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REVIEW

CHROMATOGRAPHY OF BIOGENIC AMINES. I. GENERALLY APPLICABLE SEPARATION AND DETECTION METHODS

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CONTENTS

1. INTRODUCTION

Cells and extracellular fluids of all living organisms contain a complex mixture of compounds containing amino groups such as amino acids, peptides, amines and alkaloids. The study of structural, metabolic and functional relationships between these compounds frequently requires the determination of several individual compounds in biological samples. For instance, the study of the metabolism of tryptophan in the brain normally involves analytical monitoring of tryptophan, 5-hydroxytryptophan, tryptamine, N,N-dimethyltryptamine, 5-hydroxytryptamine, bufotenin (N,N-dimethyl-5-hydroxytryptamine), melatonin (N-acetyl-5-methoxytryptamine), indolylacetic acid and 5-hydroxyindolylacetic acid, and frequently also metabolites of the kynur-

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enine pathway of tryptophan, conjugates (sulphates, acetates, glucuronides, etc.) of the tryptophan metabolites and peptides. A recent trend in analytical biochemistry was therefore the development of methods that allow the determination of functionally and metabolically related compounds from the same sample. However, although in certain instances interest may be focused exclusively on a single amine, one must devise methods that allow the determination of the individual amine in the presence of many other related compounds of comparable concentration. In methods of this type chromatography is particularly useful.

The concentration of biogenic amines in tissues is, with few exceptions, low, i.e., generally lower than 10 nmole per gram of wet tissue. The necessity for the determination of low concentrations of biogenic amines in discrete areas of the brain, in small cell populations and in single cells stimulated the improvement of the sensitivity of the methods. The possibility of achieving the quantitative determination of picomole or even femtomole amounts of amines, amino acids and peptides is a major criterion for the suitability of a method in neurochemistry. Less sensitive methods may be applied successfully to urine analysis.

Many naturally occurring amines and amino acids have been identified and quantitated in tissues and body fluids in the last 20 years. The application of more advanced methods to their assay frequently revealed, however, that their actual concentrations were sometimes lower than was originally thought by several orders of magnitude. β-Hydroxy-γ-aminobutyric acid [1,2], choline and acetylcholine [3], piperidine [4] and putrescine [5,6] in the brain are examples of compounds for which revisions of their concentration in tissues were made of the basis of advanced methods. Improvements in the specificity of the methods was as important as an increase in sensitivity. The still increasing application of mass spectrometry in combination with other separation methods in analytical biochemistry is one of the latest trends aimed at improving specificity. The exploitation of the specificity of enzymic reactions, mostly combined with the use of radioactive substrates, and immunological methods are alternatives to separation methods for increasing sensitivity and specificity [7,8]. Radioenzymic assays have been established for catecholamines [9—12], serotonin [13,14], β-phenylethylamines [15,16], histamine [17—19], choline and acetylcholine [20—25] and putrescine [6], among others, and radioimmunoassays are available for a few important biogenic amines [26—29].

Further insight into the molecular events associated with metabolic aberrations enormously increased the demands for routine assays of an increasing number of amines and amino acids for diagnostic purposes, and for the monitoring of therapeutic measures. Automated methods had to be developed to meet the special requirements of clinical analysis. Automated methods, with few exceptions, do not include separation steps, but utilize specific structural features of the amines for determination. They are normally of limited specificity and applicable only under thoroughly defined circumstances.

In addition to automation and the increase in sensitivity and specificity, a further trend became apparent. After two decades of nearly exclusive consideration of a small number of physiologically and pharmacologically important amines, the catecholamines, serotonin, histamine and acetylcholine, in-

terest was extended to other amines of biological origin for which the physiological significance is less apparent or less well established. Methods for the determination of these amines are being developed. The term "biogenic amine" has gradually seemed to regain its literal sense, which was originally used, for instance, by Guggenheim in his now classic book "Die biogenen Amine" [30].

Comprehensive reviews of analytical procedures for the assay of biogenic amines do not seem to exist, if we neglect the short chapters in standard books on thin-layer chromatography (TLC) and gas–liquid chromatography (GLC), and the author's short reviews [31,32]. The reason for this is clear: most papers reviewing analytical methods for biogenic amines are devoted either to a special method or to a special approach [7,8,33—45] or a certain compound [46—57]. Furthermore, established, generally accepted and generally applicable chromatographic methods for the determination of amines are, with few exceptions, not available. Any given sample of biological origin poses different problems, depending on its complexity and the concentration of the amines in the sample. It is not possible, by using a single method, to establish a complete profile of all important amines in a given biological sample. Most methods are in principle not suitable for such an analysis: with the exception of the completely non-specific detection methods used mainly in GLC, the flame-ionization detector and the more specific nitrogen-sensitive alkali detector, and the mass spectrometer, virtually no sensitive method exists for the determination of tertiary amines. They are normally not recognized, provided that the molecule has specific features for sensitive detection, as is the case, for instance, with bufotenin. Colour and fluorescent reagents for teriary amines do not meet the requirements of the sensitivity needed for tissue analysis.

Disregarding these limitations, methods exist that are sufficiently versatile to be adapted to a given analytical problem. Existing methods have been improved successfully during recent years by the application of refined chromatographic procedures or by increasing the sensitivity of detection by suitable derivative formation. It is the purpose of this paper to review these methods.

2. GENERALLY APPLICABLE METHODS

Aliphatic mono-, di- and polyamines do not exhibit structural features that permit their sensitive or specific detection. Interference refractometry was suggested for the determination of amines after elution from thin-layer chromatograms, but its low sensitivity (μg amounts of amphetamine [58]) is one of several reasons for the restricted application of this method.

Disregarding detection with non-specific flame-ionization detectors, in all methods currently in use for the assay of primary and secondary amines the amino groups are utilized for the formation of derivatives suitable for sensitive determination and/or improvement of separation. While the sensitivity of detection depends on the derivatization reaction, the specificity is limited solely by the quality of the separation procedure. In practice, nearly all detection reactions have been combined with all separation procedures.

A. Paper and thin-layer chromatography and paper and thin-layer electrophoresis of free amines

A large number of solvent and buffer systems suitable for the separation of non-derivatized amines on paper [59—74] and thin layers by chromatography [80—106] or electrophoresis [63—65, 88,107—114] have been proposed. In addition to the usual layers (cellulose, alumina and silica gel), ion-exchange paper and thin layers [88, 115] have been considered, and also separations by ligand exchange [116]. The references cited in this paragraph mainly describe separations of aliphatic amines. Although the reference list is incomplete, it demonstrates the wide application of amine separations in analytical biochemistry. Chromatographic separations of underivatized β-phenylethylamines, catecholamines, histamine, indoleamines and acetylcholine will be discussed in a subsequent paper.

For the detection and determination of separated primary and secondary amines, ninhydrin has most commonly been used, and Dragendorff reagent for tertiary amines [117]. A number of other generally applicable detection reactions are available [105]. Colour reactions of primary amines with 2,5-dimethoxytetrahydrofuran plus *p*-dimethylaminobenzaldehyde [118], and the more generally applicable reactions with 2,6-dichloroquinone 4-chloroimide [119] or potassium permanganate [77], or the fluorimetric procedure of Segura and Gotto [120] for the detection of organic compounds on thin-layer chromatograms, may prove useful in certain amine analyses, as well as the reaction of tertiary amines with α,γ-anhydroaconitic acid to give coloured products [121]. Considerable progress in the detection of primary amines was made, however, by using fluorescamine as spray reagent [122—124], which can reveal picomole amounts. The reaction of fluorescamine with primary amines is illustrated in Fig. 1. Although there are certain difficulties, quantitative evaluation of chromatograms sprayed with or dipped in fluorescamine is possible. An alternative method is the application of the *o*-phthaldialdehyde reaction in the presence of thiol-containing compounds (2-mercaptoethanol) [125] for the fluorescence staining in TLC, thin-layer electrophoresis (TLE), paper chromatography (PC) or paper electrophoresis (PE). The sensitivity of this method [126] in our hands was not as good as that of the reaction with fluorescamine, but was useful.

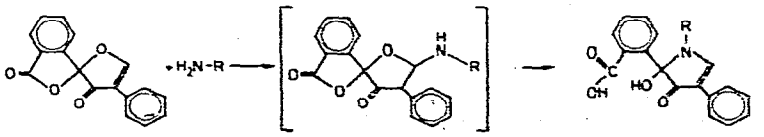

Fig. 1. Reaction of fluorescamine with a primary amine.

Despite considerable experience, there is no generally accepted approach to the analysis of the free amines in a biological sample. Disregarding the complexity of the mixture and the varying concentrations of its components, there is an additional difficulty inherent in their structural feature: the salts and the free bases of many natural aliphatic amines and β-phenylethylamines are

readily soluble in water. Homogenization with acids [0.2—0.4 *M* $HClO_4$; 10% trichloroacetic acid; acetone—0.1 *M* HCl (95:5)] is effective for their extraction from tissues, but it is difficult to concentrate large volumes of the acidic solutions without losses of trace amounts of the amines. Some of the low-molecular-weight amines are volatile. On the other hand, certain conjugates (for instance acetates) may be hydrolyzed during evaporation, and evaporation of neutralized solutions causes heavy losses. In fact, water-vapour distillation of alkaline solutions was used until recently for the separation of volatile from non-volatile amines and amino acids [62,63,127,128].

Aromatic amines and some β-phenylethylamines can be extracted from alkaline solutions with ethyl acetate, diethyl ether and similar solvents. The polyamines spermidine and spermine were mostly extracted with *n*-butanol [86,108] before chromatographic separation. A generally applicable solvent-extraction procedure for amines does not exist and, moreover, some biologically important amines are unstable in alkaline solutions. Not only catecholamines and related compounds but even simple aliphatic diamines may be partially lost due to decomposition in alkaline solutions.

A method known as ion-pair extraction may form a basis for future development. Ammonium compounds (choline, acetylcholine, etc.), primary, secondary and tertiary amines and amino acids form ion pairs with anions. The ion pairs with tetraphenylborate [129]. HgI_4^{2-} [130], anthracene-2-sulphonate [131] and di-(2-ethylhexyl)phosphate [132], among others [133—137], can be extracted with organic solvents.

Ion-pair extraction has proved useful in drug analysis [137,138] and the determination of acetylcholine [139] and of enzymes involved in acetylcholine metabolism [129, 130,140]. Ion pairs are suitable for separation by partition chromatographic methods. Column chromatographic separations utilizing this principle for the assay of some biogenic amines have recently been published [141—143].

