THIN-LAYER CHROMATOGRAPHY OF AROMATIC AMINES AND THEIR DERIVATIVES AFTER REACTIONS WITH 1-FLUORO-2,4-DINITROBENZENE

JAROSLAV FRANC and VENUŠE KOUDELKOVÁ

Research Institute of Organic Syntheses, 532 18 Pardubice-Rybitví (Czechoslovakia)

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SUMMARY

With the help of thin-layer chromatography, 128 differently substituted aromatic amines and their 2,4-dinitrophenyl derivatives have been separated. Three solvent systems were employed and in certain cases the analysis was completed by paper electrophoresis. The derivatives were prepared by reaction of the amines with 1-fluoro-2,4-dinitrobenzene.

INTRODUCTION

Aromatic amines are very important compounds in the heavy organic chemicals industry, *e.g.*, in the production of dyes. This is reflected in the number of investigations of the chromatography of aromatic amines, such as their separation on paper[1-8] and thin layers[9-21]. The identification by chromatographic methods is, however, not always simple, particularly for substituted amines.

During the last few years, the so-called reaction chromatography has been found to be successful for the identification of amines. In this technique a suitable derivative is prepared in addition to the parent compound, and both compounds are subjected to chromatographic separation to yield further data. Examples of derivatives of aromatic amines which have been employed are 3,5-dinitrobenzamides[22,23], 4-dimethylaminobenzeneazo-4-benzamides[24], 2,4-dinitrophenylamines[25] and *p*-toluenesulphonamides[26-28]. Primary aromatic amines can also be converted into arylazo-2-naphthols[29]. Amines can also be coupled with different passive components such as naphthylamine, N-1-naphthylethyldiamine, N-ethyl-1-naphthylamine or 1-phenyl-3-methyl-5-pyrazolone[30,31]. Isomeric toluidines and aniline, which are difficult to separated as salts, can be converted into bromo derivatives[32]. Dansyl chloride[33,34] has also been employed in the separation of derivatives.

The purpose of the present work is to study the separation of aromatic amines and their derivatives with 1-fluoro-2,4-dinitrobenzene. Sanger[35,36] first used the reaction with 1-fluoro-2,4-dinitrobenzene to identify different amino acids by paper chromatography. This reagent was subsequently employed for volatile aliphatic amines, which, after their conversion into 2,4-dinitrophenyl derivatives, can easily be separated

by thin-layer chromatography (TLC)[37]. Recent studies based on the use of this reagent were reported by Tammiletho[38-41], who was interested in the analysis of pharmaceuticals. The only aromatic compounds he considered were 2,4-dinitrophenyl derivatives of aminophenols and aminobenzoic acids.

The reaction scheme is as follows:

Since 1-fluoro-2,4-dinitrobenzene contains electronegative substituents in positions 2 and 4, the fluorine in position 1 is very reactive, and it is expected that this reagent will react with most aromatic amines.

EXPERIMENTAL

Synthesis of derivatives

To 1 ml of a *ca.* 0.5 % ethanolic solution of the amine or an aqueous solution in the case of amines with a sulphonyl group, was added 0.5 ml of the reagent, prepared by dissolving 1 ml of 1-fluoro-2,4-dinitrobenzene in 100 ml of ethanol. A 1-ml volume of 0.1 *M* sodium hydrogencarbonate was also added. The mixture in the test-tube was stirred and heated on a water-bath at 60° for 20 min. Four millilitres of 0.2 *M* sodium hydroxide in 60 % dioxane were then added and the solution, which had darkened, was again heated on a water-bath at 60° for 60 min. On cooling, in certain cases a precipitate was formed which was subsequently dissolved by adding ethanol. It was possible to use such solutions directly in the chromatography. An disadvantage of this approach is that the reagent is partially hydrolyzed to 2,4-dinitrophenol, so that two spots occur on the chromatogram, one due to 2,4-dinitrophenol and the other to the reagent itself.

Certain amines did not react, due to steric hindrance, and the following more rigorous method was adopted for preparing the derivatives. Several milligrams of the amine were dissolved in 3–5 ml of ethanol (water in the case of aminosulphonic acids) in a flask and 0.5 ml of the reagent were added. The mixture was boiled on a water-bath under a reflux condenser for 30–40 min. If a precipitate appeared on cooling, several millilitres of ethanol were added to obtain a clear solution. The resulting solution was used for the chromatography.

