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IDENTIFICATION AND QUANTITATION OF AMINES BY THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The analysis of biogenic amines requires highly sensitive methods owing to the low concentrations of these compounds in tissues. Since the detection sensitivities of the methods available are limited, relatively large amounts of tissue have to be worked up normally. This implies the necessity of using methods with a high separatory capacity.

The reaction with 1-dimethylamino-naphthalene-5-sulphonylchloride (DANS-Cl) is recommended for the sensitive determination of primary and secondary amines and phenols. If extracts from more than 100 mg tissue are to be analysed pre-separation of the derivatised amines by gel column chromatography is necessary. Thin-layer chromatography is used as the final separation method.

For the unambiguous identification of the DANS-amides, mass spectrometry has turned out to be the most powerful tool. Quantitation of the DANS-amides is achieved by *in situ* fluorescence determinations, or by conventional fluorescence measurements after their elution.

INTRODUCTION

Identification and quantitation of amines can be achieved, at present, by all the standard methods of separation and determination: paper chromatography (PC)[1–11] and thin-layer chromatography (TLC)[12–44], paper and thin-layer electrophoresis (TLE)[3,27,45–50], ion-exchange[4–9,51–54], ligand exchange[55,56], and gel chromatography[57] of the free amines in combination with colour or fluorescence reactions; derivative formation of primary and secondary amines and separation of these derivatives[12,16,58–112]; gas–liquid chromatography (GLC) of free amines[113–117], or of certain amine derivatives[63,118–126], and finally, utilisation of the separatory capabilities of the mass spectrometer[84,86]. All these methods will give reasonable results under favourable conditions.

However, if the amines to be determined are in tissues, body fluids or secretions they generally occur as very complex mixtures. An additional difficulty arises from the fact that endogenous amines occur over a wide concentration range, namely between 10^{-6}–10^{-11} moles/g tissue. Thus analytical methods with high detection

sensitivity and which, in addition, allow the separation of relatively large samples are required. As would be expected no method exists at present which meets all these requirements, since no method alone—with perhaps the exception of high-resolution mass spectrometry (MS)—yields enough information about an unknown amine for its unambiguous identification, as well as allowing its quantitative determination. The solution to this problem would appear to come only from the combined application of several methods.

Combinations of methods have often been applied in the past. Ion-exchange chromatography and PC or TLC have been used most frequently for the identification of naturally occurring amines (see for instance refs. 4–9). More recently GLC directly coupled with MS has been applied to the identification of amines[124] and combinations of TLC with GLC and MS gain more and more attention (see e.g. refs. 127–130).

The method to be described is also a combination of existing methods, and in general gives sufficient qualitative and possibly quantitative information on any primary or secondary amine. The possibilities and limitations of this combination of methods are discussed and are compared with related methods.

CHOICE OF THE AMINE DERIVATIVE

At first glance the separation of free amines seems to be preferable as compared to the separation of amine derivatives, since formation of the derivative is unnecessary and differences between the components of a given amine mixture are diminished because of the increase in the molecular weights. However, separated free amines can only be detected after their transformation into coloured, fluorescent or other suitable derivatives, since they lack in general the attributes necessary for sensitive determination. Interference refractometry, as proposed by CHOULIS[131], would normally not be sensitive enough for biological problems.

At present ninhydrin is still the reagent most frequently used for the detection and quantitation of primary and secondary amines (see for instance refs. 1–9, 12, 18, 25, 32, 45 and 47). Tertiary amines are mostly detected with Dragendorff's reagent or other reagents of similar sensitivity[132], or recently by reaction with α,γ-anhydroaconitic acid[133]. Apart from the fact that there is no sensitive colour or fluorescence reaction which is applicable to the detection of primary, secondary and tertiary amines, these reactions also exhibit a common drawback, viz. the separated amines are generally lost for further characterisation, e.g. by further separation methods. Of course the value of the more or less specific colour of fluorescence reactions for the determination of certain amines (diazo coupling of catecholamines[31,42,44], condensation of indoleamines and of β-phenylethylamines with formaldehyde[13,39–41], etc.) still remains unquestioned by this statement. However, it must be pointed out that the specificity of these reactions is not high enough for unambiguous identification, and even their sensitivity is not at all that high, in the analysis of endogenous amines.

Derivative formation circumvents some of the disadvantages of the analysis of uncoupled amines. Since no suitable derivatives of tertiary amines are known at present, one must accept that only primary and secondary amines can be determined by this method, together with those substances which also react with the

selected reagent. This is a considerable drawback to the methods which employ derivatives and it is cold comfort that this disadvantage affects the ninhydrin reaction as well.

A reagent suitable for the analysis of amines and amino acids should fulfil the following conditions:

(1) Rapid quantitative reaction under mild reaction conditions in water or at least in water-containing media.

(2) Specificity for primary and secondary amino groups, without side reactions.

(3) Excess reagent easily separable from its reaction products.

(4) High detection sensitivity for the reaction products by high extinction coefficients in visible or UV light, by intensive fluorescence, by radioactivity, or other suitable attributes.

(5) Relatively low polarity of the reaction products, which permits their isolation and concentration from the water phase by extraction with organic solvents.

(6) Favourable chromatographic behaviour of the reaction products allowing complete separation.

If the reagent is intended for use in determining the end groups of peptides and proteins, its amino acid derivative must be stable under acid hydrolysis conditions.

