IDENTIFICATION OF AROMATIC SUBSTANCES BY "ELECTROPHORETIC SPECTRA" USING PAPER ELECTROPHORESIS

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The majority of papers dealing with electrophoresis in papers are concerned with the behaviour of different higher-molecular-weight organic substances, predominantly of animal or vegetable origin. On the other hand, the number of papers on the separation of low-molecular-weight substances is comparatively small, and, further, a considerable number of them deal with organic acids.

The number of publications on electrophoresis of simpler aromatic substances[1-11] is still smaller, although, as already previously pointed out[12], this method is very suitable as a supplement to chromatographic procedures, since it was found that some factors which play an important role in chromatography on paper exert no influence upon the mobility in the electric field. This mainly relates to some functional groups and to the mobility of isomeric compounds (however, in as far no interaction in the form of hydrogen bridges takes place). On the other hand, electrophoresis on paper permits the estimation of the number and type of functional groups, whose contribution to the total mobility is roughly additive[12].

In the present work we attempted to use paper electrophoresis for identification purposes, but from another viewpoint. Determination of the mobilities for pH values of 1-10 for one and the same compound enables the construction of a graph (electrophoretic spectrum), from whose curve certain conclusions can be drawn as to the arrangement of the molecule. This result is analogous to that obtained in earlier papers where either paper or gas chromatography[13,14] is used for a similar purpose. In paper chromatography, the R_F values are determined for twenty different solvent systems, and in gas chromatography on four columns with various stationary phases. The chromatographic behaviour and the structure of the substances subjected to chromatography are closely interconnected, and this manifests itself in the course of establishing the curves (chromatographic spectra).

In order to attain easy reproducibility of the mobilities measured at various pH values, buffers of a certain pH value and a certain ionic strength were selected as conductive media. In addition to this, a special piece of simple equipment was designed which permits simultaneous electrophoretic separation at various pH values in a single experiment. For control of the function of the instrument and, if necessary, correction of mobilities, a standard substance was added to each compound subjected to electrophoresis.

For determination of the electrophoretic spectra, a number of compounds of the benzene, naphthalene and diphenyl series were selected.

EXPERIMENTAL

Apparatus

The apparatus for the determination of the electrophoretic spectra is illustrated in Fig. I. The essential part of the equipment is the arrangement of the cathode (A_1) and the anode (A_2) section, which consists of ten chambers, each of which is filled with an electrolyte (100 ml) of different pH value. All ten chambers have a platinum electrode in common. The entire apparatus is made of Plexiglas. In principle it is an instrument for electrophoresis of current type with a so-called humid chamber.
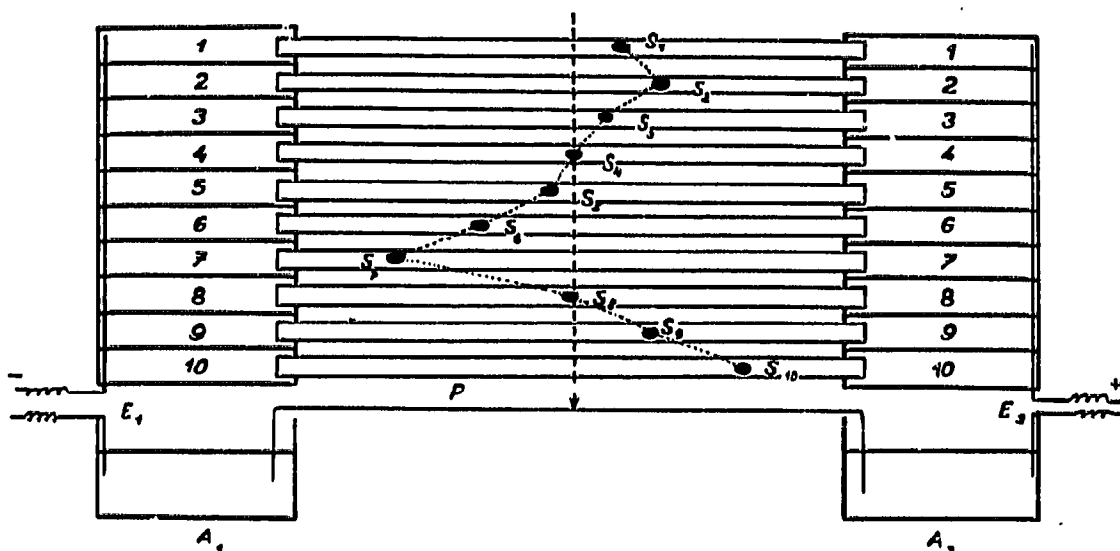
Fig. I. Schematic representation of equipment for determination of electrophoretic spectra.

Each of the ten anodic chambers is connected with the corresponding cathodic chamber by a strip of Whatman No. 1 paper (size 2.5 × 55 cm), onto which, at the starting place in the middle, the substance under investigation is applied together with the standard (3-nitrophthalic acid), which makes it possible to apply corrections to the mobilities and, thus also, to compare individual experiments.

The voltage used is 250 V, the duration of electrophoresis is 3 h, the current intensity amounts to about 6 mA.

Electrolytes

A number of electrolytes of different pH values were prepared in sufficient amounts; the pH value of the solutions thus obtained was measured by a hydrogen electrode at 18°.

Composition and measured pH values of the electrolytes are given in Table I.

Evaluation

Detection of unknown constituents is first undertaken by a number of detection procedures such as were described in the paper dealing with chromatographic spectra obtained by paper chromatography[13]. This detection is also a part of the identification.

This is followed by a check of the mobility of the standard (3-nitrophthalic acid) to make sure that all ten chambres are intact and that no exchange of buffers has

TABLE I

COMPOSITION AND MEASURED pH VALUES OF THE ELECTROLYTE

Electrolytes Nos. 2–10 were further diluted with distilled water in the ratio 1:1.