Separation methods

The parent amines and their derivatives were separated either by TLC or by paper electrophoresis.

For the separation on Silufol UV-254, three developing systems were used, which also served as a classification of the amines to be identified: S_1 = diethyl ether-cyclohexane (4:3). S_2 = ethyl acetate–*n*-propanol–ammonia (5:4:1); S_3 = ethyl acetate–*n*-propanol–ammonia (2:1:2).

In the case of paper electrophoresis the following conditions were employed: electrolyte, 1 *N* acetic acid; paper, Whatman No. 1; voltage, 320 V for 2 h.

Detection

The detection of aromatic amines was carried out by spraying with a 1 % solution of p-N,N-dimethylaminobenzaldehyde in 1 M hydrochloric acid (Ehrlich agent). The derivatives with 1-fluoro-2,4-dinitrobenzene were detected by spraying with 5 % tin(II) chloride in hydrochloric acid, to reduce the relevant nitro groups to amino groups, and after drying the chromatogram (electrophoreogram) was sprayed with the Ehrlich agent.

A total of 128 different substituted aromatic amines was used. The R_F values, ΔR_M values and mobilities u obtained are listed in Tables I–IX. For amines marked by asterisks, the second method of preparation was used to obtain their derivatives.

RESULTS AND DISCUSSION

As mentioned in the Introduction, the aim of this work was to obtain a better differentiation of aromatic amines during their identification, by comparing the R_F values of the amine and of its 2,4-dinitrophenyl derivative.

The results of the separation by TLC together with that by paper electrophoresis are summarized in Tables I to IX.

In the system diethyl ether–cyclohexane (4:3), S_1, in most cases it is possible to separate the amine from its derivative, and the free amines usually have lower R_F values than their derivatives. However, there are cases where the opposite situation is observed, e.g., 2-iodoaniline, 2-chloroaniline, 2-chloro-4-nitroaniline, 2-methoxy-4-chloroaniline, 2-carboxyaniline and 2-hydroxy-1-naphthylamine.

TABLE I

SIMPLE ARYLAMINES

Solvent systems S_1, S_2 and S_3 as in Experimental. In S_1 and S_3 the amines and their derivatives moved with the solvent front. A = Amine; D = derivative.

Aromatic amine	S_1		
	$R_F(A)$	$R_F(D)$	ΔR_M
Aniline	0.39	0.66	0.48
3-Methylaniline	0.40	0.72	0.58
2,3-Dimethylaniline	0.47	0.73	0.48
2,4-Dimethylaniline	0.40	0.74	0.63
2-Iodoaniline*	0.69	0.55	−0.26
2-Hydroxyaniline	0.18	0.32	0.33
3-Hydroxyaniline	0.09	0.34	0.71
2-Ethoxyaniline	0.50	0.61	0.19
4-Ethoxyaniline	0.17	0.63	0.92
4-Aminobenzaldehyde	0.13	0.76	1.32
1,3-Diamino-4-methylbenzene	0.48	0.81	0.66
1,2-Diaminobenzene	0.04	0.33	0.99
1,4-Diaminobenzene	0.00	0.12	—
1,4-Diamino-2,5-dimethylbenzene	0.00	0.16	—
1,2-Diamino-4-ethoxybenzene	0.00	0.33	—
p-Xylylenediamine	0.00	0.11	—

* The amine derivative was prepared by the second, more rigorous method.

TABLE II

CHLORINATED AND NITRATED ARYLAMINES

Details as in Table I.