A series of reagents have been recommended for the determination of amines and of amino acids. The formulae of the most important of these reagents are shown in Fig. 1. The most frequently used compound among these is 2,4-dinitrofluoroben-
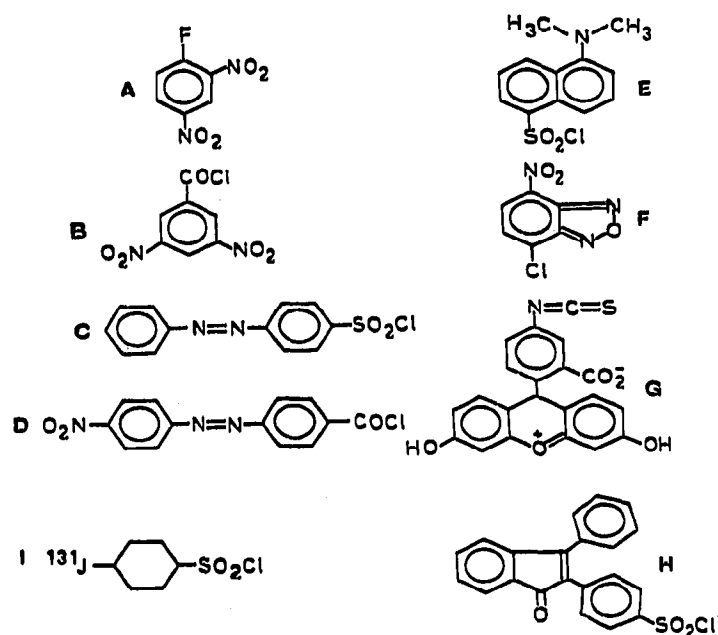

Fig. 1. Structural formulae of some of the reagents recommended for derivative formation with amino acids and amines. A = 2,4-Dinitrofluorobenzene (DNP-F)[134]; B = 3,5-dinitrobenzoylchloride[13]; C = 4-(phenylazo)benzenesulphonylchloride[195]; D = 4-(4'-nitrophenylazo)benzenecarbonylchloride[196]; E = 1-dimethylaminonaphthalene-5-sulphonylchloride (DANS-Cl)[196,197]; F = 4-chloro-7-nitrobenzo-[c]-(1,2,5)-oxadiazole (NBD-Cl)[182]; G = fluorescein isothiocyanate[180]; H = 2-p-chlorosulphophenyl-3-phenylindone[181]; I = p-iodo[131I]benzenesulphonylchloride[136].

zene (DNP-F), which was introduced by SANGER et al.[134,135] as an end group reagent. Its yellow reaction products with amines and amino acids are detectable on chromatograms in amounts down to $5 \cdot 10^{-9}$ moles/spot[64]. A somewhat improved sensitivity is gained by the application of the azo-compounds (C and D in Fig. 1)[106-109], but they are not used to much extent in practice. Reagents E–H (Fig. 1) give intensely fluorescing reaction products with amines and amino acids, which can be detected on chromatograms in amounts of 10^{-10}–10^{-12} moles/spot. Among the radioactive reagents p-iodo[^{131}I]benzenesulphonylchloride (pipsylchloride) is the one most prominent in amino acid analysis[136-138], although, its application in amine analysis seems to have been restricted[139], since no separation systems for pipsylamides have been published as far as is known. [1-^{14}C]dimethylamino-naphthalene-5-sulphonylchloride (DANS-Cl) has also been recommended for quantitative end group analysis of proteins[140], but since the sensitivity of the radioactivity measurements with radioactive DANS-Cl of usual specific activity (1–10 mCi/mmole) is no greater than that which can be obtained by fluorescence measurements (see p. 103), very little can be said in favour of this expensive method other than the convenience of determining the radioactivity by liquid scintillation counting.

None of the compounds discussed so far fulfils the conditions we have laid down for an ideal amine reagent, though it is true that reaction can be achieved at room temperature in water–acetone, water–dioxane or in similar systems at pH 8–10 rapidly and quantitatively, so long as the amine is not autoxidised or hydrolysed under the reaction conditions. However, the reagents mentioned not only react with primary and secondary amino groups, but also with phenols and alcohols. In order to increase the specifity of the dinitrophenylation reaction, different authors have recommended the use of 2,4-dinitrobenzenesulphonic acid instead of DNP-F[141,142]. This reagent has, apart from its non-reactivity with alcohols, the advantage that the excess reagent is easily removed from the reaction products, since it is not extracted from the water–alkali phase. Therefore it is not necessary to destroy it by hydrolysis as is the case with DNP-F, DANS-Cl, etc. A disadvantage of this reagent is its slow reaction speed with secondary amines.

A high detection sensitivity is only characteristic for those reagents which give fluorescent derivatives. Fluorescent derivatives are preferable to coloured compounds, although numerous studies[143-164] have shown that coloured or UV absorbing substances can be quantitatively determined by densitometry, reflectance measurements or by determinations of fluorescence diminution of emitting plates, as well as the photometric determination of the eluted spots. The prerequisites for the quantitative evaluation of paper or thin-layer chromatograms by *in situ* methods are, severe and linear proportionality between the amount of substance and the recorded curve area is only obtained within a narrow concentration range. In contrast to this, *in situ* fluorescence measurements are not only more sensitive but are, as mere light intensity measurements, largely independent of spot size and shape. As has been demonstrated by different authors, linear relationships exist over a wide range between the amount of substance and the recorded area of the curve, so long as concentrations of the substance are avoided such as would cause fluorescence quenching by self-absorption[13,60,157,165-170]. The criterion of extractability is fulfilled for the amine derivatives of all the reagents given in Fig. 1.