No.	Electrolyte composition	pH
1	100 ml 0.1 N HCl	1.75
2	0.40 ml 0.2 M K_2HPO_4 + 19.60 ml 0.1 M citric acid	2.35
3	4.11 ml 0.2 M K_2HPO_4 + 15.99 ml 0.1 M citric acid	3.1
4	7.71 ml 0.2 M K_2HPO_4 + 12.29 ml 0.1 M citric acid	4.2
5	10.30 ml 0.2 M K_2HPO_4 + 9.70 ml 0.1 M citric acid	5.2
6	12.63 ml 0.2 M K_2HPO_4 + 7.37 ml 0.1 M citric acid	5.8
7	16.47 ml 0.2 M K_2HPO_4 + 3.53 ml 0.1 M citric acid	7.2
8	19.45 ml 0.2 M K_2HPO_4 + 0.55 ml 0.1 M citric acid	8.7
9	0.05 M borax	8.9
10	6.0 ml 0.1 N borax + 4.0 ml 0.1 N NaOH	9.7

occurred. 2-Nitrophthalic acid was chosen as standard because it is very easy to detect in U.V. light as a dark spot, and also because it has favourable mobility values in nearly all the buffers employed. Then the mobilities of the identified substance are calculated on the basis of the mobility of the standard, most advantageously in buffer No. 4 ($u = 11.4 \cdot 10^{-5}$ cm$^2 \cdot$V$^{-1} \cdot$s^{-1}). The electrophoretic spectra are established at least twice. The mobilities of various aromatic compounds in the individual buffers are presented in Table II.

Aromatic substances with zero mobility

Since in paper electrophoresis only those substances that are capable of carrying a positive or a negative charge migrate, there is a long list of compounds containing miscellaneous functional groups which exhibit zero mobility in all the buffers employed.

Among the measured substances are the following aromatic compounds: 1-amino-2,3-dichlorobenzene, 4-chlorobenzaldehyde, 4-nitrotoluene, 1,4-dinitrobenzene, 3-nitrophenol, 2-nitrophenol, 1-nitronaphthalene, 1,5-nitrochloronaphthalene, 4,4'-N-tetramethyl-2,2'-dinitrobenzidine, 4-nitrodiphenyl, 2,2'-dinitrobenzidine, 4-hydroxydiphenyl, 2-hydroxydiphenyl, 4,4'-dichloro-2-nitrodiphenyl, 4,4'-dinitrodiphenyl, 2-nitrodiphenyl, 3-nitrodiphenyl, 4,2'-dinitrodiphenyl, 3,3'-dinitrophenyl, 2-nitrophenanthrene, 9-nitrophenanthrene, 2-nitro-4,4'-dichlorodiphenyl, 3,3'-dimethoxy-4,4'-dichlorodiphenyl, 3,3'-dimethyldiphenyl, and 3,3'-dimethoxydiphenyl.

DISCUSSION

From Table II it is obvious that the electrophoretic spectra (Fig. 2) of compounds of the diphenyl series were those predominantly determined. Our attention was devoted to these substances, since they are for the greater part strongly carcinogenic and their determination in biological material is of great importance. For this reason, any identification method is desirable.

If a reference standard is available, identification of an unknown substance is relatively easy, and comparison of the R_F values by paper chromatography and of the curves of electrophoretic spectra permits correlation of the data obtained. The
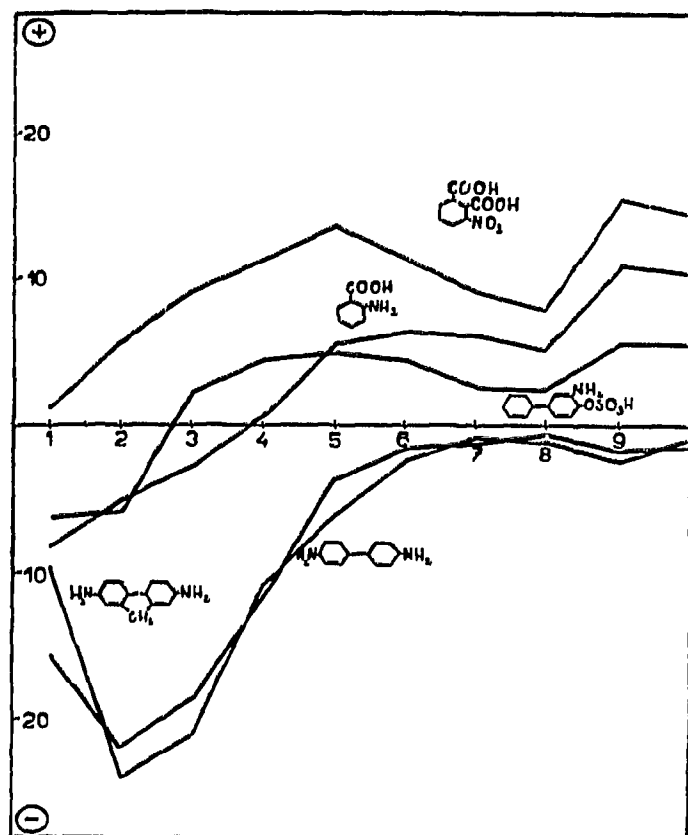
Fig. 2. Electrophoretic spectra of various aromatic compounds; on X-axis: pH values of buffers employed; on Y-axis: mobility $u \cdot 10^5$.

identity can be confirmed in this case also by means of various detection reagents[13].

Use of the mobility values u (Table II), either for comparison with the standard or for further treatment in cases where no reference standard is available, necessitated the establishment of the experimental error for the individual buffers.
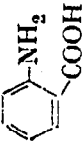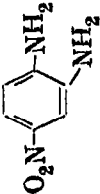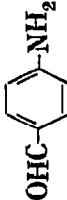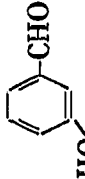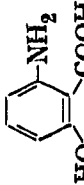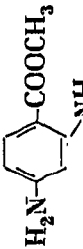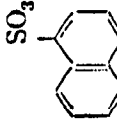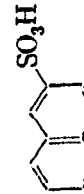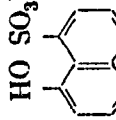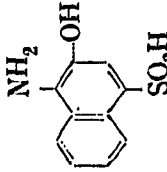
Although simultaneous electrophoresis in various buffers leads to considerable reproducibility, a certain scattering of the values of u is encountered, mainly caused by personal errors (in particular in determination of the centres of diffuse spots etc.). For this reason, statistical evaluation of errors was carried out, and it was found that the variation coefficient for all buffers amounts to 8.4%. Consequently, comparison of results necessitates taking into account a scatter corresponding to. this error.

