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5-DI-*n*-BUTYLAMINONAPHTHALENE-1-SULPHONYL CHLORIDE — A NEW REAGENT FOR FLUORESCENCE LABELLING OF AMINES, AMINO ACIDS AND PEPTIDES

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SUMMARY

The replacement of the dimethylamino group of 5-dimethylaminonaphthalene-1-sulphonyl chloride (DANS-Cl) by the di-*n*-butylamino group eliminates some of the shortcomings of DANS-Cl as a reagent for the fluorescence labelling of compounds that contain primary or secondary amino groups .The derivatives of the modified reagent (BANS-Cl) and of DANS-Cl are formed with similar reaction velocities. As the BANS derivatives are less polar than the DANS derivatives, even BANS-Glu, BANS-Asp and similar amino acid derivatives can be extracted with ethyl acetate from the reaction mixture, and less polar chromatographic systems can be used for their separation. The activation and fluorescence of DANS and BANS derivatives do not differ greatly, but the fluorescence quantum yields of the BANS derivatives exceed those of the DANS derivatives by *ca.* 15% (in ethyl acetate solution). As the modified reagent does not split off dimethylamine under the usual reaction conditions, but dibutylamine instead, it is useful for the microdetermination of methylamine and dimethylamine, which could not be determined quantitatively by the dansylation procedure. A further useful attribute of the BANS derivatives is the high abundance of a typical fragment, which increases the detection sensitivity of the mass spectrometric amine analysis.

INTRODUCTION

5-Dimethylaminonaphthalene-1-sulphonyl chloride (DANS-Cl) is a reagent frequently used for the determination of end-groups in peptides and proteins[1–3] and for the identification and quantitative determination at the nmole or pmole level of amines and amino acids[3–6]. However, as the DANS derivatives of methylamine and dimethylamine are formed from the reagent under the usual reaction conditions, either by direct electrophilic attack of the dimethylamino group, or by a mechanism analogous to that of the Bucherer reaction[3], the dansylation procedure is not suitable for the determination of these amines, which are of considerable biochemical interest. Furthermore, the sensitivity of the mass spectrometric determination of the DANS derivatives[7] is restricted by the relatively extensive cleavage of the C–S bond; the fragment ion of DANS derivatives with the highest relative abun-

dance is *m/e* 170 or 171, with the exception of DANS-indoleamines and some other derivatives. These ions correspond to dimethylaminonaphthalene[8–12].

We have attempted to eliminate these shortcomings of the dansylation method without at the same time changing the principles of the procedure by replacing the dimethylamino group in the reagent with the di-*n*-butylamino group. In this paper we describe the first results obtained with the modified reagent, and a method for the determination of methylamine and dimethylamine in tissue at the pmole level.

MATERIALS AND METHODS

The reagents and solvents used were of AR grade unless otherwise stated.

5-Di-n-butylaminonaphthalene-1-sulphonyl chloride (BANS-Cl)

BANS-Cl was prepared from 5-di-*n*-butylaminonaphthalene-1-sulphonic acid by reaction with PCl_5 in a similar manner to the preparation of DANS-Cl, according to a procedure originally described by Weber[13]. The reaction product was extracted with ethyl acetate and after washing several times with water, the ethyl acetate phase was dried with sodium sulphate and evaporated to dryness *in vacuo*. Purification of BANS-Cl and DANS-Cl was achieved by chromatography on silica gel columns (Silica Gel H, E. Merck, Darmstadt) with toluene as eluent. In contrast to DANS-Cl, BANS-Cl crystallizes very slowly, so that the orange-red oily product was used. This was chromatographically uniform in several solvents.

Reaction of BANS-Cl and DANS-Cl with amines and amino acids

This reaction was carried out in acetone–water (3:1) saturated with sodium carbonate at room temperature in the manner described in detail previously[3,6]. The reaction products were extracted from the reaction mixture, depending on their polarity, either with cyclohexane or *n*-heptane for aliphatic amine derivatives, with toluene for more polar amine derivatives, or with ethyl acetate for the derivatives of amino acids.

Purification of derivatives

The purification of preparative amounts of the derivatives of methylamine, dimethylamine, pyrrolidine and piperidine, was achieved, as in the case of the reagent, by column chromatography on silica gel with toluene as eluent. The chromatographically purified compounds moved as uniform spots in all of the solvent systems tested so far, which were solvents suitable for the separation of a large number of DANS-amides[6,14]. We were not able to crystallize 200–300 mg amounts of the compounds, with the exception of BANS-dimethylamide. After thorough drying over P_2O_5 *in vacuo*, the oily reaction products were used.