Among the aliphatic amines, a group of di- and polyamines (Fig. 2) deserves special mention. These amines are ubiquitous in the natural world and probably play basic roles in cell biology [144,145]. Moreover, they might be useful as markers of neoplastic growth and indicators of the effectiveness of cancer chemotherapy [146—148]. These amines can serve as a typical example of the present situation in the analysis of amines in tissues and body fluids, and to

① $H_2N-(CH_2)_3-NH_2$

② $H_2N-(CH_2)_4-NH_2$

③ $H_2N-(CH_2)_5-NH_2$

④ $H_2N-(CH_2)_3-NH-(CH_2)_4-NH_2$

⑤ $H_2N-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$

Fig. 2. Structural formulae of the natural di- and polyamines. 1 = 1,3-Diaminopropane; 2 = putrescine (1,4-diaminobutane); 3 = cadaverine (1,5-diaminopentane); 4 = spermidine; 5 = spermine.

demonstrate the various strategies applied to the solution of a most important analytical problem.

The theoretical and practical importance of the polyamines has stimulated the establishment of a large number of analytical methods. Bachrach [145] summarized about 30 solvents for PC, 11 solvents for TLC and 12 different buffers for PE of the non-derivatized polyamines. Most workers used extraction of alkaline solutions with *n*-butanol in order to accumulate the polyamines and to separate them from amino acids. It should be emphasized that the chromatographic systems used for the separation of aliphatic amines are also generally suitable for amino acid separations. Ninhydrin was nearly always used for detection and determination. Some laboratories preferred, however, to determine spermidine and spermine by staining with amido black [108].

Most of these methods never gained much attention, but certain versions of PE [107,108] found wide application until recently. It turned out, however, that the urinary spermine concentrations, as measured with the PE method, were higher than those found with other procedures [148]. Putrescine concentrations in tissues were even higher by an order of magnitude than the results obtained with more advanced methods [5,6,149]. Therefore, virtually all of these methods have been abandoned as far as tissue, blood and urine analysis are concerned. For the establishment of metabolite patterns of radioactive precursors, PE and preferably TLE [150, 151] are, however, the most suitable techniques. For this purpose, it is advisable to apply trichloroacetic acid or neutralized perchloric acid extracts (neutralized with $KHCO_3$, in order to remove perchlorate) instead of *n*-butanol extracts on the chromatographic plates. Putrescine, and probably other diamines, might form degradation products in alkaline solutions, as was mentioned before.

A recent version of TLC, namely separation on silicagel sintered-glass plates combined with in situ fluorimetry after reaction with fluorescamine, was suggested as an improvement of the determination of polyamines on a micro-scale [123]. N-3-Aminopropylheptane-1,7-diamine was used as the internal standard. The sensitivity of detection with this method is of the order of 100 pmole. Its specificity and applicability in tissue and body-fluid analysis have not yet been established. However, this example shows that the TLC of free amines is still under development.

B. Ion-exchange chromatography of amines

The ion-exchange column chromatographic pre-separation of complex biological mixtures, and subsequent determination of amines in the fractions by using specific methods, has a long history. This approach is still one of the most important in the chemical analysis of biogenic amines.

The construction of automated devices for amino acid analysis following the work of Spackman et al. [152] suggested the utilization of these devices for amine analysis in the same way as for amino acid analysis. The work of Perry and co-workers [67—72,153] is exemplary in this respect. This group, and others [154—160], used almost exclusively columns packed with sulphonated polystyrene resins and either pH or salt gradients, or combined pH and salt gradients, for the successive elution of the different amines. As aromatic amines

in particular show considerable interactions with the polymer matrix, the elution patterns do not follow exactly the ion competition equilibria. The complete dissolution of a complex mixture of biogenic amines cannot be expected with this method, but starting from large tissue samples it was possible to identify a number of aliphatic amines and phenylethylamines in the brain if additional separation methods (PC, PE) were applied [72,153,154]. The same approach was successful for the detection of amines in urine [68,71,155,161] and cerebrospinal fluid [69]. On of the drawbacks of this approach is that identification of the amines is based exclusively on chromatographic criteria, which are insufficient in principle and may lead to erroneous conclusions.

Technical improvements to commercial amino acid analyzers have been considerable during the last decade. The development of spherical resins and polymer-coated glass spheres with a narrow range of diameters of 10 μm and less increased the resolution and sensitivity. Even with ninhydrin as the detection reagent, the sensitivity is now in the nanomole range. An improvement in sensitivity came from the application of continuous fluorescence monitoring, using fluorescamine [162,163] or *o*-phthaldialdehyde [164,165] as reagents. Under favourable conditions, these methods allow the measurement of picomole amounts of amines eluted from columns with diameters of 1—3 mm. Nevertheless, the routine measurement of aliphatic amines or of β-phenylethylamines in tissues and body fluids plays only a negligible role, and even the clinically important catechol- and indoleamines and their metabolites are normally not determined by automated ion-exchange methods. However, as will be discussed in a subsequent paper, some procedures meet the requirements of routine clinical analysis.

The interest in polyamines as possible markers of malignancy induced methodical developments, which are briefly summarized here. They illustrate the general trends of the current development of ion-exchange column chromatography.

Ion exchangers for the separation of di- and polyamines from amino acids and other amines have been in use for 20 years [166] and almost all types of commercial resins have been applied[145]. Elaborate separations were achieved using cellulose phosphate columns [167]. The amines were eluted by salt or pH gradients, and were determined in the collected fractions using dinitrophenylation [166,168,169], enzymatic methods [169,170] or condensation with *o*-phthaldialdehyde [167,169,171]. The tediousness of these procedures was surmounted by the automated modifications. Commercial amino acid analyzers were adapted in several laboratories for polyamine separations using, with one exception [172], reaction with ninhydrin and colorimetry at 570 nm for quantitation [173—181a]. Separations are achieved by two-or multi-step gradient elution using pH and/or sodium chloride gradients. An example is shown in Fig. 3. Recently, an attempt was made to apply ligand-exchange chromatography to the separation of polyamines [182]. Cellulose ion exchangers and different polystyrene sulphonated resins were loaded with Cu^{2+}, Zn^{2+} and Ni^{2+}, but the results were not adequate to meet current standards.

The sensitivity of detection was increased from about 500 to 0.2 nmole when fluorescamine was used [172]. The standard deviation of the procedure is of the order of ± 5% and the recovery is better than 90% [177—179].

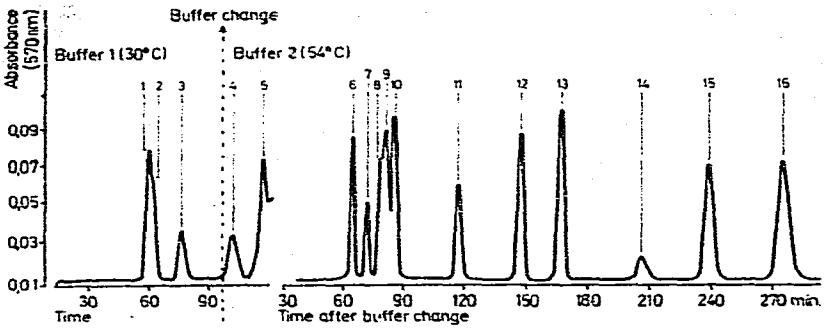

Fig. 3. Elution pattern of amines from a sulphonated polystyrene ion-exchange column using a two-step buffer system with an exponential pH and NaCl gradient in the second step. For details, see ref. 176. Buffer flow-rate, 70 ml/h. 1 = Ammonia; 2 = monoacetyl-1,3-diaminopropane; 3 = monoacetyl-1,4-diaminobutane; 4 = monocarbamyl-1,4-diaminobutane; 5 = arginine; 6 = 1,4-diamino-2-hydroxybutane; 7 = diaminopropane; 8 = N^1-monoacetylspermidine; 9 = N^8-monoacetylspermidine; 10 = 1,4-diaminobutane (putrescine); 11 = 1,5-diaminopentane (cadaverine); 12 = N-(3-aminopropyl)-1,3-diaminopropane; 13 = spermidine; 14 = agmatine; 15 = N, N'-bis-(3-aminopropyl)-1,3-diaminopropane; 16 = spermine (20 nmol of each amine). According to Tabor et al. [176].

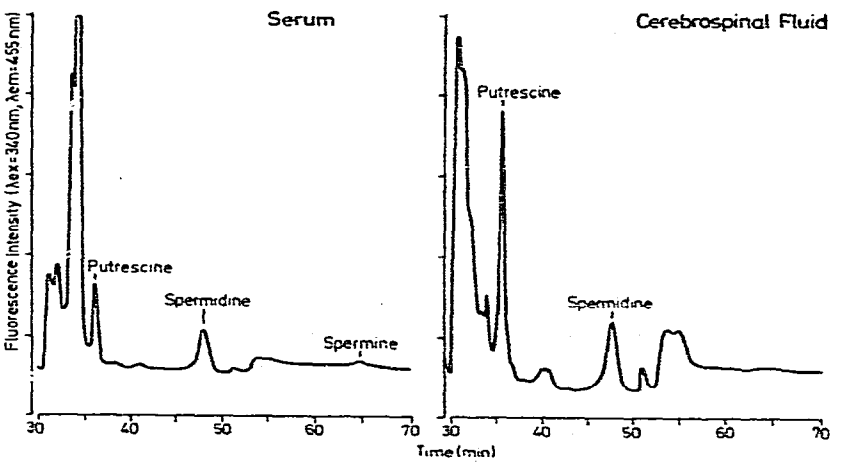

Fig. 4. Separations of serum and cerebrospinal fluid polyamines using an automated high-performance liquid chromatographic technique in combination with the reaction of amines with *o*-phthaldialdehyde. (After acid hydrolysis the equivalents of 1.25 ml of serum and cerebrospinal fluid were separated). According to Marton and Lee [183].

Recently, small high-pressure columns were utilized in combination with highly developed fully automated equipment with computerized peak-area calculation. This device allows the routine determination of 3—6 pmoles of putrescine and spermidine and 12—15 pmoles of spermine if the *o*-phthaldialdehyde method is adapted to the system [183]. Fig. 4 shows separations of polyamines from 1.25-ml serum and cerebrospinal fluid samples. Further improvements can be expected from improvements to the ion-exchange resins.

C. Coloured derivatives of amines for thin-layer chromatographic separation

A reagent suitable for the analysis of small amounts of amines (and amino acids) should fulfill the following criteria: (a) Rapid quantitative reaction under mild conditions in water or water-containing media; (b) specificity for primary or secondary amino group; (c) high sensitivity of detection; (d) favourable chromatographic properties of the derivatives; and (e) low polarity of the reaction products in order to permit the accumulation of the reaction products by solvent extraction.

Several compounds are available that react either with primary amino groups or with primary and secondary amino groups, but no reagent is known to be specific for secondary amino groups, nor is there a derivative-forming reaction known for tertiary amines that is suitable for quantitative analysis.

The reagents summarized in Fig. 5 react, with one exception, with primary and secondary amines in weak alkaline solutions to give coloured derivatives in high yields. The derivatives are stable, can be extracted from the reaction mixture with organic solvents and are suitable for chromatographic separation and spectrophotometric determination in the nanomole range. Hence they meet at least partially the above-mentioned criteria.