Some compounds form diffuse spots in certain buffers. Although this fact leads to less accurate mobility data, it is, on the other hand, a reproducible and thus a characteristic property of these substances. In some cases, the spots are even diffusely distributed on both sides of the start. These substances are not given in Table II (aniline, N-dimethylaniline, 2-methyl-6-chloroaniline, 2,5-dimethylaniline, 4-chloroaniline, 3-chloroaniline, 4-methylaniline).

Comparison of the curves of the electrophoretic spectra of compounds with various functional groups, and also with different numbers of them, requires the mobilities obtained to be related on a uniform basis. If all mobilities are divided by one of them, the curve of the electrophoretic spectrum is simplified to such an extent that the influence of the number of functional groups in the molecule disappears and

TABLE II
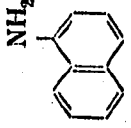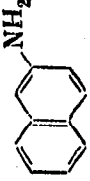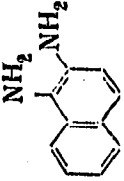
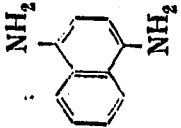### MOBILITY u IN INDIVIDUAL BUFFERS AND COLORATION OF SPOTS
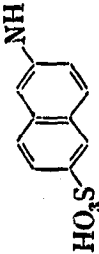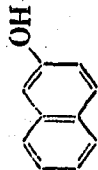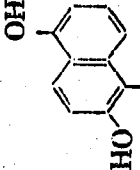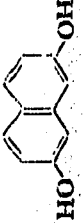
	Buffer No.										After spray with Ehrlich reagent		Without spray
	1	2	3	4	5	6	7	8	9	10	Vis.	UV	UV
Stand.	1.3	5.8	9.1	11.4	13.8	11.7	9.4	8.1	15.6	14.9	och	yg	d
	+	+	+	+	+	+	+	+	+	+			
1	10.4	18.6ᵃ	14.0ᵃ	12.6	3.3	0.4	0.4	0.3	0.5	0.9	ry	1–4 y / 5–10	d
	—	+	—	+	+	+	—	—	—	—			
2	11.0	17.8	14.4	10.8	6.8	1.9	1.5	0.4	0.4	0.2	y	yg	d
	—	+	+	+	—	—	—	—	—	—			
3	1.1	1.8	1.6	1.7	1.4	1.3	1.2	1.2	3.0	3.0	ochyᵃ	ory	d
	+	+	+	+	+	+	+	+	+	+			
4	0.0	0.0	1.5	5.9	8.1	7.2	5.3	4.9	11.8	10.2	r	ror	d
		+	+	+	+	+	+	+	+	+			
5	19.8ᵃ	18.0ᵃ	17.7ᵃ	14.1ᵃ	5.6	1.9	1.7	1.7	3.3	3.0	g (dull)	gy	—
	—	—	—	—	—	—	—	—	—	—			
6	18.2	25.8	18.7	13.9	8.3	3.0	1.7	0.8	0.6	2.7	c	or	bl
	—	—	—	—	—	—	—	—	—	—			
7	0.0	0.0	0.0	0.00	0.0	2.3	3.9	3.8	9.2	8.5	y	y	d
						+	+	+·	+	+			
8	11.4	8.4	4.0	1.3	1.4	1.1	1.3	1.7	3.0	4.3	y	—	d
	—	—	—	—	—	—	—	—	—	—			

No.	Structure	Colour 1	Colour 2	Colour 3										
9	NH₂ / COOH	bl	y	bry	+10.4	+11.0	+5.4	+6.3	+6.7	+5.6	-0.8	-2.6	-5.0	-8.1 •
10	O₂N–, NH₂ / NH₂	d	—	y	-0.2	-0.8	-0.1	-0.3	-0.7	-0.2	-0.8	-3.1	-7.1	-6.2
11	OHC– / –NH₂	y	y	y	-1.3	-1.7	-0.7	-0.7	-0.7	-0.4	-1.3	-1.3	-2.5	-5.5
12	CHO / HO	d	—	—	+3.4	+2.3	+1.3	-1.2	-1.2	-1.5	-1.7	-1.4	-0.6	0.0
13	NH₂ / COOH / HO	bl	y	y	+9.5	+8.9	+4.8	+5.3	+5.1	+3.1	-1.5	-1.6	-3.9	-5.8
14	COOCH₃ / NH₂ / H₂N	blv	d	ochy	-2.6	-3.4	-1.0	-1.0	-2.0	+2.6	-4.4	+5.8	+14.5	-9.4
15	SO₃H (naphthalene)	d	—	—	+9.4	+9.3	+6.6	+6.3	+8.2	+8.8	+8.4	+9.0	+10.6	+9.4
16	SO₃H (naphthalene)	d	—	—	+8.7	+10.5	+4.2	+5.6	+6.5	+7.0	+8.0	+7.3	+11.3	+7.4
17	HO, SO₃H (naphthalene)	bl	—	—	+8.8	+7.8	+4.7	+5.2	+6.8	+7.6	+8.9	+13.1	+12.6	+7.1
18	NH₂, OH, SO₃H (naphthalene)	bl	y	c	+7.9	+7.0	+3.2	+3.2	+5.5	+3.7ᵃ	+2.9ᵃ	0.0	0.0	0.0

For footnote see p. 111.