Thin-layer chromatography

Thin-layer chromatography was carried out in standard tanks (Camag, Muttenz) lined with filter-paper in order to ensure a solvent vapour-saturated atmosphere. Glass plates, 20 × 20 cm, with 200-μm Silica Gel G layers (E. Merck, Darmstadt) or TLC ready plastic sheets F 1700, micro-polyamide (Schleicher & Schüll, Dassel) were used.

Reaction velocity of dimethylamine hydrochloride with DANS-Cl and BANS-Cl

The reaction velocity was determined by titrimetrically following the formation of HCl. A 4-ml volume of the $2.9 \cdot 10^{-2}$ *M* solution of the acid chlorides in acetone–water (3:1) was placed in the titration vessel of the Autotitrator (Metrohm AG, Herisau), which consisted of an automated 1-ml burette (Dosimat), containing 0.1 *N* NaOH in acetone–water (2:1), a pH meter (E 300 B), a glass electrode (EA 147 X) and an Impulsomat (E 373). The reaction vessel was maintained at exactly 20° by means of a thermostat. After adjusting the pH of the BANS-Cl (or DANS-Cl) solution to 7.5, the automatic titration was started. After several minutes 1 ml of a $3.0 \cdot 10^{-2}$ *M* dimethylamine·HCl solution (in acetone–water (3:1)) was added to the acid chloride solution. The velocity of the addition of NaOH at pH 7.5 was recorded by the recorder attached to the Dosimat. As hydrolysis of the acid chlorides was negligible under the reaction conditions, the total NaOH consumed could be read directly from the NaOH consumption–time curve. Reaction velocities were calculated from the steepness of this curve 0.5 min after the beginning of the reaction.

Fluorescence measurements

Fluorescence measurements were carried out with a Zeiss spectrofluorimeter (Zeiss, Oberkochen) equipped with a quartz monochromator (M4QIII), a glass double monochromator (MM12), a xenon lamp (XBO 450 W) and a 1P28 photomultiplier tube. The slits were adjusted to yield a nominal band width of 7 nm for both excitation (333 nm) and fluorescence (530 nm). Optical densities were determined with a Zeiss spectrophotometer (PMQII), the slit being adjusted to the band width below 0.3 nm.

Fluorescence quantum yields were determined of $1 \cdot 10^{-5}$ *M* solutions of the BANS and DANS derivatives by a comparative procedure[15]. A $1 \cdot 10^{-5}$ *M* solution of 5-dimethylaminonaphthalene-1-sulphonic acid in 0.1 *M* $NaHCO_3$ was used as a comparative standard, the quantum yield of this solution being 0.36 according to Chen[16]. Fluorescence was excited at a wavelength of 333 nm. The ethyl acetate used for quantum yield measurements was purified by washing it with sodium carbonate solution and by distillation. In order to minimize fluorescence quenching by oxygen, the ethyl acetate solutions were purged with a stream of nitrogen for 15 min.

Determination of methylamine and dimethylamine in rat liver

Rat liver (prepared as quickly as possible from animals decapitated while under ether anaesthesia) was homogenized with nine parts of ice-cold 0.2 *N* $HClO_4$. To 10 ml of the homogenate, 200 μl of a standard solution containing 5.42 nmole of methylamine·HCl and 23 nmole of dimethylamine·HCl were added. After 1 h, the sample with internal standard and a similar sample without internal standard were centrifuged for 15 min at approximately $800 \times g$. The supernatant liquids were washed with two 10-ml portions of benzene and with 2 ml of chloroform in order to remove lipids. To 0.5-ml portions of the liver extract, with and without internal standard, 2.5 mg of BANS-Cl (dissolved in 1.25 ml of acetone) were added and saturated with sodium carbonate. Standard samples were prepared by the addition of 200 μl of the above standard solution to 10 ml of 0.2 *N* perchloric acid, and reaction of 0.5-ml portions of this solution with BANS-Cl in the same manner as for the liver extracts.