Solvent systems for the TLC (and PC) of these derivatives have been formulated for only a few amines. Only 2,4-dinitrofluorobenzene (Dnp-F), the well known end-group reagent of Sanger [184,185], and the more specifically reacting 2,4-dinitrobenzenesulphonic acid [186], which leads to the same derivatives, have been used extensively, both for the determination of certain urinary and tissue constituents. PC [187,188] and TLC [186, 189—194] were used for separation, and it was shown that the derivatives are also suitable for ion-exchange column and GLC separation [190]. As was mentioned before, dinitrophenylation was utilized for the determination of amines in column eluates [195]. The mass spectra of the Dnp derivatives have been studied to

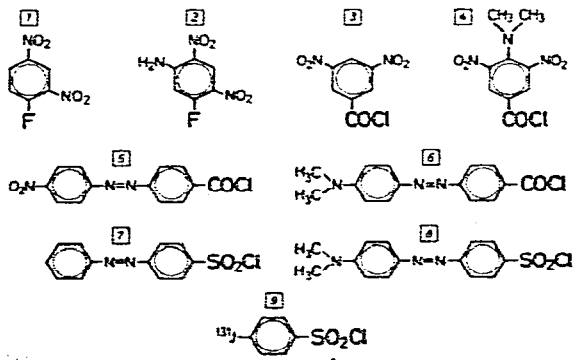

Fig. 5. Structural formulae of reagents recommended for the formation of coloured derivatives with primary and secondary amines. 1 = 2,4-Dinitrofluorobenzene [184]; 2 = 2,4-dinitro-5-fluoroaniline [197]; 3 = 3,5-dinitrobenzoyl chloride [75]; 4 = 4-dimethylamino-3,5-dinitrobenzoyl chloride [198,199]; 5 = 4-(4'-nitrophenylazo)benzenecarbonyl chloride [200]; 6 = 4-(4'-N,N-dimethylaminophenylazo)benzenecarbonyl chloride [201]; 7 = 4-(phenylazo)benzenesulphonyl chloride [202]; 8 = 4-(4'-N,N-dimethylaminophenylazo)benzenesulphonyl chloride [203,204]; 9 = [^{131}I]-4-iodobenzenesulphonyl chloride [196].

show the usefulness of this method for the unambiguous identification of amines [191].

Restriction of the sensitivity of detection to the nanomole range is the main reason for the limited use of coloured derivatives for the analysis of amines in tissues, and for the preference for fluorescent reagents of similar reactivity and separation characteristics. In order to make use of existing experience, and to increase the sensitivity of detection, tritiated Dnp-F was introduced for tissue amine analysis [128]. With this reagent, a few picomoles of a compound can be detected; however, these amounts are invisible and it is difficult, therefore, to control the quality of the separations on the thin layers. If the specificity of the separations is not adequately controlled, erroneous results might be obtained.

Increase of the sensitivity of detection by utilization of radioactive reagents is not new. [^{131}I]*p*-Iodobenzenesulphonyl chloride has been known for 30 years [196], but it was never generally applied, for the above reasons.

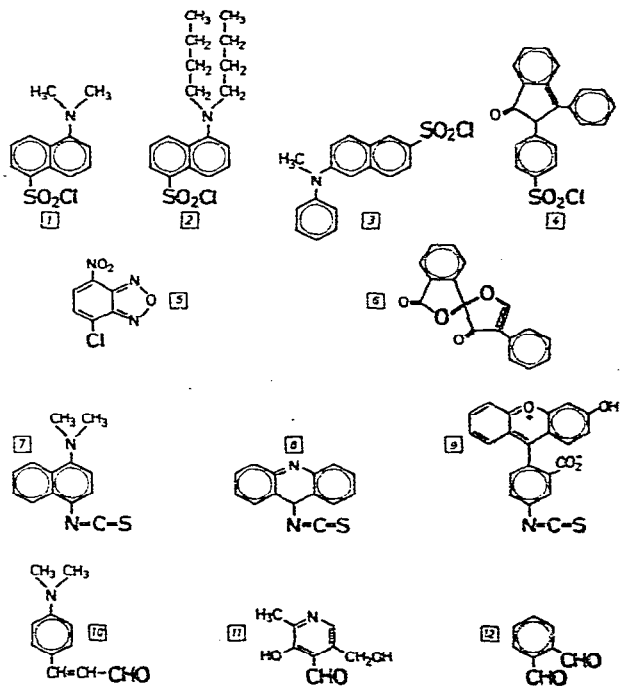

Fig. 6. Structural formulae of reagents recommended for the formation of fluorescent derivatives with primary and/or secondary amines. 1 = 5-Dimethylaminonaphthalene-1-sulphonyl chloride (Dns-Cl); 2 = 5-di-*n*-butylaminonaphthalene-1-sulphonyl chloride (Bns-Cl); 3 = 6-methylanilinonsphthalene-2-sulphonyl chloride (Mns-Cl); 4 = 2-*p*-chlorosulphophenyl-3-phenylindone (Dis-Cl); 5 = 4-Chloro-7-nitrobenzo [*c*]-1,2,5-oxadiazole (Nbd-Cl); 6 = 4-phenylspiro[furan-2(3H), 1'-phthalan]-3,3'-dione (fluorescamine); 7 = 4-dimethylaminonaphthalene-1-isothiocyanate; 8 = 9-isothiocyanatoacridine; 9 = fluorescein isthiocyanate; 10 = 4-dimethylaminocinnamaldehyde; 11 = pyridoxal; 12 = *o*-phthaldialdehyde.

D. Fluorescent derivatives of amines for thin-layer and column chromatographic separation

In Fig. 6, reagents currently used for the fluorescence labelling of amines (and amino acids) are summarized. The same reactive groups (activated halogen, sulphonyl chloride) are used both for fluorescence and colour labelling. Isothiocyanates and aldehydes are used only for special purposes. The specificity of the reactions and the formation of side-products during derivative formation are, in principle, the same for coloured and fluorescent derivatives. The obvious advantage of fluorescence labelling is the increased sensitivity of detection. Another advantage is the wide range of linearity between amount of substance and fluorescence intensity (i.e., photometer response), which simplifies and improves the in situ evaluation of TLC separated fluorescent compounds [205—208] and the continuous monitoring of column effluents.

The use of coloured and fluorescent reagents involves the same strategies: either the total tissue extracts or body fluids are made to react first, and separation procedures are applied exclusively to the derivatives, or a certain compound or a group of related compounds is pre-separated by solvent extraction, ion-pair extraction, column chromatography, etc., and the detection reaction is applied to the pre-separated compounds. In no case is a step involving purification of the derivatives dispensable, because normally side-products are formed during derivative formation; hydrolytic cleavage of the reagent is the most common side-reaction.

The information available does not permit a thorough comparison of the advantages of the different reagents. The amounts of information about the various reagents differ considerably and for some reagents is only fragmentary. Fluorescent reagents are briefly surveyed in the following sections; for a more detailed description, see ref. 209.

a. 4-Chloro-7-nitrobenzo[c]-1,2,5-oxadiazole

As an aryl halogenide with activated halogen (see Fig. 6, No. 5), 4-chloro-7-nitrobenzo[*c*]-1,2,5-oxadiazole (Nbd-Cl) is closely related to Dnp-F. It reacts readily in aqueous solutions [210,211] or in organic solvents [212—214] with primary and secondary amines, and less readily with phenols and thiols at pH 8. Thiol-containing compounds, however, react rapidly at pH 7 [215]. Usually 1—20 μg of amine dissolved in 25—500 μl of solution is mixed with four volumes of a 0.05% solution of Nbd-Cl in methanol, and 50—100 μl of 0.1 *M* $NaHCO_3$ solution are added. Completion of the reaction is achieved by heating at 55° for 60 min, and the yields are mostly > 95% [210]. Separation of the reaction product from excess of reagent is usually achieved by silica-gel column chromatography.

A thorough study of the chemical and physical properties of Nbd derivatives has not been published. They are stable in solution and on thin-layer plates, if protected from irradiation. In contrast with most other fluorescent labels, the absorption maxima of Nbd derivatives are in the visible region ($\lambda_{max(A)}$ = 460—470 nm), so that absorption and emission bands ($\lambda_{max(E)}$ = 510—530) overlap [210,215,216]. According to Klimisch and Stadler, about 50 pmol of Nbd-dimethylamine was measurable per millilitre of ethyl acetate. By direct (in situ) fluorescence measurement, direct proportionality between

the recorded curve areas and amounts of substance was observed with 70—700 pmoles [210]. Nbd derivatives are suitable for mass spectrometric identification [217].

b. Fluorescent sulphonyl chlorides

Four sulphonyl chlorides are currently in use for the fluorescence labelling of amines and amino acids: 5-dimethylamino naphthalene-1-sulphonyl chloride (Dns-Cl), 5-di-*n*-butylaminonaphthalene-1-sulphonyl chloride (Bns-Cl), 6-methylanilinonaphthalene-2-sulphonyl chloride (Mns-Cl) and 2-*p*-chlorosulphophenyl-3-phenylindone (Dis-Cl) (see Fig. 4, Nos. 1—4). These reagents react with primary and secondary amino groups under the same conditions. Their modes of application differ only insignificantly, the differences being confined to the optical and chromatographic properties of the derivatives.

Sulphonyl chlorides react with primary and secondary amino groups even under slightly alkaline conditions, and with phenols, imidazoles [44,45] and even with some alcohols at higher pH. For instance, a method for the sensitive determination of choline has been devised [218]. Thiol compounds form the corresponding disulphides [44,45,219]. The reaction of sulphonyl chlorides with amines, phenols, alcohols and thiols is shown in Fig. 7.

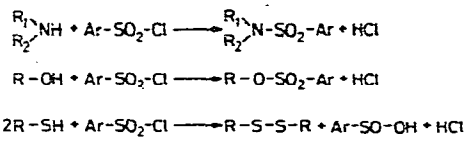

Fig. 7. Reaction of sulphonyl chlorides with amines, phenols alcohols and thiols.

In acetone—water (3:1) saturated with sodium carbonate or, in favourable circumstances, with sodium hydrogen carbonate, it is possible to obtain stoichiometric amounts of reaction products of primary and secondary amines if the reagents are applied in excess. Polyfunctional molecules, such as polyamines and aminophenols, react under these conditions with all functional groups, i.e., one obtains O,N-bis-Dns-tyramine, $N_\alpha N_\pi$-bis-Dns-histamine, tri-Dns-spermidine, tetra-Dns-spermine, tri-Dns-dopamine and tri-Dns-norepinephrine with excess of Dns-Cl.