Practical experience with fluorescein isothiocyanate (reagent G, Fig. 1)[180] and

with 2-p-chlorosulphophenyl-3-phenylindone (reagent H, Fig. 1)[181] is lacking at present. 4-Chloro-7-nitrobenzo-[c]-(1,2,5)-oxadiazole (NBD-Cl; reagent F, Fig. 1)[182] seems to have advantageous qualities with respect to its reactivity. TLC separations of its derivatives seem to give reasonable results[103-105], though extensive chromatographic studies have not been reported. DNP-derivatives of amines and of amino alcohols have been successfully separated on paper[58,59], on thin-layer[60-67] and even by GLC[67]. Some solvents for the TLC separation of the amine derivatives of the other reagents have also been published[106,108]. Since a considerable amount of experience has been gained in our laboratory on the reactivity of DANS-Cl (reagent E, Fig. 1) and the TLC of its derivatives in the course of the past few years[69-78], and since DANS-Cl has recently been used successfully by other workers for the identification of naturally occurring amines and drug metabolites[68,70,72,75,76,79,80,84-86,88,90-96,99,183,184], attention will now be focussed on this reagent.

THE DANSYLATION REACTION

DANS-Cl in acetone–water (3:1) saturated with sodium carbonate normally reacts quantitatively with primary and secondary amines[69,77,78,83], phenols[69,77,78,101,102] and imidazoles[78], as well as with some alcohols, e.g. choline[70,185,186]. Guanidine and guanidine derivatives, aminopyrimidines and aminopurines will also react with DANS-Cl[78].

Side reactions with amines seem to be rare, if autoxidation of catecholamines or similar reactions are excluded from consideration, but the partial splitting of agmatine to bis-DANS-putrescine, and the conversion of cysteamine to bis-DANS-cystamine has been observed[78]. However, DANS-Cl itself gives some fluorescent products under the reaction conditions. For example, DANS-methylamide and DANS-dimethylamide have been identified, their amount being dependent on the amount of DANS-Cl, pH and temperature of the reaction mixture and the duration of the reaction. DANS-NH_2 is also always found on the chromatograms, but the NH_3 may derive from the atmosphere. On this basis methylamine and dimethylamine cannot be determined by the dansylation procedure. Furthermore a reaction product of DANS-Cl, acetone and ammonia, DANS-2-methyl-2-amino-pentan-4- one, has been identified in dansylated tissue extracts[187]. In addition to these side products there is also a series of unidentified side products of the dansylation procedure. The formation of side products from DANS-Cl under the reaction conditions is one of the handicaps of this reagent.

The DANS-amine derivatives, with few exceptions, are easily extracted from the alkaline mixture with toluene, benzene or ethyl acetate, while the amino acid derivatives remain mostly in the alkaline phase. Therefore excess DANS-Cl in the reaction mixture is preferentially destroyed by reaction with added excess proline rather than by hydrolysis. Due to the splitting reaction of α-amino acids, however, the aldehyde with one carbon less than the parent amino acid[188] is formed, thus the corresponding aldehydes of the basic amino acids, i.e. γ-aminobutyraldehyde from ornithine[78], and δ-aminovaleraldehyde from lysine[78,95], are found together with the amines, if the parent amino acids were present in the reaction mixture. (The aldehydes corresponding to arginine and histidine have not yet been identified, though these amino acids also exhibit side reactions with DANS-Cl). Furthermore the fact
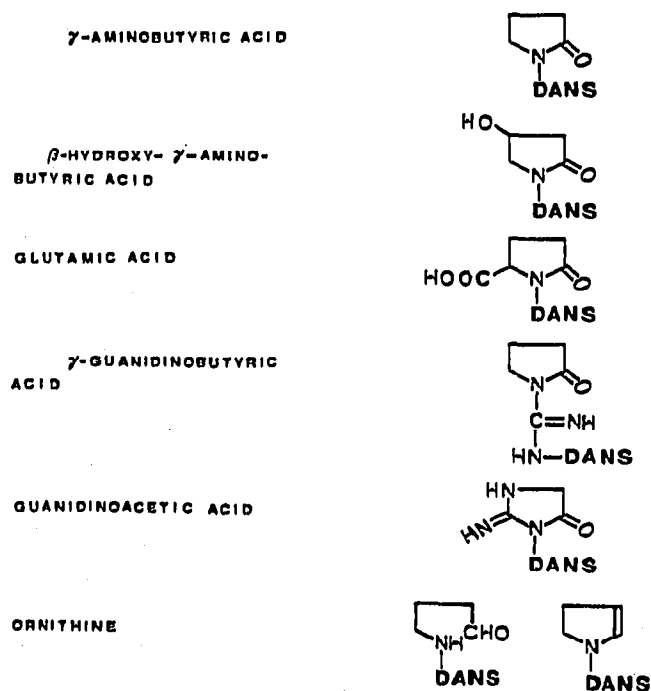
γ-AMINOBUTYRIC ACID