Then 0.5-ml portions of perchloric acid were reacted with BANS-Cl (blanks). The centrifuge tubes containing the reaction mixtures were sealed with Parafilm (American Can Co., Neenah) and placed into a Bransonic 12 ultrasonic apparatus for cleaning (Branson Instrument Co., Stamford) for 2.5 h. As was realized recently in our laboratory, sonification increases considerably the reaction velocity of the dansylation and bansylation reactions under the conditions mentioned, owing to intensive mixing and pulverizing of the sodium carbonate crystals. In order to remove excess of reagent, 5 mg of proline (dissolved in 20 μl of water) was added. After a further 15 min of sonification, the reaction products were extracted with 4 ml of *n*-heptane. The residues of the heptane extracts were applied to air-dried thin-layer plates, and the plates were divided into two halves by a line. Two samples were applied at a distance of 3 cm from two edges of the plate (one sample on each half of the plate). Development was carried out in the first direction in the usual manner by ascending chromatography with benzene (four runs). In order to immobilize the BANS derivative of γ-aminobutyric acid, the plate was then placed in an ammonia atmosphere for 30 min (ref. 17) and then, without any activation, developed with *n*-heptane–butyl acetate–triethylamine (80:30:5) (one run) in the second dimension in a horizontal tank in such a way that the solvent moved from two opposite plate edges towards the centre of the plate, thus developing the two halves of the plate at the same time. Of course, it is possible to carry out normal two-dimensional separations as well as ascending chromatography if the two halves of the plate are developed sequentially, or if only one sample is applied to the plate.

The fluorescent spots of BANS-methylamide and BANS-dimethylamide were scraped off and extracted with approximately 50 μl of ethyl acetate[18]. The extracts were evaporated and re-dissolved in 100 μl of toluene–acetic acid (99:1). Volumes of 20 μl of these solutions diluted to 100 μl were sufficient for the determination of fluorescence intensities.

Recovery was controlled by comparison of the fluorescence intensities of standard samples, liver extractes, with and without internal standard and of external standards of authentic BANS-methylamide and BANS-dimethylamide, which were chromatographed in the same manner as the tissue and standard samples.

Mass spectrometry

Mass spectra were prepared with a Varian MAT CH5 single-focusing mass spectrometer at an electron beam energy of 70 eV. The temperature of the electron source was 250°.

Quantitative mass spectrometry. A 5-μl volume of the BANS-methylamide and 2.5-μl aliquots of the BANS-dimethylamide solutions used previously for the fluorescence measurement were placed in the capillaries of the direct inlet probe of the mass spectrometer. To the BANS-methylamide samples 0.025 nmole of BANS-ethylamide and to the BANS-dimethylamide samples 0.025 nmole of BANS-diethylamide were added as internal mass spectrometric standards, and in addition 1 nmole of bis-DANS-hexamethylenediamine was added to all samples to increase the amount of evaporating compounds. Quantitation of these samples was achieved in the manner described previously[7] by measuring the ion current of the $(M-43)^+$ fragment of samples and standards.

RESULTS AND DISCUSSION

Reaction of BANS-Cl with amines and amino acids

It is well known that the reaction rates of DANS-Cl strongly depend on pH, temperature and dielectric constant of the solvent[1,3,19,20]. Under certain circumstances, for instance at elevated pH, hydrolysis of the sulphonyl chloride may become the dominating reaction, so that reactions may be incomplete despite the presence of excess of reagent.

In the present experiments, a pH was selected at which hydrolysis of the reagents was negligibly slow. However, the reactions of dimethylamine hydrochloride were quantitative with both BANS-Cl and DANS-Cl within a few minutes, as can be seen in Fig. 1, although low reagent concentrations were used and the reagent excess was not high in the reaction mixture. In Fig. 1, curves of the reactions of BANS-Cl and DANS-Cl with dimethylamine hydrochloride obtained by pH-stat titration at pH 7.5 and 20° in acetone–water (3:1) as solvent are shown. It can be derived from these curves that the (initial) reaction velocity of BANS-Cl with dimethylamine hydrochloride is 73% of that of DANS-Cl under the reaction conditions used. The second-order rate constants calculated from the experimental data (mean of four determinations) were 0.08 $l \cdot min^{-1} \cdot mole^{-1}$ for BANS-Cl and 0.11 $l \cdot min^{-1} \cdot mole^{-1}$ for DANS-Cl.

