Fluorescence induced by U.V. radiation will appear as light bands on the print, provided the energy thus emitted is higher than that reflected by the background.

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Thin-layer chromatographic separation of primary and secondary amines as 4-(phenylazo)benzenesulfonamides

The direct chromatography on thin layers of primary and secondary amines in most cases causes difficulties due to the strong adsorption of the NH-group to the adsorbents generally used. Several methods have, nevertheless, been developed for work with the free amines, but the use of derivatives is often to be preferred. Thus derivatization has been performed with reagents such as 2,4-dinitrochlorobenzene[1,2], 3,5-dinitrobenzoyl chloride[3,4], 1-dimethylaminonaphthalene-5-sulfonyl chloride[5], 4-toluenesulfonyl chloride[4,6] or benzoyl chloride[4].

In the present investigation, 4-(phenylazo)benzenesulfonyl chloride has been used for preparing derivatives of the amines. These derivatives have previously been described, and fair separations were achieved by column chromatography[7]. The 4-(phenylazo)benzenesulfonamides formed have several advantages over the free amines as well as over derivatives used before. They are thus well suited for thin-layer chromatography, *e.g.* on alumina plates, the compounds are easy to detect on the plates, as they are intensely colored, and the preparation of the derivatives can be carried out very simply even from aqueous solutions of the amines or their salts. Due to these facts the method is also very suitable for separation and characterization of volatile amines arising from the cleavage of more complicated molecules, *e.g.* of biological origin. The procedure described here was originally developed for the lower aliphatic amines, as only very few methods are available for the identification of these compounds, but results are also given for some amines of pharmacological interest. The method discussed in this paper has been included in the course in organic identification at the Technical University of Denmark.

Preparation of the derivatives

The reagent, 4-(phenylazo)benzenesulfonyl chloride, was prepared from azobenzene (Fluka, puriss.) and chlorosulfonic acid[8]. The derivatives were prepared according to the following procedure:

3 mmoles of an amine (or of an amine salt, such as the hydrochloride, sulfate or tartrate) and 2 ml of a sodium hydroxide solution (2 *N*) were poured into a centrifuge tube and 1 ml of a 1 % solution of the reagent in chloroform was added. The mixture was shaken vigorously for 3 min, centrifuged, and the upper phase (the water phase) was sucked off and rejected. The colored, organic phase was then shaken vigorously with 2 ml of a hydrochloric acid solution (4 *N*); after separation from the aqueous phase, the chloroform phase was finally shaken with 2 ml of water and used directly for thin-layer chromatography.

Chromatographic procedure

The chromatography was performed on microchromatoplates (40 × 76 mm) coated with alumina (Fluka, D5) as previously described[9]. The plates were developed in a 250 ml beaker covered with a cork. The mobile phase was a mixture of 25 ml ethyl acetate and 100 ml petroleum ether (62–82° from Shell) saturated with water. The amount of the above-mentioned chloroform solution used was about 0.1 μl. The time of the development was 10–15 min.

As the derivatives are intensely colored, they can, in most cases, be observed directly on the plates; however, the detection can be considerably improved by exposure of the plates to iodine vapour.

Results and discussion

With the procedure used, it is not possible to characterize the movement of the individual compounds by ordinary R_F values, as the chromatography is continued after the solvent front has reached the upper edge of the plate. Therefore, the data reported in this paper represent relative values, with the movement of the derivative of butylamine taken as the standard; for a few fast-moving compounds, the derivative of dipropylamine was taken as the standard. In this way, good reproducibility of the results was obtained, as deviations from the normal, due to variations in temperature, composition of the mobile phase (*cf.* however below), layer thickness etc. are eliminated to a certain degree. The reproducibility is illustrated by the results from 18 plates, chromatographed one after the other, with 2 samples of a reference mixture of the 4-(phenylazo)benzenesulfonamides (4-PABSA's) of methylamine, ethylamine, propylamine, and butylamine, one on each edge of the plate. The relative distances and the standard deviations for the 36 runs are given in Table I.

The use of relative values for the distances travelled eliminates, as mentioned, to a certain degree many of the factors of uncertainty. However, due to slight differ-

TABLE I

REPRODUCIBILITY IN CHROMATOGRAPHING 4-(PHENYLAZO)BENZENESULFONAMIDES

4-PABSA	*Average value* $\left(\pm\sqrt{\frac{\Sigma\lambda^2}{n(n-1)}}\right)$	*Standard deviation* $\left(\pm\sqrt{\frac{\Sigma\lambda^2}{n-1}}\right)$
Methylamine	0.466 ± 0.004	± 0.024
Ethylamine	0.706 ± 0.003	± 0.017
Propylamine	0.868 ± 0.002	± 0.012
Butylamine	1.00 ± 0	—

ences in the adsorption power of the stationary phase it is in most cases necessary to adjust the ethyl acetate content of the mobile phase, so as to yield an R_X value of 1.50 for dipropylamine. Both butylamine and dipropylamine have been used in this way to standardize the mobile phase employed for determining the values presented in Tables II and III.