β-HYDROXY- γ-AMINO-
BUTYRIC ACID

GLUTAMIC ACID

γ-GUANIDINOBUTYRIC
ACID

GUANIDINOACETIC ACID

ORNITHINE

Fig. 2. Amino acids with an amino group in the γ-position and their respective reaction products with 1-dimethylaminonaphthalene-5-sulphonylchloride[78,80,05,180].

that certain amino acids with an amino group in the γ-position form dansylated γ-lactams, which move together with the DANS-amides on TLC, must be taken into account. The reaction products of those γ-amino acids which have been studied to some extent previously[73,76,77,180] are shown in Fig. 2. The formation of dansylated lactams from γ-aminobutyric acid, β-hydroxy-γ-aminobutyric acid and γ-guanidino-butyric acid has been used for the detection or quantitation of these amino acids in different tissues[80,81].

SEPARATION AND DETERMINATION OF DANS-AMIDES

DANS-amides have been separated by PC[82,83,87,100] and by TLC[68-81,84-86,88-102]. TLE was not very successful as a separation method[71].

The outstanding qualities of the DANS-amides for TLC separation on Silica Gel G layers are illustrated in Figs. 3 and 4. Spots are normally regularly shaped and do not tail, even on heavily overloaded plates, if solvents systems that have been worked out for the separation of the DANS-derivatives of more than 100 amines of biological or pharmacological interest[78] are used. It can be stated that nearly every separation of interest can be achieved by two-dimensional TLC on silica gel plates. It is difficult to compare the efficiency of the TLC separations of the different amine derivatives. In our opinion, however, the most important quality of the DANS-derivatives for identification and determination of amines is their excellent chromatographic behaviour.

Apart from silica gel the only other layers used have been polyamide layers for

the apparent separation of DANS-serotonin from DANS-amino acids[100], and Al_2O_3 layers for the separation of certain drug metabolites[97].

It has already been mentioned that DANS-derivatives are intensely fluorescent compounds. Their excitation (350–375 nm) and fluorescence maxima (500–550 nm), and their fluorescence intensity are, apart from environmental parameters, dependent on the structure of the amine component[69,95,100,101], so that the colour of the fluorescence gives some qualitative information on the molecular entity. Since
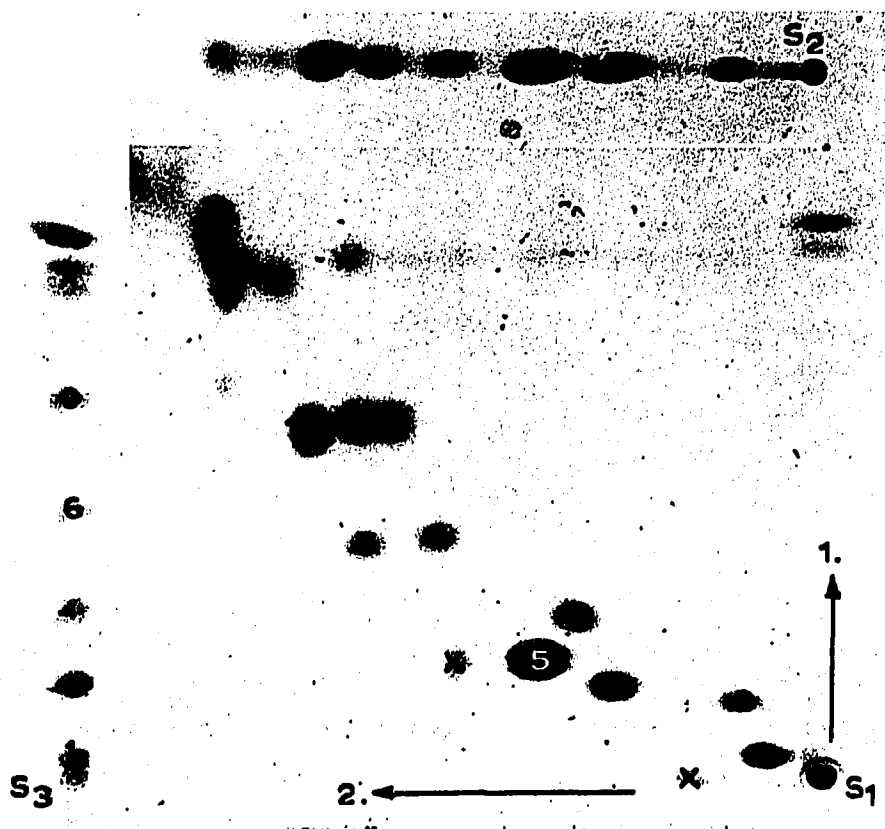

Fig. 3. Bidimensional separation of a dansylated mixture of catechols. S_1 is the starting point of the bidimensional separation and S_2 and S_3 are the starting points of the reference chromatograms on the edges of the plate. Solvents: chloroform for the first dimension and butyl acetate–cyclohexane–ethyl acetate–triethylamine (11:10:4:4) for the second. (The plate was developed twice in each direction.) 1 = Bis-DANS-3,4-dihydroxyphenylglycol; 2 = bis-DANS-N-acetyl-dopamine; 3 = tri-DANS-noradrenaline; 4 = bis-DANS-3,4-dihydroxyphenylethanolamine; 5 = tri-DANS-α-methyl-noradrenaline; 6 = tri-DANS-adrenaline; 7 = tri-DANS-α-methyladrenaline; 8 = tri-DANS-3,4-dihydroxy-β-phenylethylamine; 9 = tri-DANS-α-methyldopamine; 10 = tri-DANS-N-isopropyl-noradrenaline; X = side-reaction products of the dansylation. (The mixture contained approximately 10^{-9} moles of each substance.)

