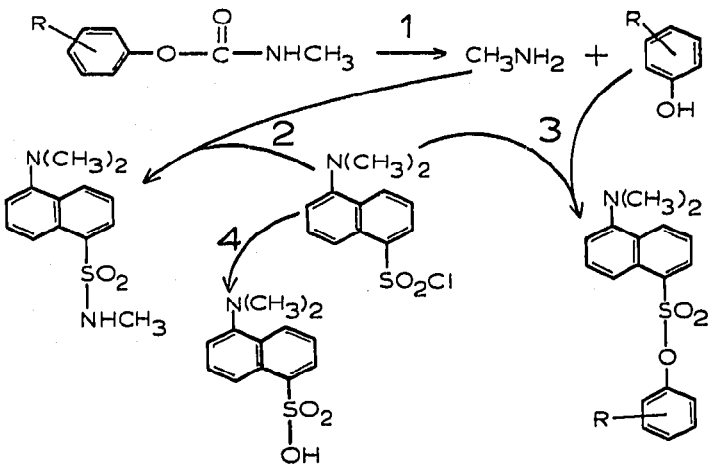


Fig. 1. The reaction of dansyl chloride with N-methyl carbamates. (1) Hydrolysis of the carbamate to the phenol and methylamine, (2) coupling of the dansyl chloride to the methylamine, (3) coupling of the dansyl chloride to the phenol, (4) hydrolysis of the dansyl chloride to the sulfonic acid.

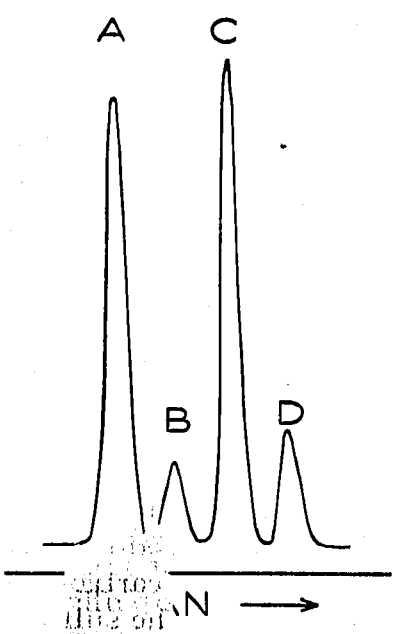


Fig. [illegible] parison of results using Baygon with and without prehydrolysis. (A) Prehydrolysis 30 m[illegible] reaction with dansyl chloride for 4 h at room temperature, (B) direct reaction w[illegible] dans[illegible]de for 4 h at room temperature, (C) 30 min prehydrolysis and reaction with dan[illegible] chlo[illegible]° for 45 min, (D) direct reaction with dansyl chloride for 1 h at 45°.

indicates that the prehydrolysis step is the critical factor in derivative formation and not the dansyl coupling.

The concentration of the dansyl chloride solution was found not to be critical. A minimum of a four-fold molar excess over the carbamates is required. Any greater excess does not affect the results. Thus 1 nmole of carbamate will react equally as well as 1 μmole when 4 μmoles of reagent are used in each case. Though only a two-fold molar excess of reagent in the stoichiometric amount, four-fold is required since the reagent is slowly hydrolysed by the buffer system and other unknown components may also react to use up some reagent.

The rate of hydrolysis of the carbamates was found to increase with an increase in buffer pH. However, at higher pHs the hydrolysis of the reagent becomes very fast causing a decrease in the yields of the dansyl derivatives. pH 9 was found to be optimum.

The rates of hydrolysis of the carbamates increased at higher temperatures as did the reaction rate of the reagent with the hydrolysis products. Figs. 3–5 show the rates of hydrolysis of three carbamates at different temperatures. These were determined by forming the dansyl derivative of the liberated phenol in the hydrolysis mixture of the parent carbamate. The amount of this dansyl derivative formed is directly proportional to the extent of carbamate hydrolysis. While the hydrolysis rate is increased at higher temperatures, there also seems to be a decomposition of the products at longer times (at 55 and 65°). It was noticed that at these temperatures

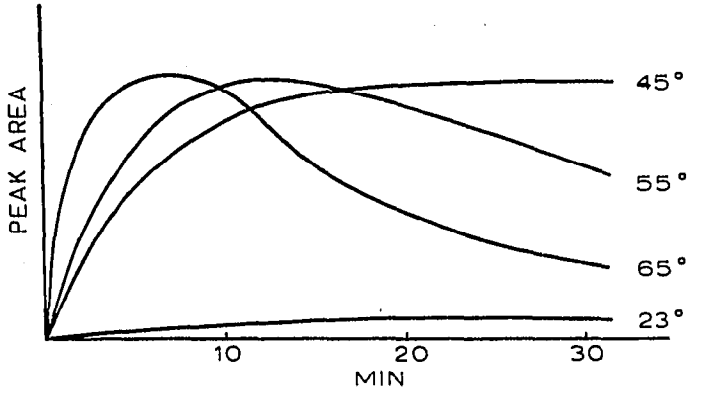


Fig. 3. The rates of hydrolysis at different temperatures for Baygon.