TABLE II (continued)

No.	Structure	Buffer No. 1	2	3	4	5	6	7	8	9	10	After spray with Ehrlich reagent Vis.	UV	Without spray UV
19		9.5[a] —	15.3[a] —	7.7[a] —	4.1[a] —	1.3 —	0.9 —	0.7 —	0.7 —	2.4 —	2.6 —	y	gy	bl
20		7.7 —	13.5 —	10.5 —	4.1 —	0.9 —	0.8 —	0.0 —	0.0 —	2.5 —	2.0 —	y	y	bl
21		4.0[a] —	7.0[a] —	5.0[a] —	2.5[a] —	1.3 —	0.7 —	1.2 —	1.3 —	1.5 —	1.2 —	y	y	bl
22		4.0[a] —	8.6[a] —	6.1[a] —	3.0[a] —	1.8 —	1.1 —	1.4 —	0.8 —	1.6 —	2.3 —	y	y	bl
23		0.0 —	0.0 —	0.0 —	0.2 +	3.8 +	3.7 +	3.2 +	2.7 +	8.2 +	8.7 +	yor	yg	blv
24		0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.00 —	0.0 —	0.0 —	1.2 +	—	—	bl
25		0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.2 +	2.2 +	—	—	d
26		0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	1.1 +	—	—	bl
27		9.2 +	18.8 +	19.0 +	14.6 +	13.0 +	12.9 +	10.1 +	10.1 +	16.7 +	18.1 +	—	—	d
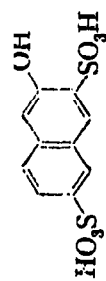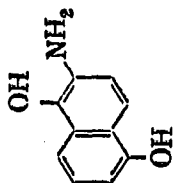
No.													
27	9.2 +	18.8 +	19.0 +	14.6 +	13.0 +	12.9 +	10.1 +	10.1 +	16.7 +	18.1 +	—	—	d
28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6 +	1.8 +	br	br	bl
29	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1 +	5.5 +	5.5 y	y (full)		d
30	1.3 +	5.8 +	1.2 +	11.4 +	6.6 +	5.8 +	4.2 +	3.4 +	8.5 +	10.7 +	—	—	d
31	8.3 +	8.3 +	6.2 +	9.0 +	8.8 +	7.5 +	6.5 +	6.2 +	11.2 +	12.7 + y	y	—	
32	9.8 −	13.5 −	9.8 −	7.8 −	3.1 −	2.0 −	0.9 −	1.3 −	1.5 −	2.2 − y	y	bl	
33	8.2 −	12.3 −	10.9 −	8.9 −	1.9 −	0.4 −	0.0 −	0.0 −	1.5 −	1.4 − y	yg	bl	
34	12.5 −	13.9 −	12.6 −	11.3 −	3.6 −	0.9 −	1.2 −	1.1 −	4.2 −	3.4 ochy	y	bl	
35	15.4 −	21.8 −	18.5 −	11.4 −	3.4 −	1.0 −	1.0 −	0.4 −	1.3 −	1.1 or	or	bl	
36	11.5 −	16.1 −	10.8 −	7.9 −	3.1 −	1.1 −	1.0 −	1.9 −	3.3 −	3.0 y	dg	bl	
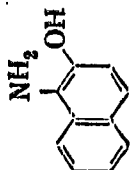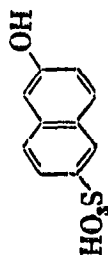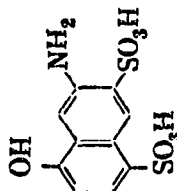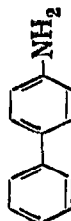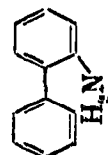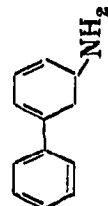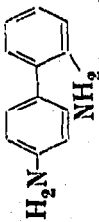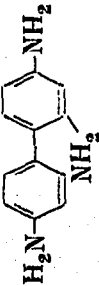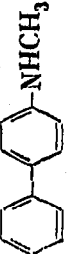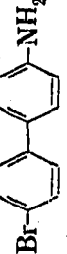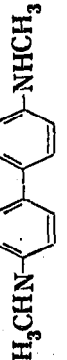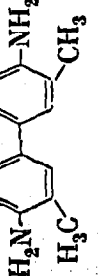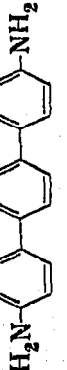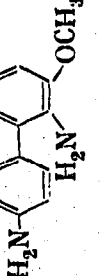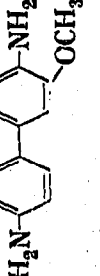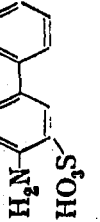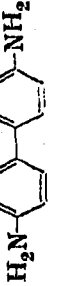
For footnote see p. 111.