humidity and low pH cause diminished quantum yields, heating of paper chromatograms[83], drying the silica gel layer, then spraying with triethanolamine[69] enhances the intensity of the fluorescence of DANS-derivatives considerably. In favourable cases as little as 10^{-12} moles/spot of a DANS-derivative can be visualised on a normal thin-layer plate and also measured quantitatively by direct scanning, or by fluorescence measurements in micro-cuvettes after elution. The usual DANS-amides can be extracted from the silica gel with benzene–triethylamine (95:5),
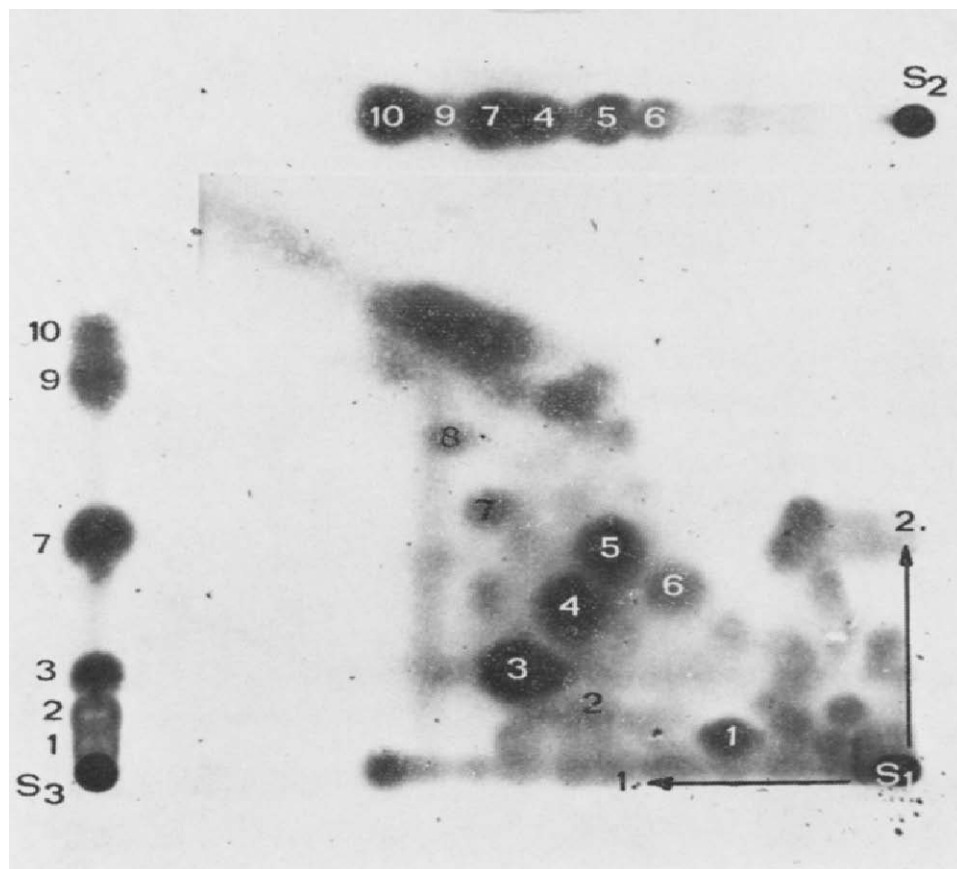
Fig. 4. Bidimensional separation of the dansylated perchloric acid extract of approximately 20 mg small gut tissue. S_1 is the starting point of the tissue sample and S_2 and S_3 are the starting points of the reference samples. Solvents: ethyl acetate–cyclohexane (3:2) for the first dimension and benzene–triethylamine (5:1) for the second. 1 = DANS-ethanolamine; 2 = bis-DANS-serotonin; 3 = DANS-NH$_2$; 4 = tri-DANS-spermidine; 5 = tetra-DANS-spermine; 6 = bis-DANS-histamine; 7 = DANS-methylamine; 8 = DANS-aminovaleraldehyde (from lysine); 9 = DANS-β-phenylethylamine; 10 = DANS-piperidine.

benzene–acetic acid (99:1), or dioxane; DANS-derivatives with tertiary amino groups (*e.g.* DANS-butofenine) or other polar DANS-derivatives (*e.g.* DANS-amino acids) are extracted with dioxane–conc. ammonia (9:1) or with methanol–conc. ammonia (19:1)[69].