Amino acids react first with the amino group. With excess of reagent, however, mixed anhydrides are formed, especially at elevated pH. The anhydrides of α-amino acids are partially fragmented under the usual reaction conditions to carbon monoxide, the aldehyde with one carbon atom less than the parent amino acid and the ammonia derivative [220]. Under the same conditions γ-amino acids form substituted γ-lactams, as was shown with Dns-Cl as reagent. This reaction is the basis for a sensitive and specific method for the determination of the biologically important γ-aminobutyric acid [1,57,221]. Tertiary amines may be attacked to a significant extent, especially at elevated pH and temperature, forming the derivative of a secondary amine by elimination of an alkyl or aryl group [222].

Increasing reaction velocities are normally paralleled by increased rates of hydrolysis. The formation of the corresponding sulphonic acid as a by-product

of the reaction is inevitable and methods applied for the further separation of the fluorescent derivatives have to take this into account. In the amine analyses the separation of the bulk of the sulphonic acid can usually be achieved by solvent extraction from alkaline solution.

The reaction of amines with sulphonyl chlorides in water-free organic solvents is possible [223], but has rarely been applied. This possibility of derivative formation deserves more attention.

Virtually all types of chromatographic procedures can be applied to the separation of the fluorescent sulphonamides. The most effective separations were performed on active surfaces, which have the advantage of the applicability of a wide range of separation methods, from pure adsorption systems to pure partition systems. In fact, there are few separation problems that cannot be solved on this basis by the selection of appropriate solvent mixtures, especially if the possibilities of two-dimensional separations by TLC are utilized. Numerous solvents for Dns-amide separations on thin layers have been published [44,45,209]. Bns derivatives are less polar than the corresponding Dns derivatives, but the general experience gained with Dns derivatives can be applied to their separation [224]. Less experience with other fluorescent derivatives exists. In fact, Mns and Dis derivatives, with few exceptions, have been only used for amino acid determinations [225,226]. In addition to active surfaces, polyamide sheets have been used for the separation of small amounts of amino acids and amines [40,41,43]. The high sensitivity of detection for fluorescent spots on polyamide sheets is mainly a consequence of the small diameters of the spots that can be obtained owing to the small particle size of the polyamide layers. This advantage is offset by the restriction to partition chromatographic systems, the relatively high background fluorescence of commercial sheets and especially the low capacity of the layer. This low capacity seriously limits the separation of complex mixtures with widely varying concentrations of the components, such as urine samples and tissue extracts. The recently developed high-performance thin-layer plates with silica gel layers combine the advantages of polyamide sheets with those of conventional active layers. They are especially useful for the detection of microamounts of biological amines in the form of fluorescent derivatives [227–228]. Fig. 8 shows an example of the separation of the Dns derivatives of the perchloric acid extract of mouse liver. The sensitivity of the method is shown by the fact that 5-pmole amounts of spermidine and spermine were measurable in 5 μg of liver tissue.

For the improvement of separations and especially to allow automated procedures to be introduced, column chromatographic methods have more recently been suggested for the separation of Dns derivatives of amines [229–233]. According to unpublished results in our laboratory, Dns and especially Bns derivatives are suitable for reversed-phase high-performance liquid chromatographic separation. About 3 pmoles of the polyamines spermidine and spermine in tissues were measurable within 30 min with repeated separations by using fluorescence monitoring of the column effluent.

For the quantitative assay of fluorescent sulphonamides, at least four different methods are available: absorptimetry, fluorimetry, quantitative mass spectrometry and the application of radioactive reagents.

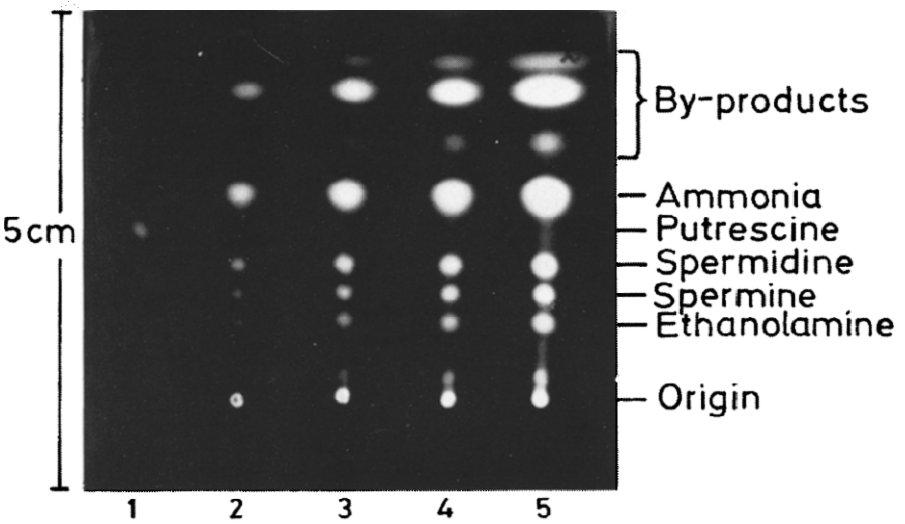

Fig. 8. One-dimensional separation of dansylated perchloric acid extracts of mouse liver on a 5 X 5 cm high-performance thin-layer plate (E. Merck, Darmstadt, G.F.R.). 1 = Bis-Dns-putrescine (reference sample); 2, 3, 4 and 5 = Dns derivatives corresponding to 5, 10, 50 and 100 μg, respectively of mouse liver tissue (about 5, 10, 50 and 100 pmoles of spermidine and spermine). Solvent, cyclohexane—ethyl acetate (1:1) (two runs). According to Seiler and Knödgen [228].

The high molar extinction of the fluorescent sulphonamides [$MnsNH_2$: $\epsilon_{255} = 4.2 \cdot 10^4$; $\epsilon_{321} = 2.3 \cdot 10^4$ (in *n*-propanol) [225]; $DnsNH_2$: $\epsilon_{252} = 1.3 \cdot 10^4$; $\epsilon_{333} = 0.43 \cdot 10^4$ (in methanol)] [44,234] allows their sensitive detection by absorptimetry, for instance in column effluents [232,235,236]. However, the most obvious method for their determination is fluorimetry. Dns derivatives are excited most effectively at 350—355 nm and the fluorescence is measured at 510—540 nm, depending on the structural features of the compound [44,45, 234]. The excitation maximum of the Bns derivatives nearly coincides with the 365-nm mercury line [224], which has considerable practical advantages as the xenon arc lamp can be replaced by the more intense and stable mercury arc lamp for fluorescence excitation. The fluorescence maxima of Bns derivatives occur at shorter wavelengths than those of the Dns derivatives (500—530 nm); their fluorescence quantum yields are comparable. Mns derivatives are excited at about 320 nm and their fluorescence maxima occur in the range 440—460 nm [225]. Dis derivatives form orange-red spots on thin-layer chromatograms, and can be detected with about the same sensitivity as Dnp derivatives, namely in the nanomole range. In strongly alkaline solutions, Dis derivatives are rearranged to strongly green fluorescing 1-phenyl-3-*p*-sulphophenyl-isobenzofuranic derivatives (Fig. 9), allowing their detection in the 0.1—1 pmole range on thin-layer plates [237]. Direct (in situ) fluorescence measurement of these spots, however, was not feasible, in contrast with all other fluorescent sulphonamides. It was therefore recommended [238] that the separated Dis derivatives be extracted with acetone, the acetone solution evaporated and the rearrangement induced by the addition of 5 ml of a solution

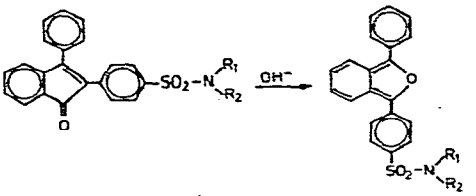

Fig. 9. Rearrangement of 2-*p*-sulphophenyl-3-phenylindone (Dis) derivatives to 1-phenyl-3-*p*-sulphophenylisobenzofuranic derivatives with sodium ethoxide [237].

of sodium ethoxide in ethanol. For the determination of pyridoxamine, the fluorescence is activated at 410 nm and emission measured at 480 nm.

Extraction of the fluorescent derivates of the adsorbent layer and in situ fluorescence scanning of the chromatograms are equally suitable procedures for the quantitative evaluation of thin-layer chromatograms [44,45,208,234]. Amounts of 100 pmoles of amines of biological origin can be determined without difficulty with a standard deviation of less than ± 5%.

In order to simplify measurements in small volumes of solvent and thus to increase the sensitivity of routine fluorescence measurements, an extraction procedure has been developed that allows the elution of TLC separated compounds with a solvent volume of about 50 µl [1,228]. In order to prevent decomposition of the Dns derivatives on the active surface, the plates are sprayed with triethanolamine—propanol-2 (1:4) immediately after chromatographic development [234]. The fluorescent spots are marked under a UV lamp (365-nm mercury line), then scraped out either using a glass capillary with a constriction [1] or a PTFE tube of about 1 mm I.D., with a cotton-wool plug fixed in the constriction (with the PTFE tube, constrictions are made with tweezers) [228]. The adsorbent is collected in one of the two compartments of the capillaries by suction with a suitable vacuum pump. For elution, the end of the tube containing the adsorbent is dipped into the solvent, and the solvent is moved through the capillary by gentle suction or, preferably, the adsorbent-filled side of the tube is connected with a motor-driven syringe, which is filled with solvent. A defined solvent volume is pumped through the capillary, the eluent being collected in the other capillary compartment, or else it is transferred directly into a small vessel, which is capped and stored at 0° until fluorescence measurements are carried out. If a spectrofluorimeter with an 8—10 µl flow-through cell or a fluorescence flow detector of a high-pressure column equipment is used for quantitative fluorimetry, an eluent volume of 50—75 µl is suitable. A few picomoles of the Dns and Bns derivatives of the polyamines spermidine and spermine can be determined routinely by using this technique, especially if high-performance thin-layer plates are used for the separations [228].

Elution of TLC separated compounds with small solvent volumes is of great importance for subsequent mass spectrometry: impurities can be kept to a minimum in the sample, and small solvent volumes simplify the transfer of the samples into the probe capillaries of the mass spectrometer. The above elution techniques, however, without prior spraying of the plates with triethanolamine, have been used in many mass spectrometric determinations of putres-

cine [149,239], piperidine [4] and serotonin [240] and for the preparation of mass spectra of TLC isolated compounds. The selection of the appropriate solvent is important. Elution should be carried out with a solvent with as low a polarity as possible, in order to minimize elution of contaminants in the adsorbent. For the elution of Dns- and Bns-amine derivatives from silica-gel plates and similar active layers, ethyl acetate is normally suitable. For subsequent radioactivity measurements, dioxan may be preferable. For derivatives of higher polarity, acetone or even methanol can be used. Dns-amino acids are extracted with methanol—25% ammonia solution (95:5) [44,234]. Silica gel G plates are preferable to plates with organic binders. If mass spectra of compounds separated on polyamide sheets are prepared, it is advisable to wash the sheets with methanol—acetic acid (3:1) before use as the spectra otherwise exhibit high backgrounds [241].