TABLE II (continued)
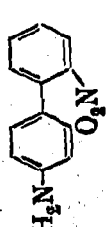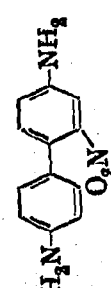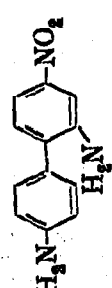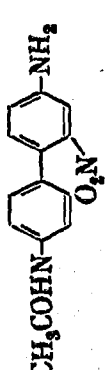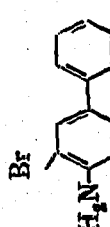
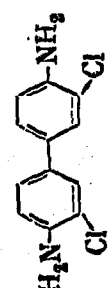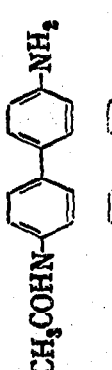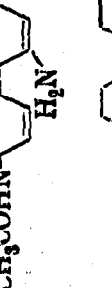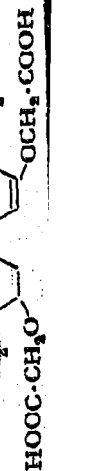| No.	Structure	Buffer No.										After spray with Ehrlich reagent		Without spray
		1	2	3	4	5	6	7	8	9	10	Vis.	UV	UV
37	H₂N–C₆H₄–C₆H₄(NH₂)	15.2 / –	20.8 / –	15.0 / –	8.7 / –	4.0 / –	0.0 / –	0.2 / –	0.6 / –	1.4 / –	1.9 / –	yoch	yg	vbl
38	H₂N–C₆H₃(NH₂)–C₆H₄(NH₂)	18.5 / –	25.2[a] / –	16.9[a] / –	9.5[a] / –	3.1 / –	2.9 / –	1.4 / –	1.4 / –	1.8 / –	2.7 / –	brr	bror	blv
39	CH₃HN–C₆H₄–C₆H₅	7.7[a] / –	15.1[a] / –	10.3[a] / –	4.5[a] / –	1.3 / –	1.1 / –	0.8 / –	0.0 / –	2.6 / –	1.5 / –	y	ochy	bl
40	Br–C₆H₄–C₆H₄(NH₂)	1.4[a] / –	0.8 / –	0.0 / –	0.0 / –	0.0 / –	0.0 / –	0.0 / –	0.0 / –	0.0 / –	0.0 / –	—	—	bl
41	H₃CHN–C₆H₄–C₆H₄(NHCH₃)	13.4 / –	25.0 / –	17.0 / –	7.3 / –	0.7 / –	0.5 / –	0.6 / –	0.1 / –	1.8 / –	1.5 / –	y	y	bl
42	H₂N(CH₃)C₆H₃–C₆H₃(CH₃)NH₂	12.1[a] / –	20.2[a] / –	12.8[a] / –	5.7[a] / –	1.5 / –	1.1 / –	0.9 / –	0.7 / –	1.3 / –	1.0 / –	ror	or	bl
43	H₂N–C₆H₄–C₆H₄(NH₂)	6.6 / –	8.0 / –	3.3 / –	1.6 / –	0.9 / –	1.0 / –	0.8 / –	0.8 / –	1.3 / –	1.7 / –	y	y	bl
44	H₂N(OCH₃)C₆H₃–C₆H₃(OCH₃)NH₂	10.5 / –	21.2 / –	14.8 / –	8.8 / –	1.7 / –	1.9 / –	0.8 / –	1.7 / –	1.5 / –	2.0 / –	y	yg	v
45	H₂N–C₆H₄–C₆H₃(OCH₃)NH₂	10.1 / –	16.8 / –	11.2 / –	3.9 / –	0.7 / –	0.7 / –	0.0 / –	0.0 / –	0.7 / –	0.7 / –	r	orr	v
46	HO₃S–C₆H₄–C₆H₄(NH₂)	0.4 / +	0.2 / +	0.1 / +	0.3 / +	3.7 / +	3.9 / +	3.0 / +	2.9 / +	5.6 / +	6.4 / +	y	gy	bl
47	H₂N–C₆H₄–C₆H₄(SO₃H)	0.1 / –	0.1 / +	0.0 / +	0.5 / +	3.1 / +	4.0 / +	2.9 / +	2.7 / +	7.1 / +	6.8 / +	ochy	yg	bl
48	H₂N–C₆H₄–C₆H₄(NH₂)	1.3 / –	0.9 / +	0.7 / +	10.7 / +	9.2 / +	7.6 / +	7.0 / +	4.4 / +	13.8 / +	11.9 / +	ochy	—	—
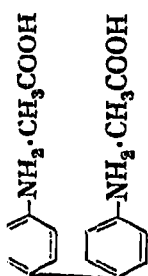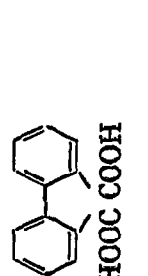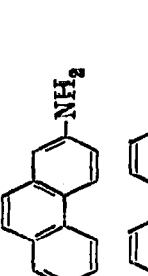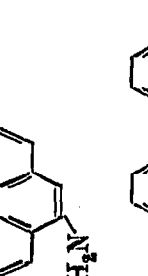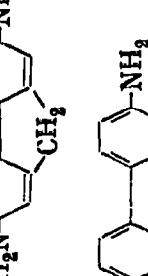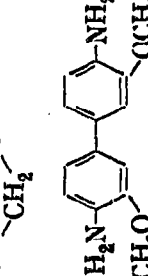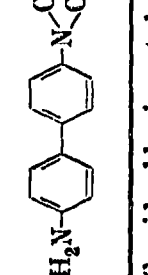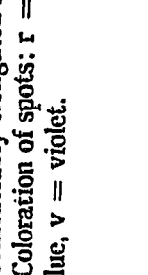
No.	Structure											Colors
48	NH₂, SO₃H / H₂N, HO₃S (aromatic)	1.3 −	0.9 −	0.7 +	10.7 +	9.2 +	7.0 +	7.0 +	4.4 +	13.8 +	11.9 +	ochy, —, —
49	NH₂ / H₂N, HO₃S SO₃H (biphenyl)	0.4 −	0.7 −	0.7 −	4.4 +	5.5 +	7.8 +	6.2 +	5.2 +	12.6 +	11.8 +	ochy, —, —
50	SO₃H / H₂N, H₂N	2.8 −	4.3 −	2.1 −	0.0	3.9 +	4.0 +	4.2 +	3.0 +	6.1 +	5.2 +	y, yg, bl
51	SO₃H / H₂N, H₂N, HO₃S	1.3 +	12.6 +	14.0 +	12.8 +	12.3 +	10.7 +	7.9 +	7.2 +	14.5 +	13.5 +	ochy, y, vbl
52	NH₂, OSO₃H (biphenyl)	6.1 −	5.8 −	2.3 +	4.8 +	5.0 +	4.6 +	2.8 +	2.9 +	5.8 +	5.8 +	y, y, —
53	NH₂, OSO₃H / H₂N	5.3 −	7.5 −	3.1 −	1.0 +	3.4 +	3.1 +	2.6 +	2.1 +	5.8 +	6.1 +	or, or, —
54	H₂N, HO₃SO, H₂N (terphenyl)	3.6 −	6.9 −	1.3 −	3.3 +	3.5 +	3.2 +	3.2 +	3.1 +	5.2 +	5.2 +	y, gy, —
55	OH, NH₂ (terphenyl)	15.8 −	21.3 −	16.5 −	8.8 −	2.8 −	0.8 −	0.9 −	0.8 −	0.8 −	0.8 −	ochy, y, —
56	OH / H₂N	4.4 −	8.4 −	6.5 −	3.3 −	1.5 −	0.0	0.0	0.0	0.0	0.0	yor, y, —
57	NH₂, OH / H₂N	12.3 −	16.3 −	12.7 −	5.9 −	1.8 −	0.7 −	0.1	0.3	0.8	0.2 −	r, ory, —
58	NO₂ / H₂N	5.0[a] −	13.5[a] −	5.1[a] −	2.8[a] −	0.9 −	0.4 −	0.0	0.0	0.0	0.0	or, dor, —

For footnote see p. 111.