As a result of ample experience[69,72,73,75,79,81,84,86,172–174,176,185,186], direct scanning and extraction procedures are reproducible with a standard error of ± 2–6%, the dansylation procedure included. This is not only true for pure substances, but also for determinations of endogenous amines in tissues, as has been demonstrated for the determination of the polyamines spermine and spermidine in different tissues, for the determination of γ-aminobutyric acid in brain, and for the determination of the activities of certain hydrolytic enzymes. Recovery was 100% in these cases. However, these substances could be separated by uni-dimensional chromatography, with the reference samples on the same plate. If spots from different plates were compared, a relative standard deviation of 10–15% was observed[173,174]. Though the reproducibility obtainable from different plates can be improved by careful selection of the plates (see ref. 13), it is nevertheless preferable to compare the tissue sample with
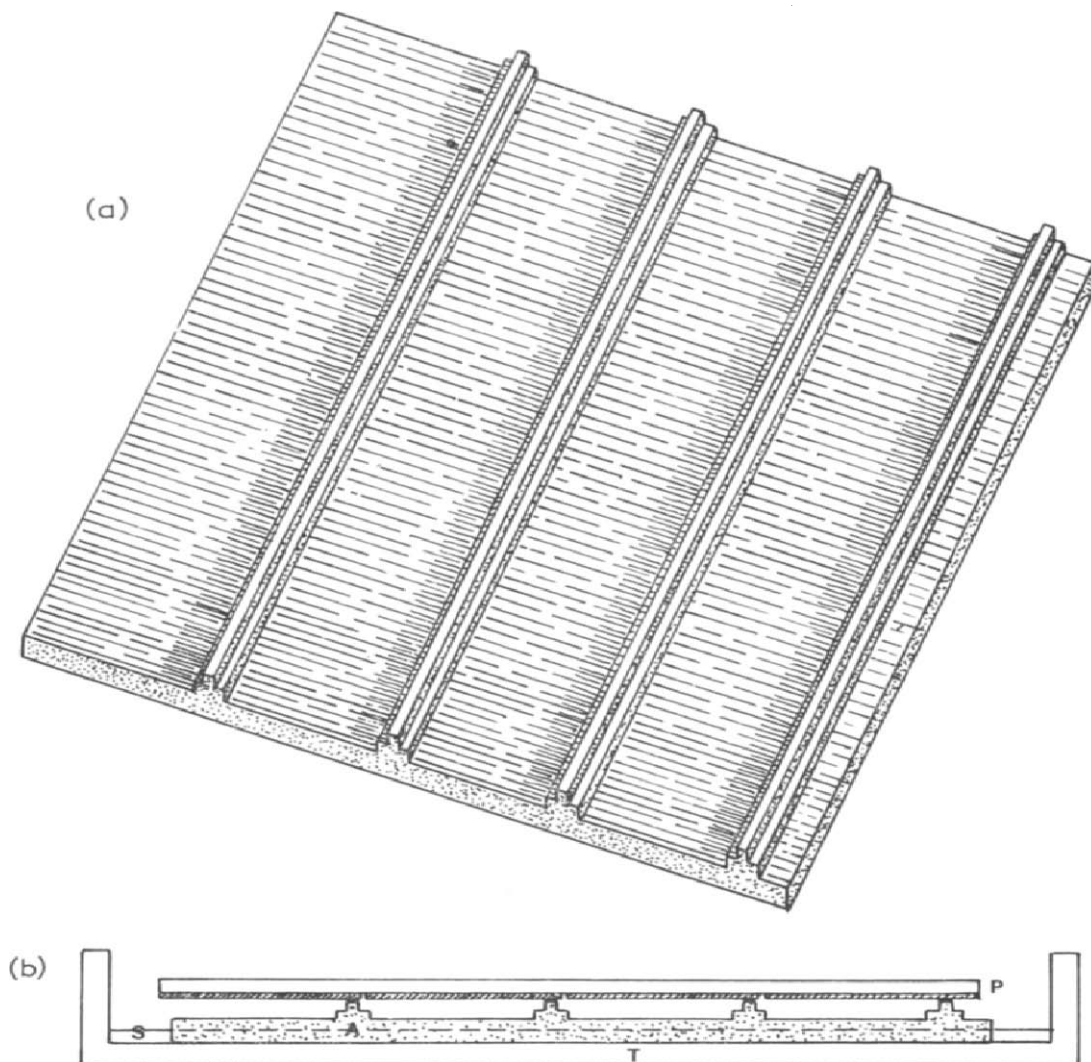
Fig. 5. (a) Porous polyethylene support for four two-dimensional separations on a single 20 × 20-cm plate. (b) A schematic diagram to illustrate its application. T = Flat chromatographic tank (tank lid omitted in the figure); S = solvent; A = porous polyethylene support; P = thin-layer plate (layer downward).

both a reference sample and a tissue sample with a known amount of the substance to be determined added, all on the same plate.