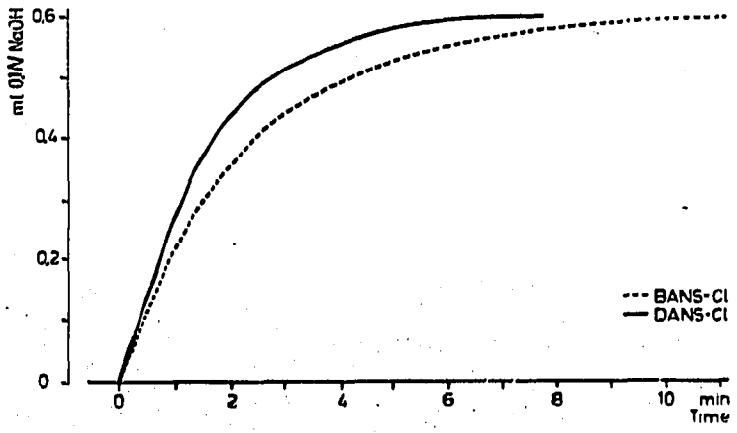


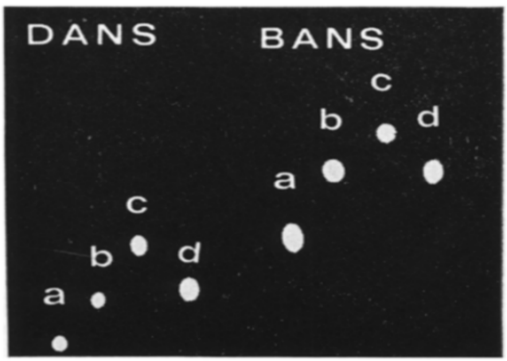
Fig. 1. Reaction velocity of dimethylamine hydrochloride with DANS-Cl and BANS-Cl (pH-stat titration curves). Reaction conditions: pH 7.5; 20°. Solvent: acetone–water (3:1). Initial concentrations of the reactants in the mixture: BANS-Cl and DANS-Cl 0.0233 *M*; dimethylamine·HCl 0.006 *M*.

The lower reaction rate of BANS-Cl compared with that of DANS-Cl is not a serious drawback of the use of this reagent, because under the conditions normally used for the fluorescence labelling of amines and amino acids, the reaction velocities are considerably higher than those measured in the above experiments, in which the pH conditions were relatively unfavourable. In order to obtain complete reaction with small amounts of substances within reasonable times, acetone–water mixtures saturated with sodium carbonate, or similar systems, are recommended if weak bases (for instance anilines) and phenols are reacted with BANS-Cl, under reaction condi-

tions corresponding to those used for the dansylation of these compounds[19]. With stronger bases and also with the fluorescence labelling of peptides and amino acids, the replacement of sodium carbonate with sodium or potassium hydrogen carbonate may be useful, especially as BANS-Cl reacts with amino acids to form BANS-NH_2 and the aldehyde with one carbon atom less than the parent amino acid if the reagent is present in excess in the reaction mixture. In accordance with the slower reaction velocity of BANS-Cl, the fragmentation reaction of this reagent with amino acids is, however, less pronounced than with DANS-Cl[3,21].

Chromatographic behaviour

The chromatographic behaviour of a particular compound is largely dictated by the dipole moment and by specific interactions of the compound with components of the solvent mixture and/or the supporting medium. In Figs. 2 and 3, chromatograms are shown of amine and amino acid derivatives of BANS-Cl and DANS-Cl, respectively. In accordance with expectations, BANS derivatives exhibit increased mobilities compared with the analogous DANS derivatives, owing to the exchange of the methyl group against longer aliphatic chains. This holds provided that systems are used in which the mobile phase is less polar than the stationary phase, but does not depend on the chromatographic system. Whether the stationary phase is a solid medium of a pure adsorption chromatographic system (which is approximately realized in the example shown in Fig. 2), or an adsorbed solvent component of a distribution chromatographic system (which is approximated in the chromatography on polyamide layers, Fig. 3), the relative order of mobilities of the different BANS derivatives is the same as that of the DANS derivatives. Hence, the extensive experience already obtained with chromatographic separations of DANS deriva-



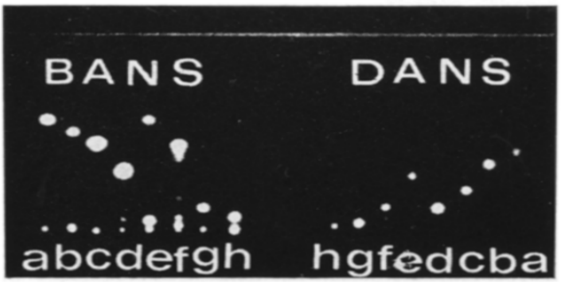


Fig. 2. Thin-layer chromatogram (200-μm Silica Gel G) of some DANS- and BANS-amides. Solvent: cyclohexane–*n*-butyl acetate (8:3)[14]. a, Methylamide; b, dimethylamide; c, piperidide; d, pyrrolidide.