In Table II, relative values are given for all the 4-PABSA's dealt with. Table III contains the derivatives which have R_X values greater than 1.50 relative to butylamine. As such large values are only poorly reproducible relative to butylamine, it is preferable to use a faster running compound as the standard. Dipropylamine has been chosen as standard for these compounds, and the R_X values determined on this basis are given in Table III. All the compounds were chromatographed on 3–6 different plates with a standard sample of the four 4-PABSA's, dealt with in Table I, on each edge.

TABLE II

R_X VALUES (RELATIVE TO THE DERIVATIVE OF BUTYLAMINE) ON ALUMINA FOR 4-(PHENYLAZO)-BENZENESULFONAMIDES

Primary amines			*Secondary amines*		
No.	*4-PABSA*	R_X	*No.*	*4-PABSA*	R_X
1	Methylamine	0.47	22	Dimethylamine	1.16
2	Ethylamine	0.71	23	Diethylamine	1.43
3	Propylamine	0.87	24	Dipropylamine	1.50
4	Isopropylamine	0.92	25	Dibutylamine	>1.50
5	Butylamine	1.00	26	Diisobutylamine	>1.50
6	*sec.*-Butylamine	1.04	27	Diamylamine	>1.50
7	Isobutylamine	1.06	28	Diallylamine	1.47
8	*tert.*-Butylamine	1.05	29	Dipropargylamine	1.28
9	Amylamine	1.08	30	Dibenzylamine	1.41
10	Isoamylamine	1.13	31	β-Phenylisopropylmethylamine	1.26
11	Hexylamine	1.15	32	Ephedrine	0.57
12	Octylamine	1.20	33	Pyrazole	1.38
13	Decylamine	1.27	34	Imidazole	0.94
14	Allylamine	0.81	35	Morpholine	0.99
15	Cyclopentylamine	1.00			
16	Cyclohexylamine	1.05			
17	Benzylamine	0.87			
18	α-Phenethylamine	0.86			
19	β-Phenethylamine	0.83			
20	Amphetamine	0.94			
21	Mescaline	0.15			

TABLE III

R_X^+ VALUES (RELATIVE TO THE DERIVATIVE OF DIPROPYLAMINE) ON ALUMINA FOR 4-(PHENYLAZO)-BENZENESULFONAMIDES

No.	*4-PABSA*	R_X^+
24^+	Dipropylamine	1.00
25^+	Dibutylamine	1.05
26^+	Diisobutylamine	1.06
27^+	Diamylamine	1.10

With the method described, a good separation of the first six straight-chain, saturated primary amines as well as of octylamine and decylamine was achieved, the distance travelled increasing with increasing length of the carbon chain. A branched chain causes the compounds to move faster, *e.g.* isopropylamine runs faster than propylamine. The effect caused by increased branching of the carbon chain tends to be more pronounced the closer the branch is situated to the amino group; the great difference between the distances travelled by primary and secondary amines with the same number of carbon atoms is most striking (*cf.* diethylamine and butylamine). Double bonds retard movement, and so also do alicyclic groups; phenyl groups have a very strong retarding effect.

The preparation of derivatives of compounds containing amino groups attached directly to a benzene ring is not possible. Further, it has not been possible to prepare derivatives from diamines or amines containing acidic groups (phenols etc.). Finally it has proved impossible to obtain derivatives from di-*sec.*-butylamine and phentermine, possibly due to some sort of steric hindrance.

It should be noted that alcohols, hydrazine compounds, tertiary amines, and ammonia do not interfere in the procedure outlined above.

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Charge-transfer complexes of 2,4,6-trinitrophenyl-N-methylnitramine (tetryl) and 2,4-dinitrochlorobenzene with some amines

Thin-layer chromatography has been efficiently employed for the investigation of various charge-transfer complexes. For example studies on complexes of terpenes[1–4], glycerides[5], hydrocarbons[6] with silver and polynitro aromatic compounds with hydrocarbons[7] have been reported. Recently the π-complexes of a number of aromatic amines with 2,4,6-trinitrotoluene (*s*-TNT) and *m*-dinitrobenzene (*m*-DNB) have been investigated[8] by this technique.

In the present paper, studies of the π-complexes of 2,4,6-trinitrophenyl-N-methylnitramine (tetryl) and 2,4-dinitrochlorobenzene (DNCB) with some amines are