Journal of Chromatography, 84 (1973) 214–217

CHROM. 6839

Note

An investigation of fluorigenic labelling of chlorophenols with dansyl chloride*

M. FREI-HÄUSLER and R. W. FREI
Department of Chemistry, Trace Analysis Research Centre, Dalhousie University, Halifax, Nova Scotia (Canada)
and
O. HUTZINGER
Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia (Canada)

(First received February 21st, 1973; revised manuscript received May 7th, 1973)

A micro-scale method for the quantitative determination of chlorophenols is of interest not only because several members of this family of compounds are wood preservatives and fungicides, but also because the hydrolysis of a number of widely used pesticides and herbicides yields compounds such as 2,4-dichlorophenol and 2,4,5-trichlorophenol. It has also been shown recently that several chlorophenols are among the metabolites formed from lindane in rats[1].

Chlorophenols have been evaluated semiquantitatively on thin-layer chromatographic (TLC) plates by using different spray reagents[2]. The detection limits reported are in the submicrogram range. An approach to the development of a more sensitive and selective method, which involves the formation of dansyl derivatives separated by TLC and *in situ* fluorescence measurements, is outlined in this note. Although derivatization of free hydroxyl groups is a common procedure in gas chromatographic analysis, it has not been the usual practice to do so in TLC.

EXPERIMENTAL

Analytical grade dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride) obtained from Aldrich Chemicals was dissolved in acetone to form a 0.1% solution.

The compounds tested were 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 3,4-dichlorophenol, 2,4,5-trichlorophenol, pentachlorophenol (all from Aldrich Chemicals), 3-chlorophenol (BDH Chemicals Ltd.) and phenol. Solutions of these compounds were prepared in acetone at $1 \cdot 10^{-3}$ *M* concentration. The buffer used consisted of a 0.1 *M* solution of sodium carbonate in distilled water and the sodium hydroxide solution was 1 *N*. Triethanolamine and 2-propanol in the proportion 20:80 (v/v) were used as a spray solution. The *n*-hexane for extraction and the chromatographic solvents were of reagent grade.

---

[*] Issued as NRCC No. 13374.

Reaction conditions

The reaction conditions were those described previously[3] except that the reaction was allowed to proceed overnight. A 20-μl volume of the hexane extract was spotted on to a thin-layer plate.

Chromatography

Benzene–chloroform (1:1) was used as the solvent for the chromatographic separation of the dansyl derivatives of chlorophenols[4]. Merck-prepared Silica Gel 60 thin-layer plates were used throughout this investigation. After development, the plates were sprayed with triethanolamine in 2-propanol.

Instrumental analysis

The chromatoplates were evaluated with a Zeiss PMQ II Chromatogram Spectrometer in the fluorescent mode. The instrumental settings were the same as those described earlier[3].

Synthesis and mass spectrometric studies

The dansyl derivatives of trichlorophenol and pentachlorophenol were synthesized in the manner outlined previously[3]. Their structures were confirmed by mass spectrometry. The spectra were obtained with a DuPont/CEC 21-491 instrument, using the standard probe for direct introduction of the samples into the ion source.

RESULTS AND DISCUSSION

Chromatography

Some chromatographic data are presented in Table I. The separation of the dansyl derivatives of chlorophenols is influenced by the number of chlorine atoms on the phenol moiety, which produces different lipophilicities and also gives rise to steric effects. The steric effect of *ortho*-substitution in the dansyl derivatives is not as pronounced as with the free chlorophenols[4].

TABLE I

R_F VALUES OF EIGHT DANSYL DERIVATIVES OF PHENOLS

Dansyl derivative of phenol	*R_F value**
4-Chlorophenol	0.60
3-Chlorophenol	0.63
2-Chlorophenol	0.59
2,4-Dichlorophenol	0.56
3,4-Dichlorophenol	0.61
2,4,5-Trichlorophenol	0.79
Pentachlorophenol	0.83

* Average of four plates. Solvent system: benzene–chloroform (1:1).

Fluorescence phenomena

The fluorescence characteristics of the dansyl derivatives of chlorophenols are shown in Fig. 1.