Fig. 3. Thin-layer chromatogram (micro-polyamide) of some BANS- and DANS-amino acids. Solvent: benzene–acetic acid (9:1). a, Isoleucine; b, leucine: c, alanine; d, glycine; e, tyrosine (O-BANS, O,N-di-BANS; O,N-di-DANS); f, lysine (α,ε-di-BANS, ε-BANS; α,ε-di-DANS); g, glutamic acid; h, aspartic acid. The DANS derivatives were authentic samples (Sigma Chemical Co., St. Louis); the BANS derivatives were prepared by reaction of the amino acids with BANS-Cl under the usual reaction conditions. Aliquots of the reaction mixtures were applied to the chromatogram. (The spot at the origin is 5-di-*n*-butylaminonaphthalene-1-sulphonic acid.)

tives[2–4,6,14,22] is still useful if DANS-Cl is replaced with BANS-Cl, and the principles for the selection of the solvent systems for particular separations are the same.

Chromatography with reversed-phase systems has not been applied so far to DANS derivatives, as far as we know. Such systems, however, may have some advantages, particularly with regard to the conservation of the naphthalenesulphonamides, which are destroyed on active surfaces in the presence of oxygen and light. Separations by high-pressure liquid chromatography using stationary phases of non-polar compounds firmly bound to the surface of a solid matrix will presumably be especially effective. However, derivatives of low polarity are generally advantageously separated also by adsorption chromatography, and non-polar compounds can be isolated conveniently from tissue preparations by solvent extraction. In this respect, BANS derivatives are preferable to DANS derivatives in the analysis of amino acids and peptides and other compounds of similar polarity.

Fluorescence

When viewed under UV light (365 nm), DANS derivatives appear on a silica gel plate as greenish to deep orange spots. The fluorescence colour depends strongly on the structure of the reaction component of DANS-Cl and the solvent adsorbed on the thin layer[3,6,19].

Chen[23] has demonstrated that fluorescence maxima are shifted toward longer wavelengths with increasing dielectric constant of the solvent, and a hypsochromic shift of absorption and fluorescence maxima has been observed[19] in alkaline solutions of DANS derivatives of primary amines due to dissociation of the NH group. BANS derivatives cover the same wide range of fluorescence colours, but the corresponding derivatives always show a shift towards the blue region, *i.e.*, BANS-amides appear more greenish than the analogous DANS-amides under identical conditions. This difference in fluorescence colour has an adequate hypsochromic shift in the fluores-

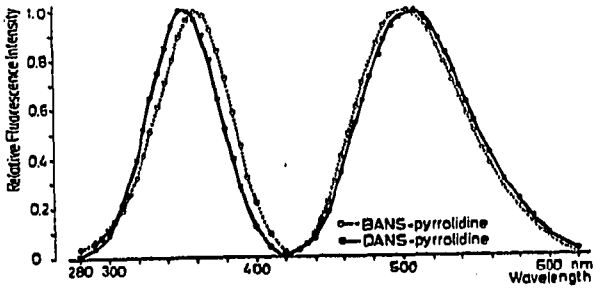


Fig. 4. Activation and fluorescence spectra of BANS-pyrrolidide and DANS-pyrrolidide in ethyl acetate solution (uncorrected instrument readings). Ordinate, relative fluorescence intensities; abscissa, wavelength (nm). Instrument: Zeiss spectrofluorimeter with slits adjusted to yield a nominal band width of 7 nm.

cence maxima, as can be seen in Fig. 4. All of the spectra of BANS derivatives measured so far exhibited fluorescence maxima 2–10 nm shorter in wavelength than the analogous DANS derivatives. In contrast to the hypsochromic shift of the fluorescence maxima, the activation maxima are shifted toward longer wavelengths when the dimethylamino group of the DANS-amides is replaced with a dibutylamino

TABLE I

ACTIVATION AND FLUORESCENCE MAXIMA OF SOME 5-DIMETHYLAMINONAPHTHALENE-1-SULPHONYL-(DANS-) AND 5-DI-*n*-BUTYLAMINONAPHTHALENE-1-SULPHONYL-(BANS-) AMIDES IN ETHYL ACETATE SOLUTION

Uncorrected instrument readings.

Amine	*DANS*		*BANS*	
	Activation $\lambda_{max.}$ (*nm*)	*Fluorescence* $\lambda_{max.}$ (*nm*)	*Activation* $\lambda_{max.}$ (*nm*)	*Fluorescence* $\lambda_{max.}$ (*nm*)
Methylamine	350	502	360	500
Dimethylamine	355	510	360	505
Pyrrolidine	352	508	360	503
Piperidine	352	508	362	505

group, the difference between the activation maxima being more pronounced than that between the respective fluorescence maxima (Table I).