Aromatic amine	S_1		
	$R_F(A)$	$R_F(D)$	ΔR_M
2-Chloroaniline	0.57	0.52	−0.09
3-Chloroaniline	0.37	0.59	0.39
4-Chloroaniline	0 25	0.65	0.74
2-Methyl-4-chloroaniline	0.36	0.76	0.75
2-Methyl-3-chloroaniline	0.46	0.73	0.50
2-Methoxy-5-chloroaniline*	0.53	0.47	−0.10
2,4-Dichloroaniline*	0.49	—	—
2,5-Dichloroaniline*	0.58	—	—
3,4-Dichloroaniline	0.29	0.64	0.64
2-Chloro-4-nitroaniline	0.31	0.26	−0.07
2-Nitro-4-chloroaniline	0.37	—	—
2-Nitroaniline*	0.43	—	—
3-Nitroaniline*	0.30	—	—
4-Nitroaniline	0.19	0.11	−0.27
2-Nitro-4-methylaniline*	0.46	--	—
1,2-Diamino-4-nitrobenzene	0.05	0.11	0.37
2,4-Dinitroaniline*	0.67	—	—
2-Methyl-5-chloroaniline	0.46	0.71	0.46
1,4-Diamino-2-methyl-5-chlorobenzene	0.00	0.35	—

* The amine derivative was prepared by the second, more rigorous method.

TABLE III

SULPHONATED AMINES (AMINOSULPHONIC ACID)

E = Paper electrophoresis: all the amines and their derivatives were separated by chromatography in the system S_1 where they remained at the start. Other details as in Table I.

Aromatic amine	S_2		S_3		E	
	$R_F(A)$	$R_F(D)$	$R_F(A)$	$R_F(D)$	$u(A) \cdot 10^5$	$u(D) \cdot 10^5$
2-Nitro-4-sulphoaniline*	0.08	—	0.22	—	11.4	9.6
3-Sulphoaniline	0.03	0.26	0.11	0.29	1.7	7.9
4-Sulphoaniline	0.03	0.13	0.10	0.23	2.5	5.9
2-Sulpho-4-nitroaniline*	0.35	0.24	0.35	0.27	8.9	—
2,4-Disulphoaniline*	0.00	0.04	0.00	0.15	14.9	12.2
2-Sulpho-4-methylaniline	0.11	0.35	0.21	0.30	4.8	7.1
2-Sulpho-5-methylaniline	0.10	0.20	0.13	0.23	3.1	7.4
3-Methyl-4-sulphoaniline	0.10	0.21	0.13	0.24	3.1	7.3
2-Methyl-5-sulphoaniline	0.10	0.19	0.14	0.24	2.5	7.1
2-Methyl-4-sulphoaniline	0.09	0.21	0.13	0.23	4.6	7.3
3-Sulpho-4-methylaniline	0.08	0.26	0.18	0.25	0.0	6.4
1,3-Diamino-2-methyl-5-sulphobenzene	0.00	0.22	0.08	0.26	0.0	4.8
2-Hydroxy-3-sulpho-5-nitroaniline	0.00	0.00	0.07	0.22	9.7	6.9
2-Hydroxy-3-nitro-5-sulphoaniline	0.00	0.00	0.00	0.23	10.6	6.9
2-Hydroxy-5-sulphoaniline	0.00	0.02	0.00	0.13	0.00	5.7
		0.09		0.19		

* The amine derivative was prepared by the second, more rigorous method.

TABLE IV

AMINOCARBOXYLATED ACIDS AND THEIR ESTERS

E = Paper electrophoresis; ÷, the compounds were moving with the solvent front. Other details as in Table I.

Aromatic amine	S_1		S_2		S_3		E	
	$R_F(A)$	$R_F(D)$	$R_F(A)$	$R_F(D)$	$R_F(A)$	$R_F(D)$	$u_A \cdot 10^5$	$u_D \cdot 10^5$
2-Carboxyaniline*	0.32	0.13	0.09	÷	0.21	÷	−2.5	−0.8
3-Carboxyaniline	0.09	0.20	÷	÷	÷	÷		
4-Carboxyaniline*	0.10	0.12	0.03	0.15	0.11	0.22	−3.6	−0.7
2,5-Dicarboxyaniline*	0.09	0.00	0.00	0.00	0.00	0.14		
3,5-Dicarboxyaniline*	0.00	0.30	0.00	0.00	0.00	0.13	−2.5	−0.8
3-Carboxy-4-hydroxyaniline*	0.00	0.32	÷	÷	÷	÷		
2,4-Dihydroxy-5-carboxyaniline*	0.10	0.00	0.00	0.00	0.03	0.19		
Methyl 2,4-dinitrobenzoate	0.12	0.60	0.00	0.00	0.03	0.52		
Ethyl 2,4-dinitrobenzoate*	0.13	0.41	0.00	0.00	0.04	0.48		

* The amine derivative was prepared by the second, more rigorous method.