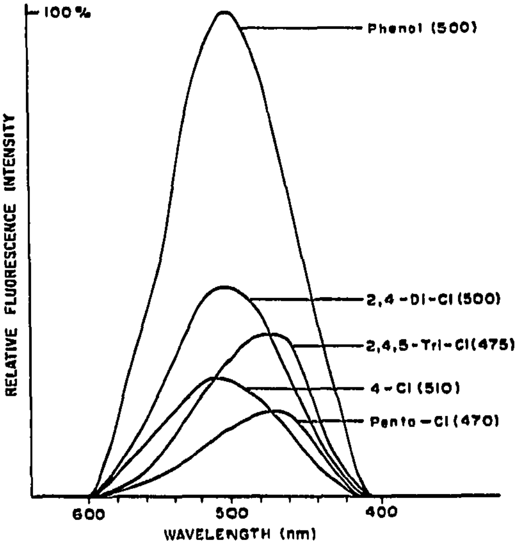

Fig. 1. Fluorescence emission spectra and relative intensities for dansyl derivatives of phenols. Equal amounts of phenols ($4 \cdot 10^{-10}$ mole per spot) were reacted. The spectra are given for the dansyl derivatives of phenol, 2,4-dichlorophenol (2,4-Di-Cl), 2,4,5-trichlorophenol (2,4,5-Tri-Cl), 4-chlorophenol (4-Cl) and pentachlorophenol (Penta-Cl). Emission maxima are given in parentheses.

With regard to the dansyl derivatives of phenol and lower-substituted chlorophenol, a hypsocromic shift in the emission maxima of the higher chlorinated derivatives is observed (Fig. 1).

The fluorescence intensities of the different dansyl derivatives of the phenols were compared. Equal concentrations of the phenols were used for the dansylation reaction. The large difference between the fluorescence intensities of the dansyl derivative of phenol and those of chlorinated phenols may be partially due to the lower reactivity of the latter compounds towards dansylation. Also, the phenolic chlorine atoms exert a considerable quenching (heavy-atom) effect. This is evident from the fact that the same concentrations of synthesized and subsequently chromatographed dansyl derivatives of trichlorophenol and pentachlorophenol show the same difference in fluorescence intensity, *viz.*, fluorescence intensity of the dansyl derivatives (trichlorophenol:pentachlorophenol = 2:1).

The stability of the derivatives under UV light is low. They decompose readily, yielding dansylsulfonic acid, which results in an increase in the fluorescence of the spot to be measured. Hence care must be taken to prevent UV decomposition during development and measurement. UV decomposition of other dansyl derivatives of phenols has been studied by Lawrence *et al.*[5].

Identification of the derivatives

The dansyl derivatives of trichlorophenol and pentachlorophenol were synthesized as previously described[3]. Their mass spectra (*cf.*, Fig. 2) clearly show the molecular ion (trichlorophenol derivative, *m/e* 429; pentachlorophenol derivative, *m/e* 497) as well as the characteristic fragmentation pattern of dansyl derivatives

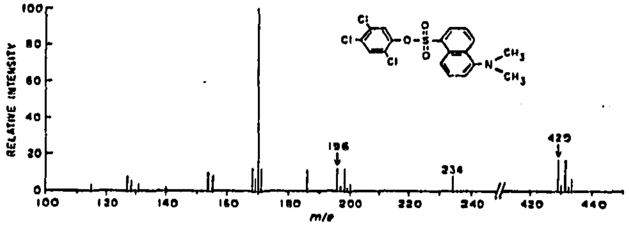

Fig. 2. Mass spectrum (70 eV) of the dansyl derivative of 2,4,5-trichlorophenol.

(*m/e* 170, 234). Ions corresponding to the chlorophenols (trichlorophenol, *m/e* 196; pentachlorophenol, *m/e* 264) were also present in the spectra.

REFERENCES

1 R. W. Chadwick and J. J. Freal, *Bull. Environ. Contam. Toxicol.*, 7 (1972) 137.
2 G. Terdema and P. H. Batelman, *J. Chromatogr.*, 33 (1968) 460.
3 M. Frei-Häusler, R. W. Frei and O. Hutzinger, *J. Chromatogr.*, 79 (1973) 209.
4 B. Smith, *Acta Chem. Scand.*, 16 (1962) 843.
5 L. J. F. Lawrence, D. S. Legay and R. W. Frei, *J. Chromatogr.*, 66 (1972) 295.