Apart from the fact that it is far easier to achieve complete separation by two-dimensional chromatography than by several uni-dimensional separations, quantitative evaluation is often handicapped or totally prevented in uni-dimensional chromatography by fluorescing streaks along the length of the plate or by overlapping spots. In those cases where separations along a short distance in the second direction have already been found possible, several two-dimensional separations can be run, at the same time, on a single 20 × 20-cm plate with the aid of a porous polyethylene support, as shown in Fig. 5. After the usual development of the plate in the first dimension, lines are scraped out between the different samples to inhibit the solvent flow in one direction. The plate is then positioned layer downward on the support, and placed in a horizontal tank containing solvent (see Fig. 5). Separations of DANS-bufotenine obtained in this way, from the components of a dansyl-
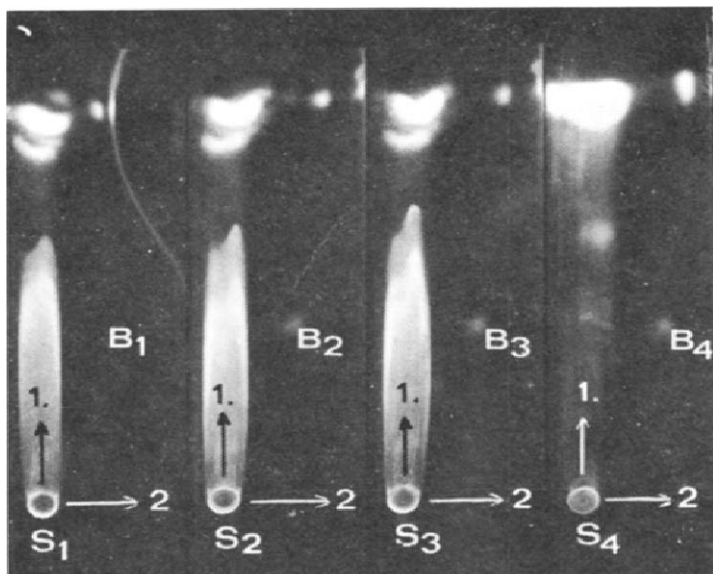
Fig. 6. Bidimensional simultaneous separation of four samples on a 20 × 20-cm plate with the aid of the porous polyethylene support of Fig. 5. S_1, S_2 and S_3 are starting points of the dansylated tissue extracts (300 mg mouse brain with small amounts of bufotenine added); S_4 is the starting point of the reference sample (DANS-bufotenine). B_1–B_4 are bidimensionally separated bufotenine spots. Solvents: chloroform–methanol (3:2) for the first dimension and chloroform–triethylamine (5:1) for the second.

ated mouse brain extract, are shown in Fig. 6. This method permits two-dimensional separations to be carried out and also has the advantage of quantitation of the tissue and reference sample on the same plate.

DETERMINATION AND IDENTIFICATION OF AMINES IN TISSUE

Serious problems arise if the amines to be identified or determined occur in low concentrations in tissue, because TLC separation of dansylated tissue extracts of more than 100 mg tissue is not normally possible. Generally the plates are overloaded by the large amounts of DANS-spermine, DANS-spermidine, and the slightly soluble DANS-NH$_2$. The same thing occurs with DANS-pyrrolidone if brain or retina extracts are dansylated.

There are several ways to circumvent this, and indeed any of the separation methods suitable for the determination of free amines could be applied. Some of them have already been successfully used, e.g. the determination of tyramine[84–86], putrescine[102], and the identification of 3,4-dimethoxy-β-phenylethylamine in the urine of schizophrenics[94].

However, we were looking for a generally applicable pre-separation method which could be adapted to different analytical problems. In our opinion gel column chromatography of the DANS-amines on columns with gels swelling in organic solvents would fulfil the requirements. The best separations have been obtained hitherto with Sephadex LH 20 with chloroform as solvent[103]. As is shown in Fig. 7, there is, unfortunately, no direct correlation between the log of the molecular weights of the DANS-amides and their elution volumes. Such a relationship seems to exist only for closely related compounds. However, the method is very useful as even a column of
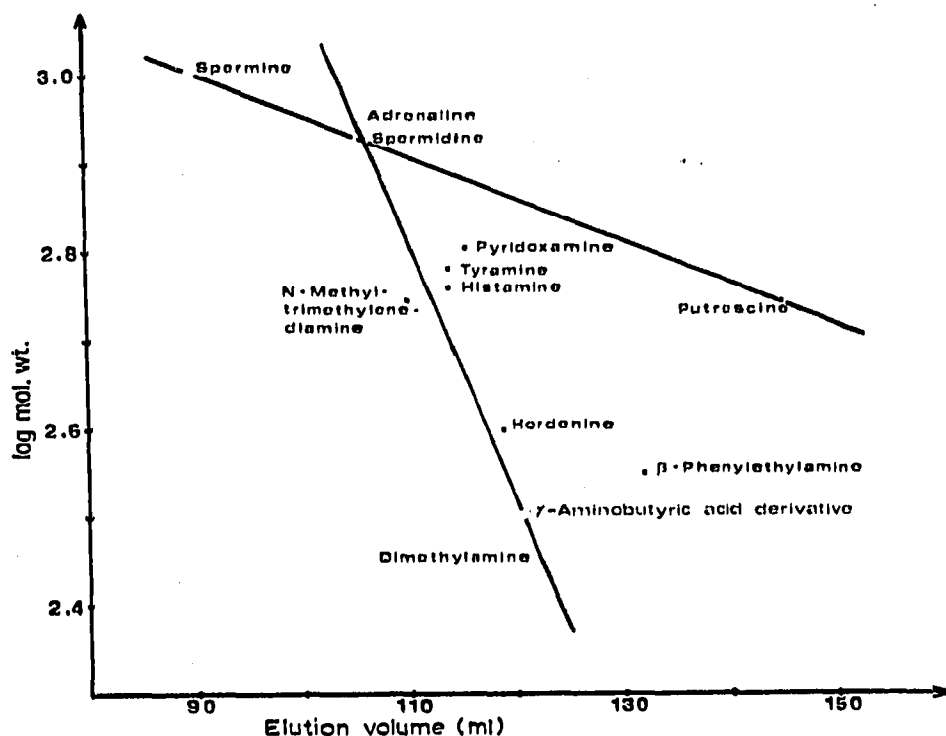
Fig. 7. Gel column chromatography of DANS-amides. Relationship between molecular weights of some DANS-amides of different structure and their elution volumes. Gel, Sephadex LH 20; column diameter, 20 mm; gel bed volume, 220 ml; solvent, chloroform; flow rate, 30 ml/h.