As the activation maxima of the BANS-amides are even closer to one of the most intensive emission lines of a mercury lamp (365 nm) than are the activation maxima of the DANS-amides (both compounds dissolved in ethyl acetate), the use of a mercury lamp is most advantageous for the excitation of fluorescence of these compounds, if they are dissolved in suitable solvents. Superior fluorescence intensities of solutions of BANS derivatives in comparison with corresponding solutions of DANS derivatives result, however, not only from the favourable situation of the activation spectra under certain experimental conditions, but are also based on higher fluorescence quantum yields of the BANS derivatives in comparison with those of the DANS derivatives. On average, the quantum yields of the BANS derivatives exceed those of the DANS derivatives by *ca.* 15% (in ethyl acetate solutions), as can be seen from the figures in Table II.

TABLE II

FLUORESCENCE QUANTUM YIELDS IN ETHYL ACETATE SOLUTION OF 5-DIMETHYLAMINONAPHTHALENE-1-SULPHONYL- (DANS-) AND 5-DI-*n*-BUTYLAMINONAPHTHALENE-1-SULPHONYL- (BANS-) AMIDES

Temperature 22°; exciting wavelength 333 nm. Mean standard deviations ±5%.

Amine	*DANS*	*BANS*
Methylamine	0.61	0.72
Dimethylamine	0.53	0.64
Pyrrolidine	0.59	0.68
Piperidine	0.59	0.68

Mass spectra

As chromatographic behaviour alone in many instances cannot give sufficient criteria for the unambiguous identification of a compound, mass spectrometry is an almost indispensable method in micro-analysis, because other methods available at present are normally not sensitive enough to enable amounts in the range 1 nmole–1 pmole to be measured. Furthermore, mass spectrometric methods for the quantitative determination of small amounts of solid compounds have been developed[7,24–27], so that fragmentation characteristics are of interest, which favour identification and quantitation.

The evaporation profiles of BANS and DANS derivatives are similar, which is of interest for the application of the integrated ion current technique as a quantitative method.

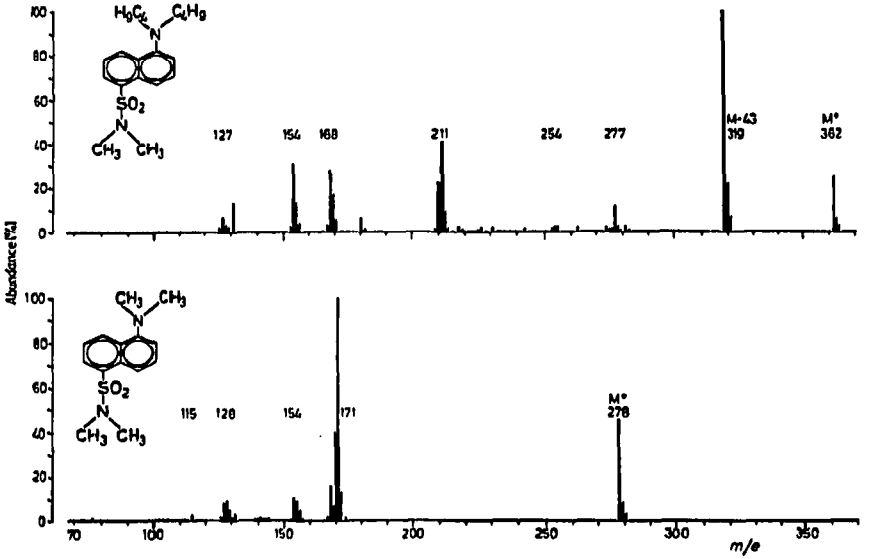


Fig. 5. Mass spectra of BANS-dimethylamide and DANS-dimethylamide. Instrument: Varian MAT CH5 mass spectrometer; electron beam energy 70 eV.

Mass spectra of BANS and DANS derivatives are shown in Figs. 5 and 6. It is obvious that the fragment with the highest relative abundance is the ion at *m/e* (M–43) for the BANS derivatives. This fragment is formed by the usual *β*-fragmentation (metastable transition) of one of the *n*-butyl chains of the dibutylamino group. The molecular ions of the BANS derivatives normally exhibit relative abundances of 25% of the $(M-43)^+$, their intensity being comparable with the intensity of the molecular ions of the corresponding DANS derivatives.

It is well known that with DANS derivatives, the ion with the highest relative abundance is that formed by fission of the C–S bond[8–12]. There are only few exceptions to this rule[9]. Hence the fragment at *m/e* 170 (or 171) is indicative of a DANS derivative. However, this fragment is useless for the identification or the quantitative determination of a particular compound. The replacement of the dimethylamino group with the di-*n*-butylamino group introduces a new "weak link" into the molecule.