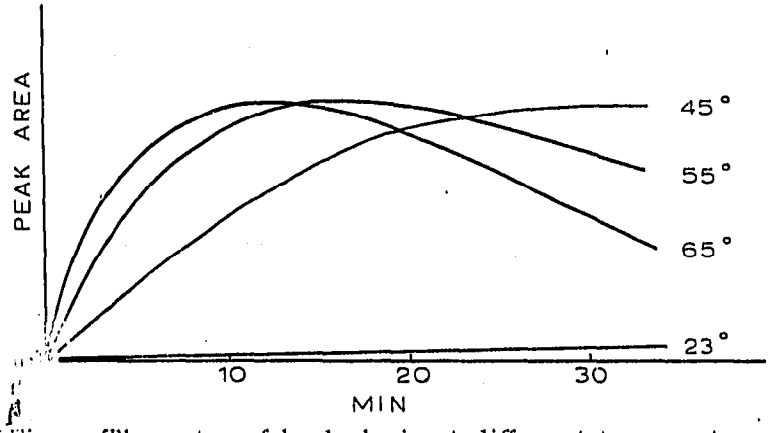


Fig. 4. The rates of hydrolysis at different temperatures for Landrin.

the reaction volume significantly decreased at these times. This was not evident at 45° which, accordingly, was used for all further work since the reaction time is not critical once maximum yield has been attained. The behaviour of Bux and Matacil is similar to Baygon, and Mesurol shows the same hydrolysis properties as Sevin.

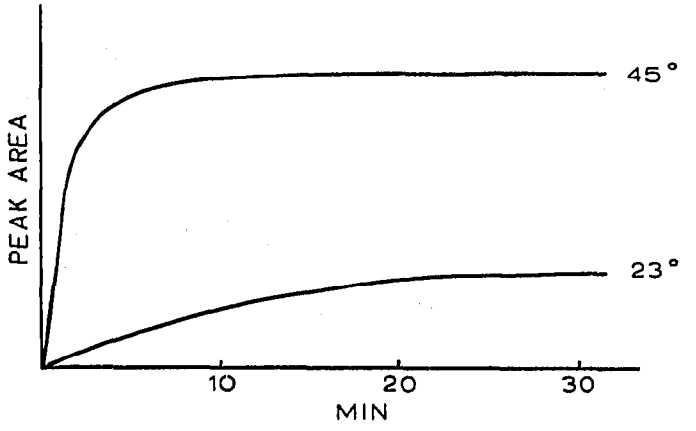


Fig. 5. The rates of hydrolysis at different temperatures for Sevin.

The methylamine derivatives of the carbamates gave similar results to the phenol derivatives, but the phenol derivatives being characteristic of each carbamate were chosen for instrumental evaluation.

At 45° the reagent was found to be completely hydrolysed in 30 min. This was evidenced in the TLC of the reaction mixture. After this time no excess reagent ap-

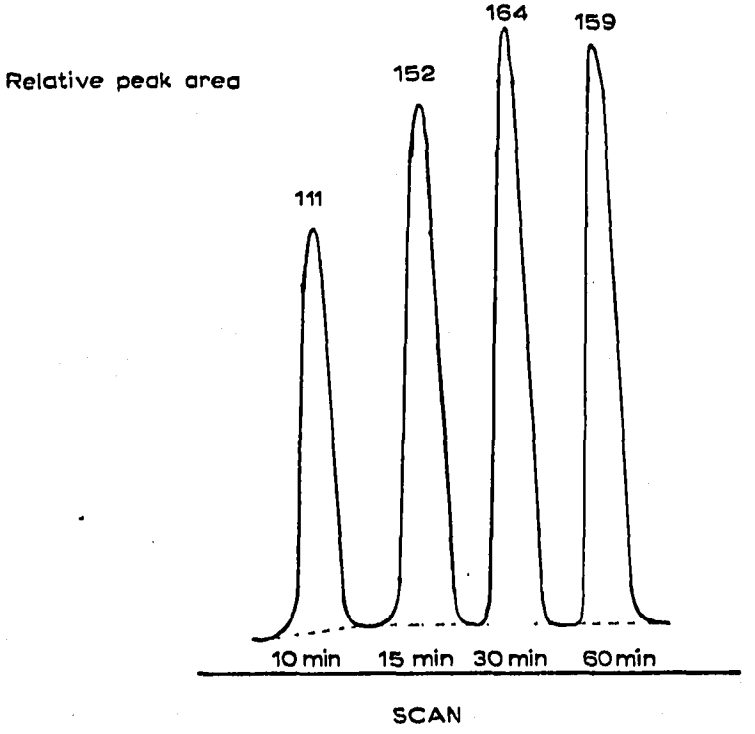


Fig. 6. An *in situ* fluorescence scan of the spots obtained at different times of the dansyl coupli reaction with the phenol portion of 0.1 μg Landrin after 30 min prehydrolysis. Complete formati of the dansyl derivative is obtained after 15–20 min of reaction at 45°.

peared in the chromatograms. However, the coupling with the carbamate hydrolysis products is not affected and is complete in 15–20 min for all carbamates studied (Fig. 6).

Conclusion

Optimum temperature for the hydrolysis and fluorigenic labelling of N-methyl carbamates was found to be 45°. A prehydrolysis step between 10 and 30 min is needed depending upon the carbamate used. A buffer of pH 9 was found best for the reaction. With optimum conditions less than 1 ng per spot can be analysed instrumentally.

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