Usually molecular ions are observed in the electron-impact mass spectra of Dns and Bns derivatives and other fluorescent derivatives. The quantitative evaluation of a molecular ion or a typical fragment ion is much more specific than is fluorimetry or any other quantitative method that is currently available. Although underivatized compounds can be determined by the integrated ion current technique [242], it is advantageous to use fluorescent derivatives: (a) all separation steps in advance of the quantitative evaluation can be controlled visually; (b) owing to the high molecular weight of the derivatives, the background at the mass range of the molecular ions is normally low; and (c) erroneous peak identification is much less probable than with low-molecular-weight free amines owing to the low background.

Usually, the sample eluted from a thin-layer chromatogram is evaporated together with a suitable standard (generally the corresponding derivative of a homologue of the amine to be determined [243] or a deuterated sample [244]) from the direct probe of the mass spectrometer. The ion current of the molecular ion (or fragment ion) of the sample and standard are recorded alternately during evaporation and subsequently integrated. The ratio of the integrated ion currents of the sample and standard is a measure of the amount of sample, and is nearly independent of changes in instrumental sensitivity during the measurement. Fig. 10 shows the recorded ion currents of the molecular ions of varying amounts of bis-Dns-putrescine (*m/e* 554) and constant amounts of the internal standard bis-Dns-hexamethylenediamine (*m/e* 582). It is one of the pre-requisites of the method that the sample and standard should have similar evaporation profiles. The integrated ion current technique, using Dns or Bns derivatives, allows the precise determination of picomole or, in favourable cases, even of femtomole amounts of biogenic amines. The sensitivity of the method is dependent on the instrumental sensitivity.

Among the currently available fluorescent derivatives, Bns derivatives are especially suitable for qualitative and quantitative mass spectrometry. Dns derivatives form the ion corresponding to dimethylaminonaphthalene (*m/e* 170 or 171) with the highest abundance [244—248]. In contrast, Bns derivatives are split preferentially in the *n*-butyl side-chain, forming a fragment ion $(M-43)^+$ that still contains the complete information of the derivatized molecule [224]. Because, in addition to the $(M-43)^+$ ion, the molecular ion (M^+) is observed with about the same relative intensity as that of Dns derivatives, derivative

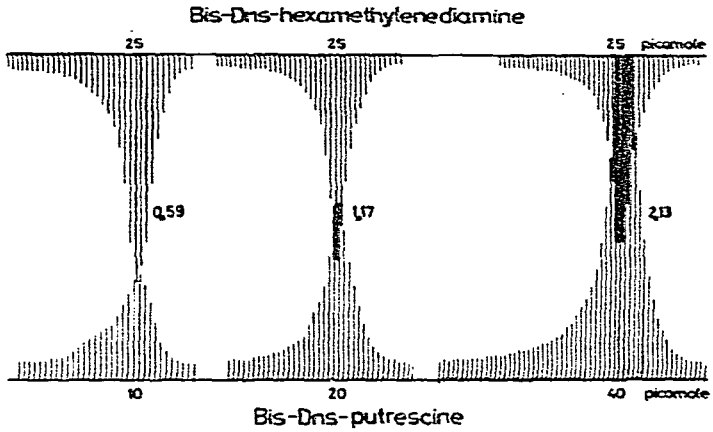

Fig. 10. Ion current curves of mixtures of 25 pmoles of bis-Dns-hexamethylenediamine (*m/e* 582) with 10—40 pmoles of bis-Dns-putrescine (*m/e* 554). The numbers are calculated peak-area ratios. Evaporation time, 30 sec. (Schematic drawing of two channel recordings from an electrostatic recorder). (For details see ref. [243]).

formation with Bns derivatives not only permits the determination of smaller amounts, but also facilitates the identification of molecular ions in the mass spectra of mixtures of compounds by means of the two characteristic ions M^+ and $(M-43)^+$, the intensities of which are observed in a fixed ratio. In addition to electron impact, field desorption may provide useful ionization methods. In combination with multiple-ion detection it may allow the quantitative analysis of complex mixtures of amines in the form of their Bns derivatives, without prior separation. A method calles metastable defocusing was recently suggested as being applicable to mixtures of Dns derivatives [249]. At a low electron beam energy (12 eV), Dns derivatives form almost exclusively the fragment ion at *m/e* 171. The instrument is focused on this fragment ion and the acceleration voltage is then varied while the analyzer voltage is kept constant. This procedure has the effect of focusing successively on the collector, during the travel from the source to the analyzer, the precursor ions that give rise to the formation of this fragment ion. Hence the method uses the determination of ions formed by metastable decomposition in the field-free region of the mass spectrometer. Applications of the method to biological samples have not yet been published.

An increase in specificity is the main characteristic of mass spectrometric methods, apart from their sensitivity. An increase in the sensitivity of detection, although not of specificity, is obtained by using radioactive reagents. N-Methyl-[^{14}C]Dns-Cl (specific radioactivity 10—30 Ci/mole) and [G-^{3}H] Dns-Cl (specific radioactivity 3—10 Ci/mmole) are commercially available. The labelled reagents can be applied in a similar manner to the unlabelled reagent, but they are normally restricted to applications with small reaction volumes (1—50 μl) because of their high cost. Their application allows the replacement of fluorescence measurements with automated liquid scintillation counting. The intense fluorescence of the derivatives is used only to reveal the separated spots on the chromatograms. If the reagent with the highest available specific

radioactivity is used, the sensitivity of the method allows the determination of a few picomoles. A further increase in the sensitivity of detection is gained by the preparation of autoradiographs from the chromatograms, which can be evaluated by microscope photometry [250]. Unfortunately, this method has disadvantages: high costs and erroneous interpretation of the autoradiographs and quantitative measurements owing to the presence of non-fluorescent but radioactive impurities and degradation products. The application of labelled reagents is improved by using double-isotope methods:

A known amount of the compound to be determined is added to the sample in the form of its ^{14}C-labelled analogue as an internal standard (in analogy with the use of deuterated standards in quantitative mass spectrometry). After derivative formation with the ^{3}H-labelled reagent, the derivative is extensively purified. Incomplete derivative formation, or losses during the purification steps do not affect the quantitative result, provided that sufficiently large amounts of the purified sample are isolated to allow precise radioactivity measurements. The amount of the non-radioactive compound present in the sample can be calculated from the $^{3}H/^{14}C$ ratio. The coefficient of variation of this method is of the order of 6% [251].

c. Isothiocyanates

Isocyanates and isothiocyanates react with primary and secondary amines to give urea and thiourea derivatives, respectively. Several isocyanates have been suggested in the past as fluorescence probes [209]. However, as they react readily with water and alcohols to give urethanes, they were replaced with the less reactive isothiocyanates.

At present, three main fluorescent labels are in use for the labelling of low-molecular-weight amino-containing compounds that bear the isothiocyanate moiety: 9-isothiocyanatoacridine [252] (Fig. 6, No. 8), fluorescein isothiocyanate [253] (Fig. 6, No. 9) and 4-dimethylaminonaphthalene-1-isothiocyanate [254] (Fig. 6, No. 7). The last two compounds seem to have been used only for end-group analysis of peptides and for the detection of free amino acids.

9-Isothiocyanatoacridine leads to the formation of several fluorescent products, and the fluorescence of one of these products could be related to the amount of amine present in the sample [252]. On chromatographic evidence, the fluorophore is a cyclization product that is formed by photo-oxidation from the primarily formed thiourea derivative (Fig. 11) [255]. A few thin-layer chromatographic separations have been carried out with isothiocyanatoacridine derivatives. A linear relationship has been observed between fluorescence intensity and amount of sample on the plates (excitation of fluorescence at 295—310 nm; emission measurement at 500—525 nm). The usefulness of 9-

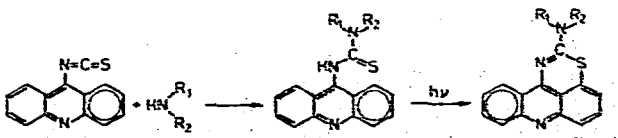

Fig. 11. Reaction of 9-isothiocyanatoacridine with an amine.

isothiocyanatoacridine as a reagent for the assay of small amounts of biogenic amines has not yet been demonstrated convincingly.

d. Fluorescamine

Fluorescamine (4-phenylspiro[furan-2(3H),1'-phthalan]-3,3'-dione) (Fig. 6, No. 6) reacts with compounds that contain nucleophilic functional groups (primary and secondary amines, alcohols, water, etc.). However, only primary amines form fluorescent products according to Fig. 1. Fluorescamine is therefore a specific reagent for compounds with primary amino groups [256]. For the reaction of aliphatic amines, a pH of 8—8.5 is adequate [257,258].

Fluorescence measurements are normally carried out in the range of maximal stability of the fluorophore between pH 4.5 and 10.5. In this range the absorption maximum is at 390 nm and the fluorescence maximum at 475 nm [257].

Fluorescamine is normally used for the assay of amines, amino acids and peptides in column effluents [162,163], or as a spray reagent for the detection of these compounds on thin-layer chromatograms [122—124]. However, in preliminary work, Imai et al. [259] ran the fluorophores of some amines (dopamine, norepinephrine and their corresponding 3-O-methylation products, polyamines) on silica-gel plates. They showed that about 250—500 pmoles of these compounds could be detected. Nakamura and Pisano [260] suggested that the compounds should be derivatized with fluorescamine at the origin of the thin-layer plates, prior to separation. As the quantitative evaluation of the fluorescent spots did not seem to be completely satisfactory, high-performance liquid chromatographic systems have been devised for the separation of these amines [261,262]. The fluorescamine derivatives were measurable at the 100-pmole level. Further experience will be necessary in order to evaluate the usefulness of this promising reagent fully.

e. Pyridoxal and pyridoxal-5-phosphate

Pyridoxal (Fig. 6, No. 11) and pyridoxal-5-phosphate form Schiff bases with primary amino-containing compounds. Complete reaction is usually achieved at pH 9.3 in phosphate buffer (yields > 90%). After 30 min, the Schiff bases are reduced to the corresponding pyridoxyl derivatives (Fig. 12). The excess of $NaBH_4$ is removed by acidification [263, 264]. Ion-exchange column chromatographic systems have been used exclusively for the separation of pyridoxyl derivatives, which were monitored in the effluent by absorptiometry (absorption maxima at 255 and 328 nm) or by fluorescene measurement (fluorescence emission maximum at 400 nm). Amounts of 10—100 pmol of an amino acid are detectable.

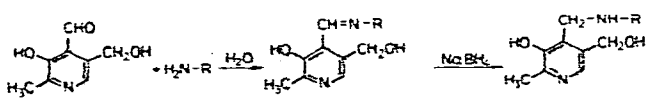

Fig. 12. Derivative-forming reaction of a primary amine with pyridoxal.