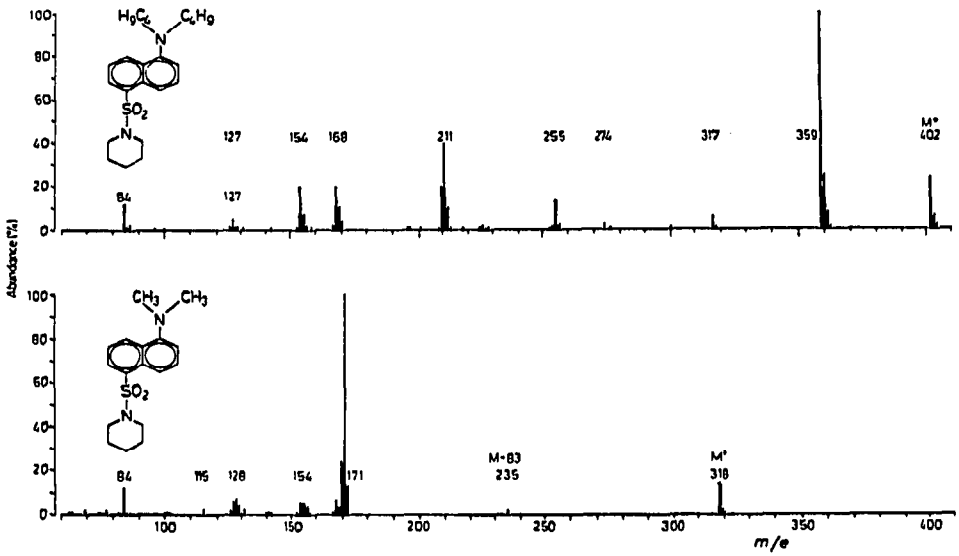


Fig. 6. Mass spectra of BANS-piperidide and DANS-piperidide. Instrument: Varian MAT CH5 mass spectrometer; electron beam energy 70 eV.

The fission of this link, however, produces a fragment that is useful both for identification and for quantitation, as its formation occurs with a higher relative abundance than the formation of the molecular ion of the corresponding DANS derivative. Furthermore, the occurrence of $(M-43)^+$ in addition to M^+ facilitates the discovery of the molecular ions in the spectra of mixtures of substances, especially as the intensities of the M^+ and $(M-43)^+$ ions seem to exhibit constant proportions.

The small extent of C–S bond fission of the BANS derivatives is disadvantageous in some instances as certain DANS derivatives tend to split off SO_2 during an electron impact-induced change, thus producing a typical ion at *m/e* $(M-64)^9$, which is often useful for the identification of certain compounds. For instance, N-DANS-2-oxopyrrolidine, the reaction product of γ-aminobutyric acid with DANS-Cl, and DANS-piperidine have the same molecular weight of 318. The mass spectra of these compounds differ considerably, however, owing to the elimination of SO_2 from the DANS-γ-butyrolactam, while the peak at *m/e* (M–64) is absent in the spectrum of DANS-piperidine. With the BANS derivatives of these compounds, the differentiation of the mass spectra is difficult so that chromatographic criteria or fluorescence spectra have to be used for their identification.

While the spectra of the DANS derivatives show only a few fragments formed from the DANS residue itself, the spectra of the BANS derivatives are characterized by multiple fragment formation from the dibutylaminonaphthalenesulphonyl residue. A fragmentation scheme of the BANS-amides is given in Fig. 7. In this scheme, the fragments formed from the naphthalene ring are omitted, as they are of minor significance in the present work.

While with the DANS derivatives the fission of the S–N bond of the sulphonamide group yields measurable amounts of the dimethylaminonaphthalenesulphonyl ion, yields of the corresponding ion expected from the BANS derivatives at *m/e* 318

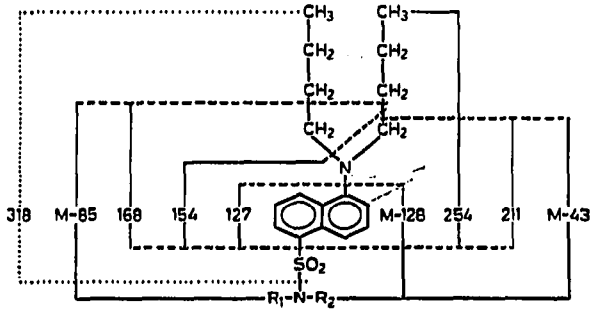


Fig. 7. Fragmentation scheme for BANS derivatives.