10 × 100 mm is large enough to separate the bulk of the DANS-amines from the most disturbing troublemakers, the DANS-polyamines and DANS-NH$_2$.

The pre-separation of the dansylated amines has several advantages as compared to the pre-separation of the free amines, viz.:

(1) Application of acid extracts to TLC from large tissue samples is difficult. Dansylated amines are easily concentrated by solvent extraction.

(2) The separation of the dansylated amines can be controlled visually. This is not possible with the underivatised amines, where one has to rely on the similarity of the chromatographic behaviour of reference mixture and tissue sample.

(3) Recovery of free amines from resin ion-exchange columns is often poor[9]. In our experience, recovery of the DANS-amine derivatives from gel columns is apparently quantitative.

(4) During the dansylation procedure certain side products are formed which have, in any case, to be separated from the amine derivatives to be determined. Since these side products are enriched only in certain fractions during column chromatography, the thin-layers spotted with fractions from the column are mostly very clean. This facilitates quantitative analysis.

(5) The effluent of small columns (diameter approximately 12 mm) can be directly applied to the thin-layer plates, since volatile solvents (e.g. chloroform) are used for their elution.

(6) The capacity of the gel columns is very large, so that normally enough material can be separated not only for quantitation but also for identification by mass spectrometry and also for radioactivity measurements.

It should be pointed out here that the possibility of isolating small amounts of pure substance without difficulty balances out the disadvantage of the lack of specificity of the derivatisation methods. Enzymatic methods, frequently used in biochemical analysis, because of their sensitivity and specificity, normally permit only the determination of the concentration of the substances, but not their radioactivity (see however, ref. 198). Since this is basically necessary in metabolic studies with radioactive compounds, the DANS-procedure and related methods satisfy a definite need.

In some cases, the unique properties of the DANS-derivatives permit their separation from other types of dansyl derivatives. This is the case, for instance, with the γ-lactams which have already been mentioned. The corresponding DANS-amino acid derivatives are obtained by hydrolytic cleavage of the lactam ring. These amino acid derivatives can then be separated from the amine derivatives by extraction. The lactam ring can easily be closed again by acylating agents (e.g. acetic anhydride) and the resulting DANS-pyrrolidone separated from trace impurities by TLC. Small amounts of γ-aminobutyric acid have been determined in this way in different tissues[78,80,81].

DANS-bufotenine and similar compounds with tertiary amino groups can be separated from the bulk of DANS-amines by extraction with acids. Other possibilities resulting from special structural features of certain DANS-derivatives may be made use of in the future.

IDENTIFICATION OF DANS-DERIVATIVES

Despite the outstanding chromatographic qualities of the DANS-amides on TLC, it is not possible to identify an unknown amide solely on the basis of its mobility in different solvents or its optical attributes. This also holds for free amines and for all other amine derivatives. In our opinion many dubious identifications have been made during the past by overestimating the potential of chromatographic methods. For an unambiguous identification independent techniques must be applied. Though in principle any physical method which gives information on molecular structure may be considered, in practice only MS is sensitive enough for the amounts of amine normally available from tissue. Fortunately DANS-derivatives are volatile enough for MS and fragmentation by electron impact is small[94,95,180,194], and consequently molecular ions are observed even from large molecules like tri-DANS-adrenaline (mol. wt. 882), or from highly hydroxylated compounds (e.g. DANS-hexosamines)[189]. Furthermore the DANS-amides are easily extracted from the silica gel. Impurities derived from the plates, often a serious handicap for MS of polar substances, play a minor role in the MS of DANS-derivatives, since the peaks of interest in the spectra of amine derivatives are in a mass range where impurities are low. This also holds for the DNP-[66] and NBD-derivatives[103] and the nitroazobenzenecarbonamides[107].

CONCLUSIONS

A combination of methods for the qualitative and quantitative analysis of biogenic amines has been described, with TLC as the essential separation technique. Since the application of MS has turned out to be a necessity—at least for qualitative

analysis—the question arises whether there are alternatives which are, if not less expensive, at least more convenient.

There are of course several possible alternatives to the combination of methods described. The most obvious seems to be GLC coupled with MS. Without going into a detailed comparison of the two methods, which is beyond the scope of this paper, two points in favour of TLC should be mentioned, viz. versatility and the possibility of applying relatively large sample volumes to the plates. TLC procedures allow, if necessary, a series of separations which can be visually controlled if the recommended fluorescent derivatives are used. Also, within certain limitations, very different amounts of substance can be separated, and the separations easily adapted to the problem by careful selection of suitable solvents.

Capillary columns as used in GLC, even with their superior separatory properties and sensitivity, have, however, a low capacity. This necessitates introducing the sample in a very small solvent volume. This normally implies that only small aliquots of a given sample can be separated. Thus it would seem that even the high sensitivities of some detectors as applied in GLC and MS are not sensitive enough for the analysis of biogenic amines. This, at least, seems to be one explanation for the hitherto rare application of this highly developed technique to amine analysis.

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