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Note

Dansylation of amines, phenolic and catecholic amines and amino acids in aprotic solvents

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Reagents which produce fluorescent derivatives have found widespread use in the qualitative and quantitative detection of amines, phenolic and catecholic amines and amino acids[1]. One of the most common of these reagents is 5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride). Derivatizations using dansyl chloride are usually carried out in aqueous acetone saturated with sodium carbonate; the derivative is isolated by concentration of the reaction solution, extraction with ethyl acetate followed by thin-layer chromatography (TLC). In this short note a procedure is described for the dansylation of some amines, phenolic amines, catecholic amines and amino acids in aprotic organic solvents (*e.g.*, dimethylformamide (DMF), acetonitrile, acetone and ethyl acetate), in which "naked" fluoride anion, solubilized by means of 18-crown-6, activates amino and hydroxyl hydrogen atoms to displacement by the dansyl group. Derivatization of as little as 25 ng (200 pmole) in 10 μl of solvent is possible; the entire reaction mixture may be applied to TLC plates without preliminary concentration or extraction.

EXPERIMENTAL

Reagents and equipment

Dansyl chloride (Calbiochem, Los Angeles, Calif., U.S.A.), 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane) (PCR Inc., Gainesville, Fla., U.S.A.), potassium fluoride and all solvents were used as obtained commercially without purification or drying. Pre-coated silica gel 60 thin-layer plates, 250 μm thick (E. Merck, Darmstadt, G.F.R.; distributed in Canada by BDH, Toronto) were used without prior treatment. Visualization of the dansyl derivatives on TLC plates was achieved by irradiation with long-wave UV light (565 nm) in a light box (Ultraviolet Products Inc., San Gabriel, Calif., U.S.A.). Mass spectra were recorded at 70 eV on an AEI-MS 902S mass spectrometer.

Dansylation procedure

Dansylating solutions were prepared by heating 18-crown-6 (40 mg/ml) and potassium fluoride (8 mg/ml) in DMF, acetonitrile, acetone, ethyl acetate and benzene followed by the addition of dansyl chloride (100 mg/ml) to the cold solution just before use.

p-Tyramine, 3-methoxytyramine, histamine, β-phenylethylamine, dopamine, dibenzylamine, N-methylbenzylamine, *n*-butylamine, aniline, *p*-octopamine, phenylethanolamine, normetanephrine, tryptamine, β-phenylalanine and γ-amino butyric acid were dansylated by the procedures described below.

Method 1. The amine, dissolved in water or ethanol, was introduced into a 0.3-ml Reacti-vial (Pierce, Rockford, Ill., U.S.A.) and evaporated to dryness in a stream of nitrogen. The dansylating solution (50–100 μl) was then added and the mixture heated at 65° in a heating block for 3 h. An aliquot (5–10 μl) of this reaction mixture was then applied to the origin zone of a TLC plate (alternatively the entire reaction mixture may be applied after being concentrated in a stream of nitrogen to *ca.* 25 μl) and developed in benzene–triethylamine (8:1). If the reaction solvent (*e.g.*, DMF) was unsuitable for easy transfer to the chromatograms it may be evaporated to dryness and dissolved in a more suitable solvent (*e.g.*, benzene).

Method 2. The amine, dissolved in acetonitrile, DMF, acetone or ethyl acetate, was introduced into a capillary tube (1.6–1.8 × 100 mm) sealed at one end, concentrated to *ca.* 5 μl and the dansylating solution (10 μl) added. The other end of the capillary was then sealed and the entire tube heated at 65° by submersion in a solvent bath. After 3 h the tube was removed, cooled and the end broken off. The contents after being removed by syringe were applied either in part or *in toto* to the origin zone of the TLC plate.

When the entire reaction mixture was applied to the TLC plates the excess dansyl chloride caused tailing which obscured the separated dansylamine zones. It was necessary in these cases therefore to separate in parallel authentic dansylamines in order to locate accurately the reaction product. The appropriate zones are then extracted with ethyl acetate and re-chromatographed. When only a small fraction of the reaction mixture was chromatographed overloading and tailing did not occur.

The yield of the derivatization reaction was determined for *p*-tyramine. Five different concentrations (6500, 2500, 650, 65 and 25 ng respectively) dissolved in alcohol were transferred to Reacti-vials, dried and derivatized in acetonitrile as described above in Method 1. Each reaction solution was then applied to the origin of a TLC plate. Superimposed on this zone was a known and similar quantity of [α,α-$^2H_2$]bis(dansyl)tyramine[2]. The plate was then developed in benzene–triethylamine (8:1) and the bis(dansyl)tyramine zones, outlined with a metal stylus, removed and placed in special capillary micro-columns and eluted with ethyl acetate as described by Philips *et al.*[2]. Quantitation was achieved by the mass spectrometric integrated ion current technique[2,3]. Yields in all cases were 50–60%.

The identities of the dansyl derivatives of all the compounds investigated were confirmed by comparison of their mass spectra with published spectra (see Table I) and by comparison of their R_F values [in benzene–triethylamine (8:1)] (see Table I) with those of authentic dansyl derivatives[3].

RESULTS AND DISCUSSION

"Naked" fluoride anion, generated from potassium fluoride and crown ethers in aprotic solvents, has been employed in a number of reactions[4–7]. In the dansylation reaction described here, the "electron-rich" fluoride anion probably acts by forming a very strong hydrogen bond with the amino and hydroxyl hydrogen atoms, thereby

TABLE I

R_F VALUES AND DETAILS CONCERNING THE MASS SPECTRA OF SOME DANSYL DERIVATIVES

Dansyl derivative	*R_F value**	*Reference to published mass spectra*
p-Tyramine (bis-dansyl)	0.51	3, 10
3-Methoxytyramine (bis-dansyl)	0.42	10
p-Octopamine (bis-dansyl)	0.17	3, 10
Tryptamine	0.19	3, 10
Histamine (bis-dansyl)	0.33	10
β-Phenylethylamine	0.62	3, 10
Dibenzylamine	0.65	—
N-Methylbenzylamine	0.77	3
n-Butylamine	0.56	10
Phenylethanolamine	0.26	3, 10
Normetanephrine (bis-dansyl)	0.11	3, 10
Dopamine (tris-dansyl)	0.47	10
Aniline	0.25	3
Phenylalanine	0.03	11
γ-Aminobutyric acid**	0.60	10, 11

* Solvent system: benzene–triethylamine (8:1).
** Cyclization to the lactam occurs during dansylation.

increasing the susceptibility of the amino nitrogen or hydroxyl oxygen to attack by the sulfur atom of dansyl chloride.

Dansylation occurred in DMF, acetonitrile, acetone and ethyl acetate, but not in benzene. Although reaction occurred most rapidly in DMF, the low volatility of this solvent rendered it unsuitable for convenient transfer to the TLC plates. Of the other three solvents studied, acetonitrile appeared to produce somewhat higher yields (based on visual comparison of the fluorescence of the zones on TLC plates).

Of the amines studied, four reacted poorly in the conditions described above. For example, about ten times more tryptamine than tyramine was required to give a spot of equal fluorescence, an even greater amount of the β-hydroxylated amines (phenylethanolamine, octopamine and normetanephrine), was required. This anomalous behaviour has not yet been explained.

Two papers by Dünges and co-workers[8,9] have described the dansylation in aprotic solvents (acetone or ethyl acetate) of some barbituric acids in the presence of potassium carbonate. Their method was not successful when applied to the amines listed in Table I. The explanation for this is probably that barbituric acids are very acidic and therefore much more susceptible to dansylation than the weakly acidic phenolic and amino groups.

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