(dotted line in Fig. 7) (dibutylaminonaphthalenesulphonyl) were extremely small; at least, we were not able to distinguish the ion formed by the fission of the sulphonamide group from the ion that originated from the fragmentation of the *n*-butyl chains. The same is true for the analogous ions formed from the fragment ion $(M-43)^+$.

With DANS derivatives, we were not able to detect the ion corresponding to naphthalenesulphonamide, while this ion (*m/e* (M–128)) can be observed in the spectra of the BANS derivatives. The ion corresponding to the amine component of the sulphonamides, however, is regularly observed for both DANS- and BANS-amides, as can be seen from the spectra in Figs. 5 and 6, and it is obvious that the fragmentations of the amine components of BANS and DANS derivatives are the same, and very similar to those of free amines.

Determination of methylamine and dimethylamine

Both methylamine and dimethylamine are naturally occurring amines[28–31]. However, there are few results available on these amines, although many methods have been suggested for their determination. Apart from some specific methods[32–34], almost all developments in chromatographic techniques over the last 30 years have been applied to the determination of aliphatic amines[5], including methylamine and dimethylamine. However, only a few methods meet the requirements of sensitivity necessary for the determination of amines in tissues. As interest is focusing more on amines other than catecholic amines, especially in cancer research, although our knowledge of the functional role of these amines is slight, there is still a need for sensitive methods for determining biogenic amines.

As DANS-dimethylamide and DANS-methylamide are side-products of the dansylation reaction[3], it was not possible to determine methylamine and dimethylamine by reaction with DANS-Cl, although this method provides a general basis for the determination of other primary and secondary amines[3,5,6]. BANS-Cl reacts in a similar manner to DANS-Cl, but instead of the derivatives of dimethylamine and methylamine, BANS-dibutylamide and BANS-butylamide are formed in the reaction mixtures.

The method of separation of BANS-methylamide and BANS-dimethylamide, described in detail above, leads to the possibility of determining methylamine and dimethylamine (together with some other amines of comparable polarity) in tissues in the range 0.001–1 nmole with reasonable reproducibility, as can be seen from the

TABLE III

COMPARISON OF METHYLAMINE AND DIMETHYLAMINE DETERMINATIONS IN LIVER EXTRACTS BY FLUORESCENCE MEASUREMENT AND BY MASS SPECTROMETRY

Sample	*Fluorescence measurement*				*Mass spectrometry*			
	Methylamine		*Dimethylamine*		*Methylamine*		*Dimethylamine*	
	Amount of standard (nmole)	*Relative fluorescence intensity*	*Amount of standard (nmole)*	*Relative fluorescence intensity*	*Amount of standard (nmole)*	*Peak area ratio*	*Amount of standard (nmole)*	*Peak area ratio*
Standard sample	0.271	35.8±3.6 (n=3)	1.15	35.6±1.4 (n=6)	0.271	0.41±0.04 (n=3)	1.15	0.98±0.09 (n=6)
Liver extract		57.0±2.3 (n=7)		60.4±6.4 (n=7)		0.68±0.045 (n=7)		1.76±0.15 (n=6)
Liver extract with added standard		87.7±4.5 (n=7)		96.5±7.2 (n=7)		1.08±0.17 (n=6)		2.74±0.08 (n=4)
External standard	0.29	54.4±2.0 (n=4)	0.74	38.8±1.1 (n=4)	0.025	1.10±0.02 (n=5)	0.025	1.30±0.02 (n=5)
Recovery from tissue (%)		94		101		98		100
Recovery of the procedure (%)		71		59		69		65
Amine concentration in liver (nmole·g^{-1})		12±2		66±12		13±2.3		64±12

data in Table III. It should be emphasized that the principles and limitations of the fluorimetric measurements of the BANS derivatives are virtually identical with those established for the DANS derivatives[3,6,19]. In order to ensure the uniformity of the spots measured, and to establish the reliability of the fluorescence method, we examined BANS-methylamide and BANS-dimethylamide separated from the liver extract not only fluorimetrically but also by a recently published mass spectrometric method[7], which is more specific than the fluorimetric method. Mass spectra were also prepared of the BANS-methylamide and BANS-dimethylamide spots. The spectra showed the uniformity of these spots and corresponding quantitative results, obtained by the two different methods of evaluation (Table III) also showed that the suggested separation method yields satisfactory results. In samples with less complex amine mixtures, simplified chromatographic systems might be useful.

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