One of the advantages of the reagent is the possibility of radioactive labelling by the application of sodium borotritide ($NaBT_4$) as reducing agent. This compound is commercially available with high specific activity.

f. ω-Formyl-o-hydroxyacetophenone and benzo-γ-pyrone

ω-Formyl-o-hydroxyacetophenone and benzo-γ-pyrone react with primary and secondary aliphatic and aromatic amines to form β-aminovinyl o-hydroxyphenyl ketones (Fig. 13). Kostka [265] utilized this reaction for derivative formation with amines, and for their sensitive detection on thin-layer chromatograms. The chromatographic characteristics of 54 enamine derivatives were studied using ethyl acetate—benzene (1:5), chloroform—xylene (4:1) and acetone—xylene (1:9) and silica gel G layers. The β-aminovinyl o-hydroxyphenyl ketones of aliphatic amines are yellowish, while the derivatives of aromatic amines are orange; they fluoresce in the UV region. Amounts of 0.1—1 μg of the derivatives can be detected on a thin-layer plate.

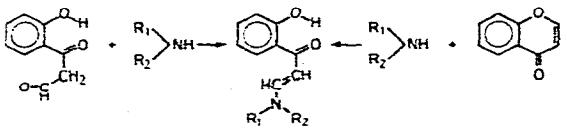

Fig. 13. Formation of β-aminovinyl o-hydroxyphenyl ketones from ω-formyl-o-hydroxyacetophenone and benzo-γ-pyrone.

As the formation of the enamine derivatives is rapid and specific for primary and secondary amines, this derivatization procedure deserves more attention than it seems to have received hitherto.

3. SUMMARY

This first part of the review on "Chromatography of Biogenic Amines" is devoted to the description of generally applicable separation and detection methods. Gas chromatographic and gas chromatographic—mass spectrometric methods, and applications of chromatographic methods to specific amines or groups of related amines and their metabolites, will be covered in Part II.

Trends in the development of separation methods (paper and thin-layer chromatography, paper and thin-layer electrophoresis and ion-exchange methods) are described, using mostly aliphatic amines as examples as they do not exhibit features that permit their specific determination. Reagents suggested for the formation of coloured and fluorescent derivatives of amines are reviewed and their applications are described. Within the limitations of the mostly inadequate information that is available, the relative usefulness of the different derivative-forming reactions are compared.

REFERENCES

1 N. Seiler and M. Wiechmann, Hoppe-Seyler's Z. Physiol. Chem., 350 (1969) 1493.
2 Y. Yoshino, F.V. DeFeudis and K.A.C. Elliott, Canad. J. Biochem., 48 (1970) 147.
3 W.B. Stavinoha, S.T. Weintraub and A.T. Modak, J. Neurochem., 23 (1974) 885.
4 N. Seiler and H.H. Schneider, Biomed. Mass Spectrom., 1 (1974) 381.
5 N. Seiler and A. Askar, J. Chromatogr., 62 (1971) 121.
6 S.I. Harik, G.W. Pasternak and S.H. Snyder, Biochim. Biophys. Acta, 304 (1973) 753.
7 M.A. Beaven, in L.L. Iversen, S.D. Iversen and S.H. Snyder (Editors), Handbook of Psychopharmacology, Vol. 1, Plenum Press, New York, London, 1975, p. 253.

8 J.M. Saavedra, Progr. Anal. Chem., 7 (1974) 33.
9 J.K. Saelens, M.S. Schoen and G.B. Kovacsics, Biochem. Pharmacol., 16 (1967) 1043.
10 W.J. Blaedel and T.J. Anderson, Anal. Chem., 43 (1971) 521.
11 L. Rentzhog, Acta Physiol. Scand., Suppl., 377 (1972) 1.
12 D.P. Henry, B.J. Starman, D.G. Johnson and R.H. Williams, Life Sci., 16 (1975) 375.
13 F.R. Matthias, Klin. Wschr., 50 (1972) 243.
14 J.M. Saavedra, M. Brownstain and J. Axelrod, J. Pharmacol. Exp. Ther., 186 (1973) 508.
15 J.M. Saavedra and J. Axelrod, Proc. Nat. Acad. Sci.U.S., 70 (1973) 769.
16 J.M. Saavedra, Anal. Biochem., 59 (1974) 628.
17 S.H. Snyder, R.J. Baldessarini and J. Axelrod, J. Pharmacol. Exp. Ther., 153 (1966) 544.
18 K.M. Taylor and S.H. Snyder, J. Neurochem., 19 (1972) 1343.
19 Y. Kobayashi and D.V. Maudsley, Anal. Biochem., 46 (1972) 85.
20 J.K. Saelens, M.P. Allen and J.P. Simke, Arch. Int. Pharmacodyn., 186 (1970) 279.
21 W.D. Reid, D.R. Haubrich and G. Krishna, Anal. Biochem., 42 (1971) 390.
22 P.A. Shea and M.H. Aprison, Anal. Biochem., 56 (1973) 165.
23 S. Wonnacott, Biochem. Soc. Trans., 3 (1975) 102.
24 L. Waldenlind, Biochem. Pharmacol., 24 (1975) 1339.
25 R. Massarelli, T. Durkin, C. Niedergang and P. Mandel, Pharmacol. Res. Commun., 8 (1976) 407.
26 B. Peskar and S. Spector, Science, 179 (1973) 1340.
27 S. Spector, Clin. Pharmacol. Ther., 16 (1974) 149.
28 D. Bartos, R.A. Campbell, F. Bartos and D.P. Grettie, Cancer Res., 35 (1975) 2056.
29 E. Knoll and H. Wisser, Z. Anal. Chem., 279 (1976) 119.
30 M. Guggenheim, Die biogenen Amine, S. Karger, Basle, New York, 1951.
31 N. Seiler, J. Chromatogr., 63 (1971) 97.
32 N. Seiler, L. Demisch and H. Schneider, Angew. Chem., Int. Ed. Engl., 10 (1971) 51.
33 F. Cattabeni, S.H. Koslow and E. Costa, Adv. Biochem. Psychopharmacol., 6 (1972) 37.
34 E. Costa and B. Holmstedt, Gas Chromatography—Mass Spectrometry in Neurobiology, Raven Press, New York, 1973.
35 D.J. Jenden and A.K. Cho, Ann. Rev. Pharmacol., 13 (1973) 371.
36 E. Costa, J. Psychiat. Res., 11 (1974) 296.
37 E. Costa, S.H. Koslow and H.F. LeFevre, in L.L. Iversen, S.D. Iversen and S.H. Snyder (Editors), Handbook of Psychopharmacology, Vol. 1, Plenum Press, New York, London, 1975, p.1.
38 F. Fonnum, Adv. Biochem. Psychopharmacol., 6 (1972) 75.
39 C.R. Creveling and J.W. Daly, Methods Enzymol., 17B (1971) 846.
40 N.N. Osborne, Progr. Neurobiol., 1, Part 4 (1973) 299.
41 V. Neuhoff, Micromethods in Molecular Biology, Springer, Berlin, New York, 1973.
42 M.L. Bastos and S.K. Mule, Progr. Chem. Toxicol., 5 (1974) 187.
43 B.E. Leonard and N.N. Osborne, in N. Marks and R. Rodnight (Editors), Research Methods in Neurochemistry, Vol. 3, Plenum Press, New York, London, 1975, p. 443.
44 N. Seiler, Methods Biochem. Anal., 18 (1970) 259.
45 N. Seiler and M. Wiechmann, in A. Niederwieser and G. Pataki (Editors), Progress in Thin-Layer Chromatography and Related Methods, Vol. 1, Ann Arbor—Humphrey Sci. Publ., Ann Arbor, Mich., London, 1970, p. 95.
46 V.P. Whittaker, in D. Eichler and A. Farah (Editors), G.B. Koelle (Subeditor), Handbook of Experimental Pharmacology, Vol. 15, Springer, Berlin, New York, 1963, p. 1.
47 D.J. Jenden and L.B. Campbell, Methods Biochem. Anal., Suppl., (1971) 183.
48 D.J. Jenden, Adv. Biochem. Psychopharmacol., 7 (1973) 68.
49 I. Hanin (Editor), Choline and Acetylcholine: Handbook of Chemical Assay Methods, Raven Press, New York, London, 1974.
50 D.F. Sharman, in R. Fried (Editor), Methods of Neurochemistry, Vol. 1, Marcel Dekker, New York, 1971, p. 83.
51 H. Weil-Malherbe, in D. Glick (Editor), Analysis of Biogenic Amines and Their Related Enzymes, Interscience, New York, 1971, p. 119.