TABLE II (continued)

No.	Structure	Buffer No. 1	2	3	4	5	6	7	8	9	10	After spray with Ehrlich reagent Vis.	UV	Without spray UV
59	H₂N–, O₂N– biphenyl	11.3	19.1	12.1	5.9	2.7	0.2	1.9	2.5	3.7	2.5	y	d	—
60	H₂N–, NH₂, O₂N–, NO₂	8.5	10.0	6.0	1.4	0.7	0.7	0.9	0.0	1.1	1.2	orr	dr	—
61	H₂N–, H₂N–, NO₂	8.5	12.6	6.9	4.0	1.2	1.0	1.8	1.6	2.8	2.8	ory	—	d
62	CH₃COHN–, NH₂, O₂N–, H₂N–	2.8	3.4	0.0	0.0	0.7	0.5	0.7	0.7	2.3	2.3	y	d	—
63	Br, Br, H₂N–	7.5ᵃ	14.5ᵃ	12.5ᵃ	7.8ᵃ	—	—	—	—	—	—	y	yg	—
64	H₂N–, NH₂, Cl, Cl	5.9	4.4	1.6	0.8	0.7	0.7	0.7	0.3	0.6	0.9	or	or	—
65	CH₃COHN–, NH₂	3.4	5.2	4.3	2.6	1.6	1.0	0.4	0.7	1.7	1.2	ory	ochy	—
66	CH₃COHN–, H₂N–	4.1	7.3	4.4	2.0	2.0	0.7	0.4	0.2	1.3	1.8	y	gy	—
67	H₂N–, NH₂, OCH₂·COOH, HOOC-CH₂O–	3.6+	2.9+	2.4+	1.6+	2.3+	3.4+	2.1+	1.8+	6.3+	7.2+	or	cr	bl

No.	Structure												
68	⌬–NH₂·CH₃COOH	16.3 (−)	23.6 (−)	19.9 (−)	8.1 (−)	0.9 (−)	0.3 (−)	0.1 (−)	0.3 (−)	0.1 (−)	0.3 (−)	0.2 brr	or	bl
69	⌬–NH₂·CH₃COOH	0.8 (+)	1.6 (+)	2.1 (+)	3.0 (+)	6.9 (+)	8.4 (+)	4.3 (+)	6.0 (+)	11.7 (+)	11.7 (+)	—	—	bl
70	HOOC COOH	0.5 (+)	0.5 (+)	0.0	0.0	0.00	0.0	0.0	0.0	0.0	0.0	0.0 y	dy	bl
71	NH₂ (phenanthrene)	5.2ᵃ (−)	8.5ᵃ (−)	2.5ᵃ	0.5	0.5	0.0	0.0	0.0	0.3	0.9	0.5 ochy	y	bl
72	H₂N– …CH₃– …NH₂	9.9 (−)	23.8 (−)	21.0	10.9	0.8	2.1	0.6	1.0	2.1	0.7 oir	or	bl
73	H₂N– …NH₂ …CH₂	3.3 (−)	8.0 (−)	5.5	3.3	1.3	0.5	0.6	0.6	1.9	1.7 y	y	bl
74	H₂N– CH₃O– …NH₂ OCH₃	10.4 (−)	19.7 (−)	13.7	3.6	1.3	0.8	0.8	1.0	2.0	1.6 brr	r	bl
75	H₂N– …N(CH₃)₂	4.4 (−)	7.1 (−)	5.4	1.1	0.0	0.0	0.0	0.0	0.0	0.0 ochy	yor	bl

ᵃ Considerably elongated spot.
Coloration of spots: r = red, och = ochre, y = yellow, g = green, or = orange, br-r = brick-red, d = dark, c = carmine, br = brown, bl = blue, v = violet.
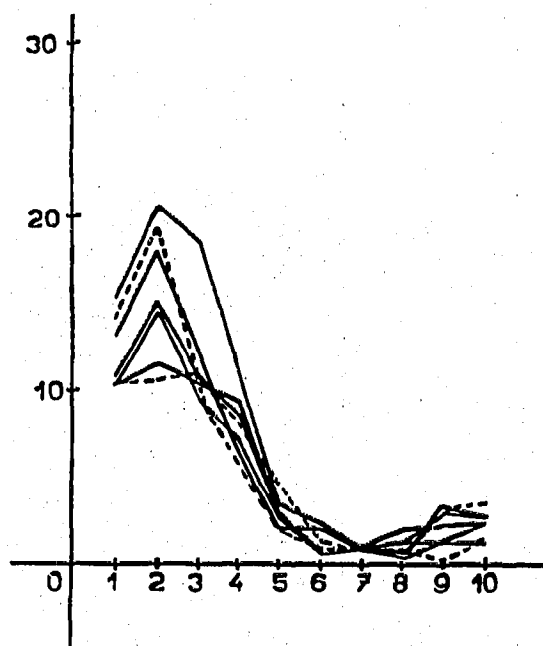
Fig. 3. Graphically represented mobility ratio u_x/u_7 for functional groups: —NH$_2$, —NH$_2$; migration region: — .