From calculations it can be seen that, on going from the amines to their 2,4-dinitrophenyl derivatives, a decrease in R_F values occurs when the other functional group in the neighbourhood of the amino group can form a hydrogen bond with the amino group. The energy of this internal hydrogen bond thus effects a shift of the R_F values.

The usual shift to higher R_F values with derivatives containing one –NH_2 group is on average $0.60–0.70 \cdot \Delta R_M$ unless an internal hydrogen bond is present or some other interaction occurs; for two –NH_2 groups this shift is of $0.80–1.10 \cdot \Delta R_M$. The presence of a methyl group *ortho* to the amino group results in a moderate decrease of the shift in R_F value to *ca.* $0.50 \cdot \Delta R_M$. The reason for this decrease is that, in the cases of the parent amines, the bulky methyl group attenuates the intermolecular hydrogen bond produced between the amino group and Si–OH of the silica gel carrier. More details can be found in Fig. 1.

The system S_1 used in the thin layer chromatography was chosen to make a classification of the amines, and the other systems were used to improve the precision only when the amine or its 2,4-dinitrophenyl derivatives did not move. Alternatively, paper electrophoresis in 1 M acetic acid was employed[42].

TABLE V

NAPHTHYLAMINES

Details as in Table I.

Aromatic amine	S_1		
	$R_F(A)$	$R_F(D)$	R_M
1-Naphthylamine	0.37	0.69	0.58
2-Naphthylamine	0.31	0.71	0.74
2-Hydroxynaphthylamine	0.43	0.30	−0.25
1,4-Diaminonaphthalene	0.00	0.21	—
1,2-Diaminonaphthalene	0.00	0.14	—
1,5-Dihydroxy-1-naphthylamine	0.00	0.39	—

TABLE VI

SULPHONAPHTHYLAMINES

E = Paper electrophoresis. Other details as in Table I.

Aromatic amine	S_2		S_3		E	
	$R_F(A)$	$R_F(D)$	$R_F(A)$	$R_F(D)$	$u(A) \cdot 10^5$	$u(D) \cdot 10^5$
2-Sulphonaphthylamine*	0.09	—	0.26	—	6.4	—
4-Sulpho-1-naphthylamine*	0.08	0.19	0.17	0.23	3.2	
5-Sulpho-1-naphthylamine	0.10	0.32	0.18	0.30	0.0	4.5
6-Sulpho-1-naphhtylamine	0.09	0.30	0.19	0.22	0.0	4.4
8-Sulpho-1-naphthylamine	0.13	—	0.23	—	0.0	—
1-Sulpho-2-naphthylamine	0.09	0.32	0.20	0.31	0.0	3.8
5-Sulpho-2-naphthylamine	0.09	0.31	0.19	0.30	0.0	4.2
6-Sulpho-2-naphthylamine	0.07	0.29	0.23	0.29	0.0	4.0
7-Sulpho-2-naphthylamine	0.06	0.30	0.24	0.28	0.3	4.2
6-Hydroxy-8-sulpho-2-naphthylamine	0.03	0.12	0.05	0.21	0.0	2.9
		0.32		0.30		
2-Hydroxy-4-sulpho-1-naphthylamine*	0.00	0.04	0.00	0.21	0.0	5.0
		0.01		0.23		
3,6-Disulpho-1-naphthylamine*	0.00	0.00	0.00	0.19	10.8	11.7
3,8-Disulpho-1-naphthylamine*	0.00	0.03	0.00	0.17	9.1	11.4
1,5-Disulpho-2-naphthylamine*	0.00	0.27	0.04	0.22	5.1	9.9
3,8-Disulpho-2-naphthylamine*	0.00	0.04	0.02	0.22	7.4	11.6
5,7-Disulpho-2-naphthylamine	0.00	0.05	0.00	0.21	5.9	8.0
4,8-Disulpho-2-naphthylamine	0.00	0.05	0.00	0.20	3.7	8.2
3,6-Disulpho-8-hydroxy-1-naphthyl- amine	0.00	0.00	0.00	0.05	5.1	7.7
		0.05		0.21		
3,6-Disulpho-8-hydroxy-2-naphthyl- amine	0 00	0.00	0.00	0.05	5.1	7.8
		0.06		0.21		
2,4,7-Trisulpho-1-naphthylamine	0.00	—	0.00	—	13.0	—
3,6,8-Trisulpho-1-naphthylamine*	0.00	0.00	0.00	0.04	12.4	14.0
3,6,8-Trisulpho-2-naphthylamine*	0.00	0.00	0.00	0.03	16.9	17.7

* The amine derivative was prepared by the second, more rigorous method.

