ACENAPHTHENE-5-SULPHONYL CHLORIDE - A NEW FLUORESCENT LABEL

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Dansyl chloride (DNS-Cl) was introduced in 1952 as a reagent for the preparation of fluorescent conjugates of proteins and has since been widely used in the identification of biogenic amines (Weber 1952). DNS-Cl reacts with primary and secondary amino groups and phenolic groups to form highly fluorescent derivatives and finds many applications in chromatographic studies. Acenaphthene is reported (Berlman 1971) to have a fluorescence quantum yield 2.6 greater than naphthalene and as such the analogous fluorescent label acenaphthene-5-sulphonyl chloride (AcNS-Cl) would be expected to have similar labelling characteristics and also provide 2-3 times more sensitivity.

1) Preparation of AcNS-OH.

The method of Dziewonski and Stollywho (1924) was modified as follows; 20g of acenaphthene was dissolved in 100g of nitrobenzene. The solution was cooled to 0^{o} using a salt ice bath. Then 9ml of chlorosulphonic acid was added dropwise, with constant stirring. The temperature was maintained below 5^{o} during the addition. The mixture was allowed to rise to 20^{o} in half an hour. After diluting with 500ml of water, the aqueous layer was separated from the nitrobenzene layer. Solid sodium carbonate was added to neutralise the aqueous solution. The neutralised solution was treated hot with sodium chloride until precipitation was observed, cooled in an ice bath for 1 hour and then filtered. Traces of nitrobenzene and water were removed by heating at 140^{o} for 48 hours in a vacuum oven. Yield 63% Mpt 230^{o} with decomposition. The ^{1}H n.m.r. spectral data corresponded with that previously reported (Cerfontain 1974).

2) Preparation of AcNS-Cl.

10g of acetaphthene-5-sulphonic acid, sodium salt was ground in a mortar with 3.5g of phosphorous pentachloride for three minutes. Following the evolution of hydrogen chloride, cracked ice and water were added and the mixture vigorously stirred to wash out phosphoric acid. The product was extracted into ethyl acetate (100ml), washed with 5%w/v sodium bicarbonate solution and then with distilled water until neutral. Following drying over anhydrous sodium sulphate, and solvent evaporation with nitrogen, acenaphthene-5-sulphonyl chloride crystals were obtained. Yield 41% Mpt $80-82^{o}$.

Comparison of Quantum yield of DNS and AcNS derivatives.

The quantum yield of the DNS-Cl and the AcNS-Cl derivatives of ephedrine and adrenaline were compared by direct fluorimetry using the method of Nachtman (1973). Excitation wavelength for the dansyl-alkaloids was set at 365nm and for the AcNS-alkaloids at 340nm. Emission wavelength for the dansyl-alkaloids was set at 520nm and for the AcNS-alkaloids at 510nm. Linear calibration graphs were obtained over the range 0-0.04mg/ml for both DNS and AcNS derivatives of ephedrine and adrenaline. Regression coefficients for the DNS derivatives of ephedrine and adrenaline were 0.996 and 0.997 respectively and for the AcNS derivatives 1.000 and 0.998. AcNS derivatives were twice as fluorescent as the corresponding DNS derivatives. A detection limit of 3ng/ml ephedrine -AcNS was determined by HPLC. A study of the effect of pH on the fluorescence of AcNS-OH showed only a small variation in fluorescence intensity over the pH range 3.60-14.0. Further work has shown that AcNS-OH fluorescence is quenched on binding to serum albumin and may be competitively displaced by phenylbutazone. As such AcNS-OH can be used as a probe molecule in drug-protein binding studies.

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