52 H. Snyder and K.M. Taylor, in N. Marks and R. Rodnight (Editors), Research Methods in Neurochemistry, Vol. 1, Plenum Press, New York, London, 1972, p. 287.
53 P.A. Shore, in D. Glick (Editor), Analysis of Biogenic Amines and Their Related Enzymes, Interscience, New York, 1971, p. 89.
54 W. Lowenberg and K. Engelman, in D. Glick (Editor), Analysis of Biogenic Amines and Their Related Enzymes, Interscience, New York, 1971, p.1.
55 N. Narasimhachari, J.M. Plaut and K.Y. Leiner, Biochem. Med., 5 (1971) 304.
56 A.A. Boulton and J.R. Majer, in N. Marks and R. Rodnight (Editors), Research Methods in Neurochemistry, Vol. 1, Plenum Press, New York, London, 1972, p. 341.
57 N. Seiler, in N. Marks and R. Rodnight (Editors), Research Methods in Neurochemistry, Vol. 3, Plenum Press, New York, London, 1975, p. 409.
58 N.H. Choulis, J. Chromatogr., 30 (1967) 618.
59 J. Gasparic, in I.M. Hais and K. Macek (Editors), Handbuch der Papierchromatographie, Vol. 1, Gustav Fischer, Jena, 1958, p. 392.
60 J.M. Bremner and R.H. Kenten, Biochem. J., 49 (1951) 651.
61 R. Schwyzer, Acta Chem. Scand., 6 (1952) 219.
62 F. Schmidt and H.V. Bauriedel, Naturwissenschaften, 43 (1956) 470.
63 F. Georgi, C.G. Honegger, D. Jordan, H.P. Rieder and M. Rottenberger, Klin. Wschr., 34 (1956) 799.
64 E.J. Herbst, D.L. Keister and R.H. Weaver, Arch. Biochem. Biophys., 75 (1958) 178.
65 C.G. Honegger and R. Honegger, Nature (London), 185 (1960) 530.
66 M. Roberts, J. Pharmacol. Exp. Ther., 14 (1962) 746.
67 T.L. Perry, K.N.F. Shaw, D. Walker and R. Redlich, Pediatrics, 30 (1962) 576.
68 T.L. Perry and W.A. Schroeder, J. Chromatogr., 12 (1963) 358.
69 T.L. Perry, S. Hansen and I. Jenkins, J. Neurochem., 11 (1964) 49.
70 T.L. Perry, S. Hansen, M. Hestrin and L. MacIntyre, Clin. Chim. Acta, 11 (1965) 24.
71 T.L. Perry, M. Hestrin, L. MacDougall and S. Hansen, Clin. Chim. Acta, 14 (1966) 116.
72 T.L. Perry, S. Hansen, J.G. Foulks and G.M. Ling, J. Neurochem., 12 (1965) 397.
73 L.H. Laasberg and S. Shimosato, J. Appl. Physiol., 21 (1966) 1929.
74 S. Ramakrishna and P.R. Adiga, J. Chromatogr., 86 (1973) 214.
75 K.H. Teichert, E. Mutschler and H. Rochelmeyer, Deut. Apoth. Ztg., 100 (1960) 283.
76 H. Grasshof, J. Chromatogr., 20 (1965) 165.
77 R. Gnehm, H.U. Reichert and P. Guyer, Chimia (Aarau), 19 (1965) 585.
78 I. Toyozawa and S. Ogura, Nippon Shokuhin Kogyo Gakkaishi, 12 (1965) 46; C.A., 64 (1966) 15021d.
79 J.R. Parrish, J. Chromatogr., 18 (1965) 535.
80 A. Lynes, J. Chromatogr., 23 (1966) 316.
81 H. Gerlach, Pharm. Zentralhalle, 105 (1966) 93.
82 I. Gemzoua and J. Gasparic, Collect Czech. Chem. Commun., 31 (1966) 2525.
83 W. Reissmann and T. Wieske, Anal. Biochem., 19 (1967) 48.
84 A. Bassl, H.J. Heckmann and E. Baumann, J. Prakt. Chem., 36 (1967) 265.
85 H. Möhrle and R. Feil, J. Chromatogr., 34 (1968) 264.
86 J.E. Hammond and E.J. Herbst, Anal. Biochem., 22 (1968) 474.
87 J.S. Ross, Anal. Chem., 40 (1968) 2138.
88 D. Cozzi, P.G. Desideri, L. Lepri and V. Coas, J. Chromatogr., 43 (1969) 463.
89 E.S. Lane, J. Chromatogr., 18 (1965) 426.
90 A.H. Beckett and N.H. Choulis, Int. Congr. Pharm. Sci., 23rd, Münster, 1963; from E. Stahl and P.J. Schorn, in E. Stahl (Editor), Dünnschicht-Chromatographie, Springer, Berlin, New York, 2nd ed., 1967, p. 470.
91 R. Segura-Cardona and K. Soehring, Med. Exp., 10 (1964) 251.
92 A. Alessandro, Bull. Chim. Farm., 104 (1965) 498.
93 W.P. De Potter, R.F. Vochten and A.F. De Schaepdryver, Experientia, 21 (1965) 482.
94 F.H. Schneider and C.N. Gillis, Biochem. Pharmacol., 14 (1965) 623.
95 D. Aures, R. Fleming and R. Håkanson, J. Chromatogr., 33 (1968) 480.
96 G.A. Johnson and S.J. Boukma, Anal. Biochem., 18 (1967) 143.
97 N.H. Choulis, J. Pharm. Sci., 56 (1967) 196.
98 J. Giese, E. Rüther and N. Matussek, Life Sci., 6 (1967) 1975.

99 J.M. Gutteridge, Clin. Chim. Acta, 21 (1968) 211.
100 E.J. Cowles, G.M. Christensen and A.C. Hilding, J. Chromatogr., 35 (1968) 389.
101 N.H. Choulis and C.E. Carey, J. Pharm. Sci., 57 (1968) 1048.
102 A. Vahidi and D.V.S. Sankar, J. Chromatogr., 43 (1969) 135.
103 R.M. Fleming and W.G. Clark, J. Chromatogr., 52 (1970) 305.
104 K. Mayer and G. Pause, Lebensm. Wiss. Technol., 5 (1972) 108.
105 H.M. Stevens and P.D. Evans, Acta Pharmacol. Toxicol., 32 (1973) 525.
106 S.P. Srivastava and V.K. Dua, Z. Anal. Chem., 279 (1976) 367.
107 F.G. Fischer and H. Bohn, Hoppe-Seyler's Z. Physiol. Chem., 308 (1957) 108.
108 A. Raina, Acta Physiol. Scand., Suppl., No. 218 (1963) 1.
109 C.G. Honegger, Helv. Chim. Acta, 44 (1961) 173.
110 G. Pastuska and H. Trinks, Chemiker Ztg., 86 (1962) 135.
111 H.G. Maier and W. Diemair, Z. Anal. Chem., 223 (1966) 263.
112 B. Lerch and H. Stegemann, Z. Naturforsch. B, 21 (1966) 216.
113 H. Bayzer, J. Chromatogr., 24 (1966) 372.
114 K. Fücker, Nahrung, 18 (1974) 663.
115 M. Roberts, J. Pharmacol. Exp. Ther., 14 (1962) 746.
116 K. Shimomura and H.F. Walton, Separ. Sci., 3 (1968) 493.
117 K.G. Krebs, D. Heusser and H. Wimmer, in E. Stahl (Editor), Dünnschicht-Chromatographie, Springer, Berlin, New York, 2nd ed., 1967, p. 813.
118 P. Haefelfinger, J. Chromatogr., 48 (1970) 184.
119 J.S. Ross, Anal. Chem., 40 (1968) 2138.
120 R. Segura and A.M. Gotto, Jr., J. Chromatogr., 99 (1974) 643.
121 A.B. Groth and M.R. Dahlen, Acta Chem. Scand., 21 (1967) 291.
122 A.M. Felix and M.H. Jimenez, J. Chromatogr., 89 (1974) 361.
123 F. Abe and K. Samejima, Anal. Biochem., 67 (1975) 298.
124 H. Nakamura and J.J. Pisano, J. Chromatogr., 121 (1976) 79.
125 M. Roth, Anal. Chem., 43 (1971) 880.
126 E. Mendez and J.G. Gavrilanes, Anal. Biochem., 72 (1976) 473.
127 Y. Kase, M. Kataoka and T. Miyata, Jap. J. Pharmacol., 19 (1969) 354.
128 R. Nixon, Anal. Biochem., 48 (1972) 460.
129 F. Fonnum, Biochem. J., 115 (1969) 465.
130 V. Glover and D.P.L. Green, J. Neurochem., 19 (1972) 2465.
131 D. Westerlund, Acta Pharm. Suecica, 9 (1972) 47.
132 I.L. Martin and G.B. Ansell, Biochem. Pharmacol., 22 (1973) 521.
133 K.O. Borg, Acta Pharm. Suecica, 8 (1971) 1.
134 B.A. Persson, Acta Pharm. Suecica, 8 (1971) 193.
135 B.A. Persson, Acta Pharm. Suecica, 8 (1971) 217.
136 R. Modin, Acta Pharm. Suecica, 8 (1971) 509.
137 B.C. Lippold, Pharmazie, 28 (1973) 713.
138 D. Westerlund, Acta Pharm. Suecica, 12 (1975) 127.
139 S. Eksborg and B.A. Persson, Acta Pharm. Suecica, 8 (1971) 205.
140 F. Fonnum, in N. Marks and R. Rodnight (Editors), Research Methods in Neurochemistry, Vol. 3, Plenum Press, New York, London, 1975, p. 253.
141 B.A. Persson, J. Chromatogr. Sci., 12 (1974) 521.
142 B.A. Persson and B.L. Karger, Z. Klin. Chem. Klin. Biochem., 12 (1974) 222.
143 B.-A. Persson and P.-O. Lagerstrom, J. Chromatogr., 122 (1976) 305.
144 S.S. Cohen, Introduction to the Polyamines, Prentice-Hall, Englewood Cliffs, N.J., 1971.
145 U. Bachrach, Function of Naturally Occurring Polyamines, Academic Press, New York, 1973.
146 D.H. Russell, C.C. Levy, S.C. Schimpff and I.A. Hawk, Cancer Res., 31 (1971) 1555.
147 D.H. Russell, B.G.M. Durie and S.E. Salmon, Lancet, (1975) 797.
148 U. Bachrach, Ital. J. Biochem., 25 (1976) 77.
149 N. Seiler and T. Schmidt-Glenewinkel, J. Neurochem., 24 (1975) 791.
150 N. Seiler, in D.H. Russell (Editor), Polyamines in Normal and Neoplastic Growth, Raven Press, New York, 1973, p. 137.