TABLE VII

AMINOANTHRAQUINONES

Details as in Table I.

Aromatic amine	S_1		S_2		S_3	
	$R_F(A)$	$R_F(D)$	$R_F(A)$	$R_F(D)$	$R_F(A)$	$R_F(D)$
2-Aminoanthraquinone*	0.23	—	÷	—	÷	—
2-Amino-3-hydroxyanthraquinone*	0.18	—	0.25	—	0.32	—
1-Amino-4-hydroxyanthraquinone*	0.38	—	÷	—	÷	—
1,4-Diaminoanthraquinone*	0.10	—	—	—	÷	—
1,2-Diaminoanthraquinone*	0.11	—	÷	—	÷	—
2,6-Diaminoanthraquinone*	0.18	0.21	—	÷	÷	÷
2,3-Diaminoanthraquinone*	0.00	0.17	÷	÷	÷	÷
1,5-Diaminoanthraquinone*	0.27	—	÷	—	÷	—
1,8-Diaminoanthraquinone*	0.26	—	÷	—	÷	—
1,6-Diaminoanthraquinone*	0.11	0.08	÷	÷	÷	÷
1,7-Diaminoanthraquinone*	0.09	0.27	÷	÷	÷	÷
2-Amino-3-chloroanthraquinone*	0 35	—	÷	—	÷	—
1-Amino-2-chloroanthraquinone*	0.67	—	÷	—	÷	—
1-Amino-2-bromo-4-hydroxyanthraquinone	0.65	—	÷	—	÷	—
Sodium 1-aminoanthraquinone-2-sulphonate*	0.39	—	0.19	—	0.27	—
1-Amino-2-sulpho-4-bromoanthraquinone*	0.00	—	0.37	—	0.36	—

* The amine derivative was prepared by the second, more rigorous method.

TABLE VIII

AMINOBIPHENYLS

E = Paper electrophoresis. Other details as in Table I.

Aromatic amine	S_1			S_2		S_3		E	
	$R_F(A)$	$R_F(D)$	ΔR_M	$R_F(A)$	$R_F(D)$	$R_F(A)$	$R_F(D)$	$u(A) \cdot 10^5$	$u(D) \cdot 10^5$
3-Aminobiphenyl	0.36	0.71	0.64	+	+	+	+		
4-Aminobiphenyl	0.34	0.73	0.72	+	+	+	+		
4,4'-Diaminobiphenyl	0.10	0.19	0.32	+	+	+	+		
4-Amino-4'-nitrobiphenyl	0.26	0.37	0.22	+	+	+	+		
4,4'-Diamino-2-nitrobiphenyl	0.06	0.16	0.48	+	+	+	+		
4,4'-Diamino-3,3'-dimethylbiphenyl	0.11	0.30	0.54	+	+	+	+		
4,4',3,3'-Tetraaminobiphenyl*	0.00	0.10	—	+	+	+	+		
4,4'-Diamino-3,3'-dinitrobiphenyl*	0.00	—	—	+	—	+	—		
4,4'-Diamino-2,2'-dinitrobiphenyl*	0.00	0.10	—	+	+	+	+		
2,2'-Dinitro-4,4'-diamino-5,5'-dimethylbiphenyl*	0.06	0.10	0.24	+	+	+	+		
2,2'-Dinitro-3,3'-dimethyl-4,4'-diaminobiphenyl*	0.09	0.10	0.05	+	+	+	+		
3,3'-Dihydroxy-4,4'-diaminobiphenyl*	0.00	—	—	+	—	+	+		
2,4'-Diamino-4-sulphobiphenyl*	0.00	0.00		0.00	0.00	0.05	0.16 0.30	7.4	10.2
2,4'-Diamino-5-sulphobiphenyl*	0.00	0.00		0.04	0.00	0.21	0.26	9.1	11.0

* The amine derivative was prepared by the second, more rigorous method.