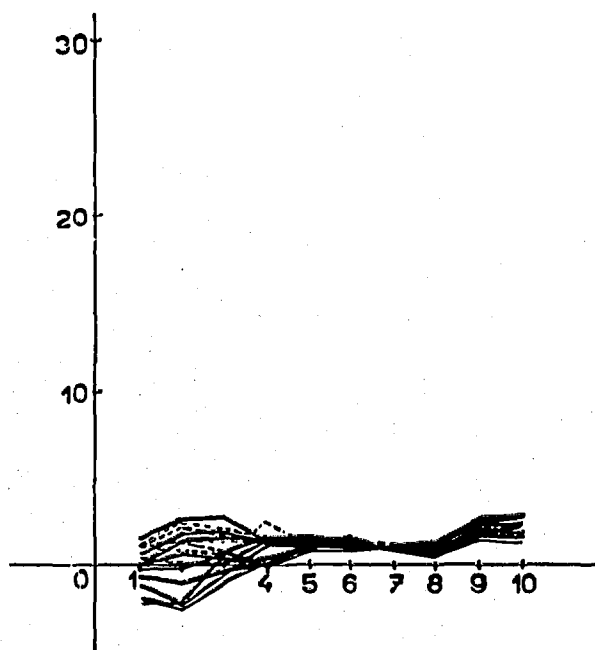Fig. 4. Graphically represented mobility ratio u_x/u_7 for functional groups: —NH$_3$, —SO$_3$H, —OSO$_3$H: migration region: — + .
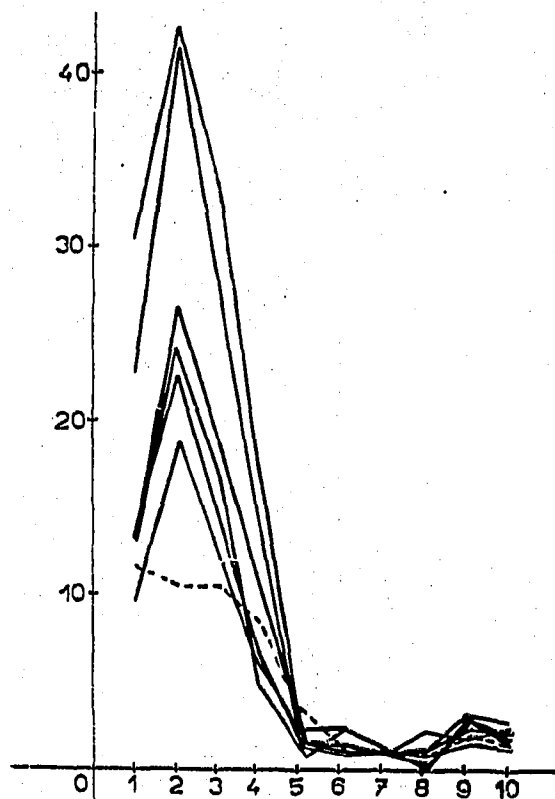
Fig. 5. Graphically represented mobility ratio u_x/u_7 for functional groups: —NH$_2$, —NHCH$_3$, —CH$_3$, —OCH$_3$; migration region: — .
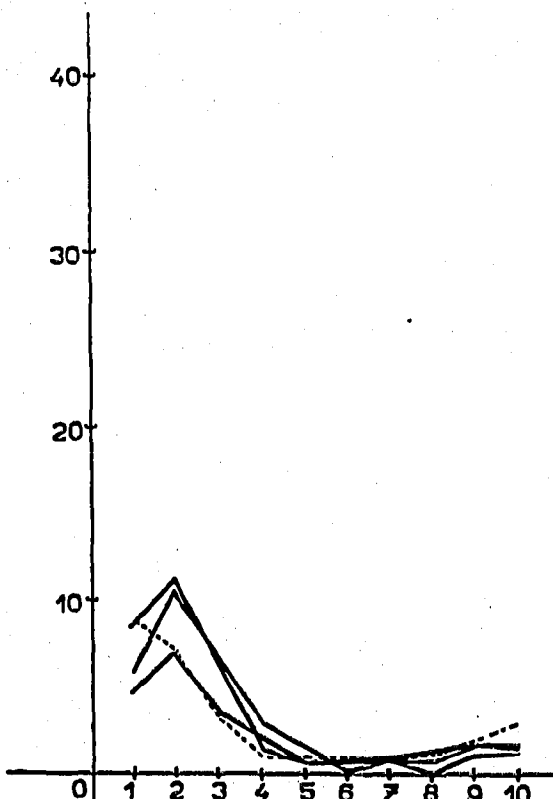
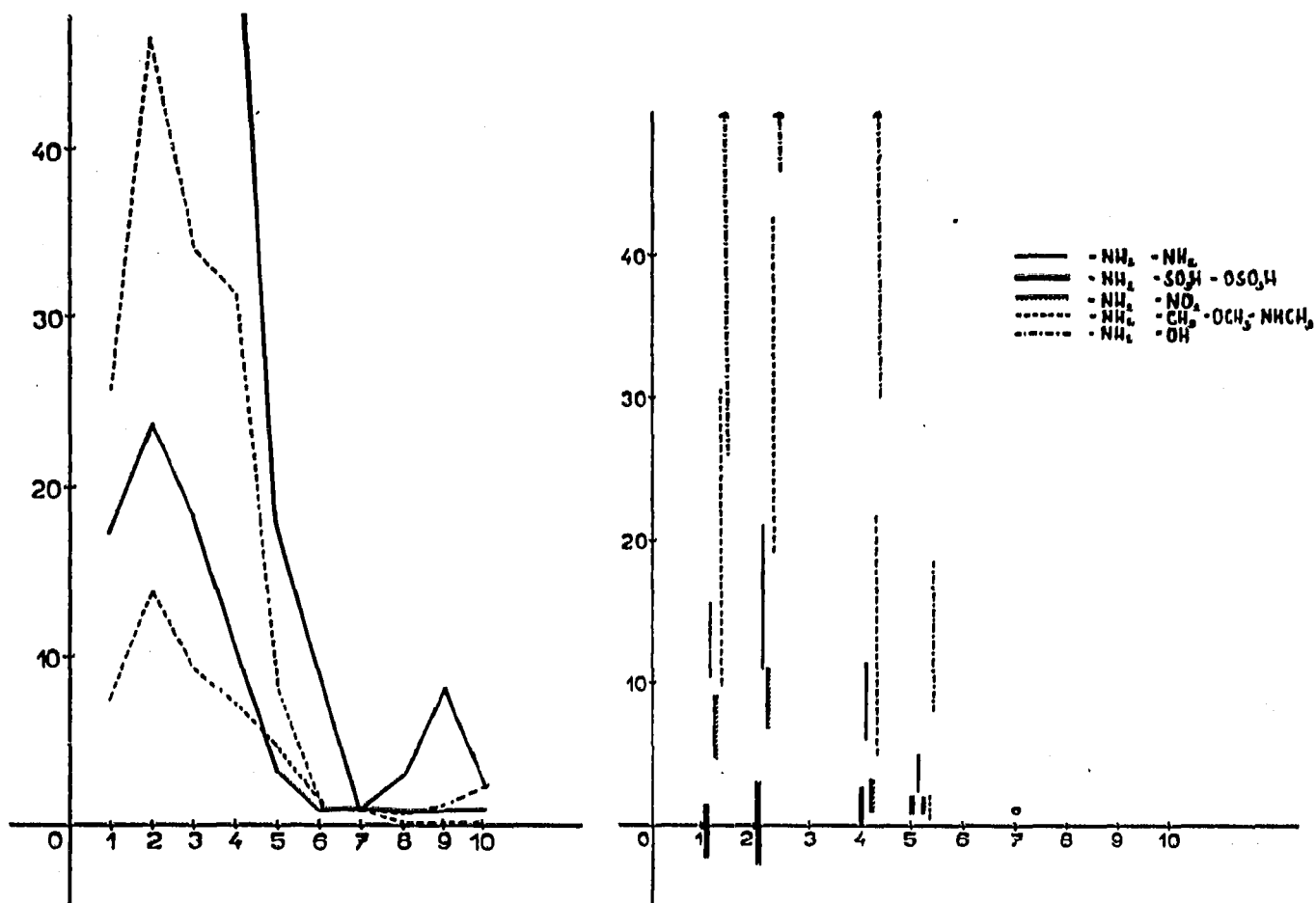Fig. 6. Graphically represented mobility ratio u_x/u_7 for functional groups: —NH$_2$, —NO$_2$; migration region: — .