151 N. Seiler and M.-J. Al-Therib, Biochem. J., 144 (1974) 29.
152 D.H. Spackman, W.H. Stein and S. Moore, Anal. Chem., 30 (1958) 1190.
153 T.L. Perry, S. Hansen and L. MacDougall, J. Neurochem., 14 (1967) 775.
154 T. Nakajima, F. Wolfgram and W.G. Clark, J. Neurochem., 14 (1967) 1113.
155 S. Baba and S. Ogiya, Yakugaku Zasshi, 89 (1969) 399.
156 H. Hatano, S. Sumizu, S. Rokushika and F. Murakami, Anal. Biochem., 35 (1970) 377.
157 R.A. Wall, J. Chromatogr., 60 (1971) 195.
158 P. Vandekerckhove and K. Henderickx, J. Chromatogr., 82 (1973) 379.
159 C.D. Scott, J. Chromatogr. Sci., 11 (1973) 379.
160 P. Jandera and J. Churáček, J. Chromatogr., 98 (1974) 1.
161 S. Baba, Yakugaku Zasshi, 89 (1969) 1712.
162 W. Leimgruber and M. Weigele, Science, 178 (1973) 871.
163 S. Stein, P. Böhlen, K. Imai, J. Stone and S. Udenfriend, Fluorescence News, 7 (1973) 9.
164 M. Roth and A. Hampaï, J. Chromatogr., 83 (1973) 353.
165 J.R. Benson and P.E. Hare, Proc. Nat. Acad. Sci. U.S., 72 (1975) 619.
166 S.M. Rosenthal and C.W. Tabor, J. Pharmacol. Exp. Ther., 116 (1956) 131.
167 L.T. Kremzner, Anal. Biochem., 15 (1966) 270.
168 L.T. Kremzner, R.E. Barrett and M.J. Terrano, Ann. N.Y. Acad. Sci., 171 (1970) 735.
169 C.W. Tabor and S.M. Rosenthal, Methods Enzymol., 6 (1963) 615.
170 T. Unemoto, K. Ikeda, M. Hayashi and K. Miyaki, Chem. Pharm. Bull. (Tokyo), 11 (1963) 148.
171 B. Elliott and I.A. Michaelson, Anal. Biochem., 19 (1967) 184.
172 H. Veening, W.W. Pitt, Jr. and G. Jones, Jr., J. Chromatogr., 90 (1974) 129.
173 D.R. Morris, K.L. Koffron and C.J. Okstein, Anal. Biochem., 30 (1969) 449.
174 H.J. Bremer, E. Kohne and E. Endres, Clin Chim. Acta, 32 (1971) 407.
175 L.J. Marton, D.H. Russell and C.C. Levy, Clin. Chem., 19 (1973) 923.
176 H. Tabor, C.W. Tabor and F. Irreverre, Anal. Biochem., 55 (1973) 457.
177 L.J. Marton, O. Heby, C.B. Wilson and P.L.Y. Lee, FEBS Lett., 46 (1974) 305.
178 C.W. Gehrke, K.C. Kuo, R.W. Zumwalt and T.P. Waalkes, J. Chromatogr., 89 (1974) 231.
179 C.W. Gehrke, K.C. Kuo, R.L. Ellis and T.P. Waalkes, J. Chromatogr., 143 (1977) in press.
180 K. Nishioka and M.M. Romsdahl, Clin. Chim. Acta, 57 (1974) 155.
181 J.A. Sturman and G.E. Gaull, Pediat. Res., 8 (1974) 231.
181a H. Adler, M. Margoshes, L.R. Snyder and C. Spitzer, J. Chromatogr., 143 (1977) 125.
182 J.D. Navratil and H.F. Walton, Anal. Chem., 47 (1975) 2443.
183 L.J. Marton and P.L.Y. Lee, Clin. Chem., 21 (1975) 1721.
184 F. Sanger, Biochem. J., 39 (1945) 507.
185 F. Sanger and H. Tuppy, Biochem. J., 49 (1951) 463.
186 A.D. Smith and J.B. Jepson, Anal. Biochem., 18 (1967) 36.
187 I.M. Lockhart, Nature (London), 177 (1956) 393.
188 A.M. Asatoor and C.E. Dalgliesh, Biochem. J., 73 (1959) 26P.
189 D.B. Parihar, S.P. Sharma and K.K. Verma, J. Chromatogr., 26 (1967) 292.
190 S. Baba and S. Ogiya, Yakugaku Zasshi, 89 (1969) 1704.
191 A. Zeman and I.P.G. Wirotama, Z. Anal. Chem., 247 (1969) 155.
192 M. Kataoka, Y. Kase, T. Miyata and E. Kawahito, J. Neurochem., 17 (1970) 291.
193 R. Nixon and R.L. Sidman, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 30 (1971) 1085.
194 H.-I. Ilert and T. Hartmann, J. Chromatogr., 71 (1972) 119.
195 D.T. Dubin, J. Biol. Chem., 235 (1960) 783.
196 A.S. Keston, S. Udenfriend and M. Levy, J. Amer. Chem. Soc., 69 (1947) 3151.
197 E.D. Bergmann and M. Bentov, J. Org. Chem., 26 (1961) 1480.
198 I.P.G. Wirotama and K.H. Ney, J. Chromatogr., 61 (1971) 166.
199 A. Zeman and I.P.G. Wirotama, Z. Anal. Chem., 259 (1972) 351.
200 K. Heyns, H.P. Harke, H. Scharmann and H.F. Grützmacher, Z. Anal. Chem., 230 (1967) 118.
201 J. Churáček, H. Pechová, A. Tocksteinová and Z. Zíková, J. Chromatogr., 72 (1972) 145.
202 A. Jart and A.J. Bigler, J. Chromatogr., 29 (1967) 255.
203 J.Y. Chang and E.H. Creaser, J. Chromatogr., 116 (1976) 215.
204 J.K. Lin and J.Y. Chang, Anal. Chem., 47 (1975) 1634.

205 N. Seiler and H. Möller, Chromatographia, 2 (1969) 273.
206 N. Seiler and H. Möller, Chromatographia, 2 (1969) 319.
207 N. Seiler and H. Möller, Chromatographia, 2 (1969) 470.
208 V. Pollak and A.A. Boulton, J. Chromatogr., 72 (1972) 231.
209 N. Seiler and L. Demisch, in K. Blau and G.H. King (Editors), Handbook of Derivatives for Chromatography, Heyden & Sons, London, 1977.
210 H.-J. Klimisch and L. Stadler, J. Chromatogr., 90 (1974) 141.
211 J.F. Lawrence and R.W. Frei, Anal. Chem., 44 (1972) 2046.
212 D. Clasing, H. Alfes, H. Möllmann and J. Reisch, Z. Klin. Chem. Klin. Biochem., 7 (1969) 648.
213 J. Montforte, R.J. Bath and I. Sunshine, Clin. Chem., 18 (1972) 1329.
214 F. Van Hoof and A. Heyndrick, Anal. Chem., 46 (1974) 286.
215 D.J. Birkett, N.C. Price, G.K. Radda and A.G. Salmon, FEBS Lett., 6 (1970) 346.
216 P.B. Ghosh and M.W. Whitehouse, Biochem. J., 108 (1968) 155.
217 J. Reisch, H. Alfes, H.J. Kommert, N. Jantos, H. Möllmann and E. Clasing, Pharmazie, 25 (1970) 331.
218 N. Seiler, Hoppe-Seyler's Z. Physiol. Chem., 348 (1967) 601.
219 E. Schulze and V. Neuhoff, Hoppe-Seyler's Z. Physiol. Chem., 357 (1976) 225.
220 D.J. Neadle and R.J. Pollit, Biochem. J., 97 (1965) 607.
221 N. Seiler and M. Wiechmann, Hoppe-Seyler's Z. Physiol. Chem., 349 (1968) 588.
222 M. Wiechmann, unpublished results.
223 W. Dünges and H.W. Peter, Excerpta Med. Found. Intern. Congress Ser., No. 286, Excerpta Medica, Amsterdam, 1972, p. 126.
224 N. Seiler, T. Schmidt-Glenewinkel and H.H. Schneider, J. Chromatogr., 84 (1973) 95.
225 R.P. Cory, R.R. Becker, R. Rosenbluth and T. Isenberg, J. Amer. Chem. Soc., 90 (1968) 1643.
226 Ch.P. Ivanov and Y. Vladovska-Yukhnovska, J. Chromatogr., 71 (1972) 111.
227 R.E. Kaiser (Editor), Einführung in die Hochleistungs-Dünnschicht-Chromatographie, Institut für Chromatographie, Bad Dürkheim, 1976.
227a A. Zlatkis and R.E. Kaiser (Editors), HPTLC — High Performance Thin-Layer Chromatography, Elsevier, Amsterdam, Oxford; New York and Institute of Chromatography, Bad Dürkheim, 1977.
228 N. Seiler and B. Knödgen, J. Chromatogr., 131 (1977) 109.
229 N. Seiler and H.H. Schneider, J. Chromatogr., 59 (1971) 367.
230 W. Dünges, G. Naundorf and N. Seiler, J. Chromatogr. Sci., 12 (1974) 655.
231 S.R. Abbott, A. Abu-Shumays, K.O. Loeffler and I.S. Forrest, Res. Commun. Pathol. Pharmacol., 10 (1975) 9.
232 M.M. Abdel-Monem and K. Ohno, J. Chromatogr., 107 (1975) 416.
233 N.E. Newton, K. Ohno and M.M. Abdel-Monem, J. Chromatogr., 124 (1976) 277.
234 N. Seiler and M. Wiechmann, Z. Anal. Chem., 220 (1966) 109.
235 H. Engelhardt, J. Asshauer, U. Neue and N. Weigand, Anal. Chem., 46 (1974) 336.
236 T. Seki and H. Wada, J. Chromatogr., 102 (1974) 251.
237 Ch.P. Ivanov and Y. Vladovska-Yukhnovska, Biochim. Biophys. Acta, 194 (1969) 345.
238 I. Durko, Y. Vladovska-Yukhnovska and Ch. P. Ivanov, Clin. Chim. Acta, 49 (1973) 407.
239 N. Seiler and U. Lamberty, Comp. Biochem. Physiol., 52B (1975) 409.
240 N. Seiler and K. Bruder, J. Chromatogr., 106 (1975) 159.
241 N. Seiler and B. Knödgen, J. Chromatogr., 97 (1974) 286.
242 A.E. Jenkins and J.R. Mayer, in R. Brummer and J.R. Penney (Editors), Mass Spectrometry, Butterworths, London, 1968, p. 253.
243 N. Seiler and B. Knödgen, Org. Mass. Spectrom., 7 (1973) 97.
244 D.A. Durden, B.A. Davis and A.A. Boulton, Biomed. Mass Spectrom., 1 (1974) 83.
245 C.R. Creveling, K. Kondo and J.W. Daly, Clin. Chem., 14 (1968) 302.
246 J. Reisch, H. Alfes, N. Jantos and H. Möllmann, Acta Pharm. Suecica, 5 (1968) 393.
247 N. Seiler, H. Schneider and K.-D. Sonnenberg, Z. Anal. Chem., 252 (1970) 127.
248 H. Egge, H. Ockenfels, H. Thomas and F. Zilliken, Z. Naturforsch. B, 26 (1971) 229.

249 F. Addeo, A. Malorni and G. Marino, Anal. Biochem., 64 (1975) 98.
250 M. Weise and G.M. Eisenbach, Experientia, 28 (1972) 245.
251 S.R. Snodgrass and L.L. Iversen, Nature (London), 241 (1973) 154.
252 J.E. Sinsheimer, D.D. Hong, J.T. Stewart, M.L. Fink and J.H. Burckhalter, J. Pharm. Sci., 60 (1971) 141.
253 H. Maeda, N. Ishida, H. Kawauchi and K. Tuzimura, J. Biochem. (Tokyo), 65 (1969) 777.
254 H. Ichiwaka, T. Tanimura, T. Nakajima and Z. Tamura, Chem. Pharm. Bull. (Tokyo), 18 (1970) 1493.
255 A. DeLeenheer, J.E. Sinsheimer and J.H. Burckhalter, J. Pharm. Sci., 62 (1973) 1370.
256 S. Stein, P. Böhlen and S. Udenfriend, Arch. Biochem. Biophys., 163 (1974) 400.
257 S. DeBarnardo, M. Weigele, V. Toome, K. Manhart, W. Leimgruber, P. Böhlen, S. Stein and S. Udenfriend, Arch. Biochem. Biophys., 163 (1974) 390.
258 P. Böhlen, S. Stein, W. Dairman and S. Udenfriend, Arch. Biochem. Biophys., 155 (1973) 213.
259 K. Imai, P. Böhlen, S. Stein and S. Udenfriend, Arch. Biochem. Biophys., 161 (1974) 161.
260 H. Nakamura and J.J. Pisano, J. Chromatogr., 121 (1976) 33.
261 K. Imai, J. Chromatogr., 105 (1975) 135.
262 K. Samejima, J. Chromatogr., 96 (1974) 250.
263 N. Lustenberger, H.W. Lange and K. Hempel, Angew. Chem., 84 (1972) 255.
264 H.W. Lange, N. Lustenberger and K. Hempel, Z. Anal. Chem., 261 (1972) 337.
265 K. Kostka, J. Chromatogr., 49 (1970) 249.