TABLE IX

AMINODIPHENYL SULPHONES

Details as in Table I.

Aromatic amine	S_1		
	$R_F(A)$	$R_F(D)$	ΔR_M
2,4'-Diaminodiphenylmethane	0.09	0.23	0.48
4,4'-Diaminodiphenylmethane	0.04	0.22	0.83
2-Aminophenyl phenyl sulphone*	0.30	—	—
3-Aminophenyl phenyl sulphone*	0.16	—	—
4-Aminophenyl phenyl sulphone*	0.18	—	—
Bis(2-aminophenyl) sulphone	0.19	0.31	0.31
2-Aminophenyl 3'-aminophenyl sulphone	0.06	0.32	0.87
Aminophenyl 4'-aminophenyl sulphone	0.05	0.31	0.87
Bis(3-aminophenyl) sulphone	0.03	0.29	1.12
Bis(4-aminophenyl) sulphone	0.00	0.11	—
3-Aminophenyl 4'-aminophenyl sulphone	0.00	0.12	—

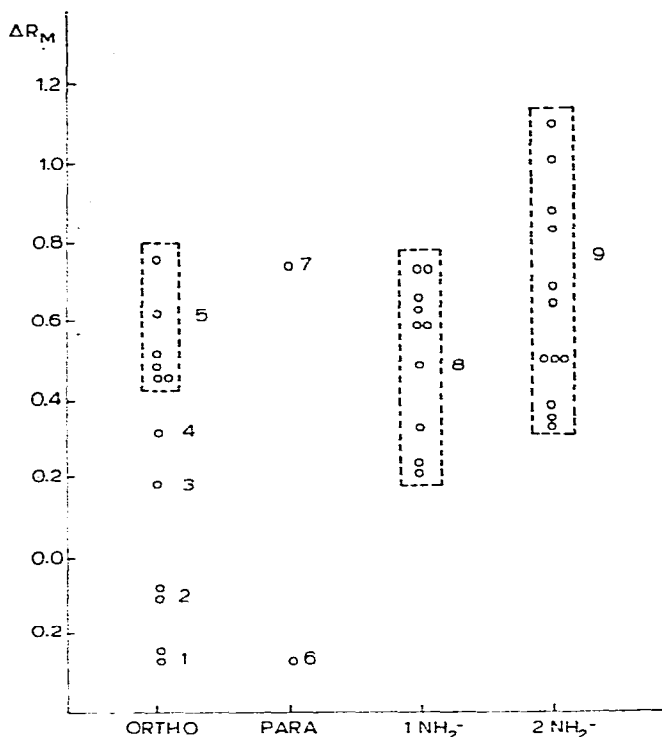* The amine derivative was prepared by the second, more rigorous method.

Fig. 1. Dependence of the difference, ΔR_M, between the R_F values of the amine and its 2,4-dinitrophenyl derivative on the type of functional group. Groups: 1 = –OH   –1; 2 = –Cl, –OCH₃; 3 = –OC₂H₅; 4 = –SO₂–; 5 = –CH₃; 6 = –NO₂; 7 = –Cl; 8 = 1-NH₂; 9 = 2-NH₂.

The reactivity of aromatic amines with 1-fluoro-2,4-dinitrobenzene was not as high as in the case of aliphatic amines and, as can be seen from the Tables, more drastic conditions had to be used in some cases. However, in a frew cases, even these conditions did not lead to the derivative desired. For example, with aminoanthraquinones the reaction did not occur when the amino group was in position 1. This can be attributed to the presence of a relatively strong internal hydrogen bond between the amino group and the anthraquinone oxygen.

In a few cases two spots occurred on the chromatogram. The relevant compounds possessed two reactive functional groups (–NH₂ and –OH) or two amino groups, both of which were able to react with the agent, so that one of the derivatives could be considered as an intermediate product of the reaction.

We conclude that, although a general rule concerning the shift of the R_F values between the amine and its 2,4-dinitrophenyl derivatives cannot be established, since the different values of the dipole moments were not taken into account, we may restrict the number of possibilities in the identification of an unknown amine.

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