Fig. 7. Graphically represented mobility ratio u_x/u_7 for functional groups: —NH_2, —OH; migration region: — . Dashed line: benzene series, two curves with the lowest values belong to compounds having a hydrogen bridge.

Fig. 8. Summarized expression of the graphs from Figs. 3-7 for individual functional groups (mobility ratio u_x/u_7).

only their character becomes manifest. In our case we selected as a base the mobility at pH 7.2 (u_7), which we divided into the other mobility values (u_x/u_7). In this way we obtained values whose connecting lines are illustrated in Figs. 3-7. The curves in each of these figures belong to substances with the same type of functional groups. At the first glance we see that the course of this curve is characteristic for different functional groups, and thus it was possible to construct a graph (Fig. 8) in which the regions of the values of u_x/u_7 are delimited for various functional groups for which sufficient experimental material was available. By means of this graph it is thus possible to determine to a certain degree the type of the functional group on the aromatic nucleus (benzene, naphthalene, diphenyl). It is obvious that various positional interactions (such as internal hydrogen bridges etc.) may cause deviation from the given data. For example, a valuable finding is that, in this way, it is possible to ascertain the presence of CH_3– and –$NHCH_3$ groups. Even the position of such a group can be determined in many cases (u_x/u_7 is higher for substitution in the p-position). Other mobility ratios may, of course, also be utilized; however, this only leads, in some cases, to a clearer conception, and not to any new conclusions (Fig. 9).

Another characteristic feature of each compound is the direction of its migration in all the buffers employed, whether it proceeds to the anode or to the catode, or, in the case of ampholytes, to both. For the last type of substance mentioned, the shape of the electrophoretic spectra allows the very easy determination of the position of the isoelectric point, which is likewise characteristic for each compound.
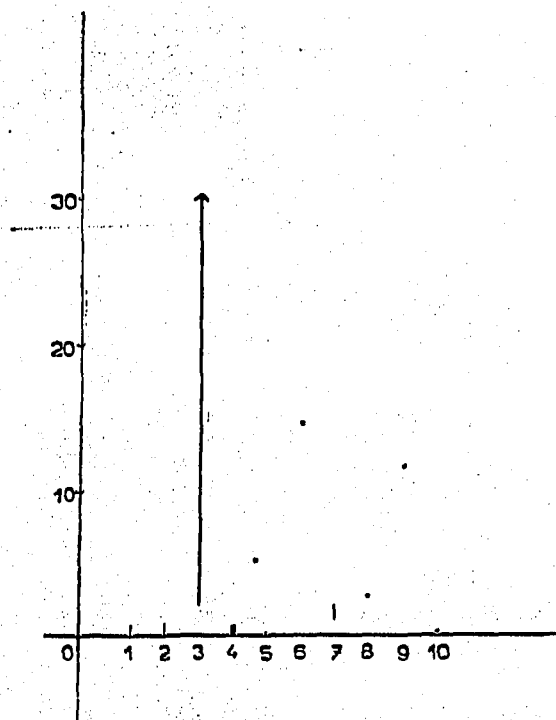

Fig. 9. Expression of mobility ratio u_{10}/u_1 for the functional groups: (1) —NH$_2$, —NH$_2$; (2) —NH$_2$, —CH$_3$, —OCH$_3$, —NHCH$_3$; (3) —NH$_2$, —SO$_3$H, —OSO$_3$H; (4) —NH$_2$, —NO$_2$; (5) —NH$_2$, —OH; (6) —COOH, —COOH; (7) —COOH, —NH$_2$, —OH; (8) —NO$_2$, —COOH; (9) —NO$_2$, 2-COOH; (10) —NH$_2$, —Cl.

The method described can also serve as an aid for determining in which buffer given compounds may be separated in the best way[15].

In general it may be stated that this method is a suitable supplement to other identification procedures.

SUMMARY

The relationship between electrophoretic migration on paper and the type, number and position of the functional groups of a series of aromatic compounds was

investigated. The compounds were mainly diphenyl derivatives, which for the greater part are strongly carcinogenic substances and whose identification in biological material causes certain difficulties.

Application of the so-called "electrophoretic spectra" offers the possibility of more detailed identification of these substances.

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