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THIRD SUPPLEMENT FOR THE PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF PHENOL DERIVATIVES AND RELATED COMPOUNDS OF BIOCHEMICAL INTEREST USING A "REFERENCE SYSTEM"

L. REIO

Wenner-Gren Institute, Department of Metabolic Research, University of Stockholm, Stockholm (Sweden)

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SUMMARY

Paper chromatographic data obtained using six solvent systems and the colour reactions using fifteen standard reagents are recorded for an additional 240 compounds. The main types of compounds presented in the tables are: monohydric, dihydric and trihydric phenol derivatives; naphthalene, quinoline and aminobenzene derivatives; aliphatic, aromatic and heterocyclic amino acid derivatives; biogenic amines and indole derivatives; alkaloids and natural products. The relationship between the mobility and the chemical structure is discussed on the basis of new information acquired. Earlier findings concerning the remarkable mobility pattern for hydroxyindoles are confirmed using more homologous compounds in this series.

INTRODUCTION

The following presentation is to supplement the series previously published in this journal[1-3] with new experimental data on another 240 compounds obtained using essentially the same procedures as described earlier. It is desirable to maintain as uniform a source of information as possible.

All the compounds were separated in 6 different chromatographic solvent systems. In addition to the mobility data, colour reactions obtained using 15 spray reagents for each of these compounds were also recorded. It was already observed at the beginning of this series of investigations that, when the R_F values for the single compounds were arranged in a special order for these solvents and when these values in turn were presented in a diagram, a number of interesting mobility patterns were obtained. Some of these patterns showed a useful and valuable relationship between the chemical structure and the type of pattern obtained. At the beginning this information was rather fragmentary; later when more compounds were systematically studied, the observed irregularities in the mobility were more well defined. The main general features concerning the earlier data have been summarised by the author a few years ago[4]. In a recent survey, employing examples also from this paper, the problems of the identification of certain substances is discussed in greater detail, only based on the characteristics in mobility[5].

The mobility was defined[5] as regular when the R_F values for the neutral compounds showed a continuous decrease from one solvent to another (from solvent F to D). A sudden elevation in the R_F value (in solvent E) was regarded as an indication of a basic compound. Vice versa, a marked drop in the R_F value (in solvent E) indicated an acidic substance. The main irregularities from these three basically standard patterns occurred between the solvents A and B, B and C, and C and D, either by an unexpected elevation or by a drop in the R_F values.

In order to disclose more irregular mobility patterns, the phenolic compounds and metabolic products from animal and vegetable origin were emphasised. The latter group of compounds included a number of alkaloids which were previously found to produce fairly characteristic R_F value patterns, clearly distinguished by the mobility of the other compounds from natural sources. In connection with these findings, it was also noticed that a few methoxylated aminophenols behaved chromatographically in a similar way. This led to an addition of aminobenzene-type compounds to discover possible similarities in the mobility and to what extent they might interfere with the interpretations. As it will be seen from the tables, both types of these compounds exhibit two maxima in their R_F values: one in solvent E (R_F values in solvents F $\langle$ E $\rangle$ A) and the other in solvent B (R_F values in solvents A $\langle$ B $\rangle$ C).

A great number of indole derivatives were also included in this study. One observation concerning another type of irregular mobility, found earlier for 5-hydroxyindole[3] and predicted for other monohydroxylated indoles, has also been confirmed by an additional investigation on several of these homologues. Here, the remarkable characteristic is that the change from the regular R_F value pattern for neutral compounds occurs between solvents B $<$ C $>$ D, which seems to be unique as far as the Ehrlich-positive compounds are concerned.

MATERIALS AND METHODS

For one-dimensional descending chromatography, rectangular glass jars 20 × 30 × 60 cm were used. Whatman No. 1 filter paper (chromatography grade, 24 × 48 cm) was used throughout the experiments. The solvent front was allowed to travel 40 cm from the start. The composition of the six solvent systems is given in the section *Abbreviations used in Tables I–XII*.

Spray reagents

The following twelve standard spray reagents were used to detect the compounds: diazotised sulphanilic acid (obtained from Th. Schuchart Co., G.F.R.); diazotised 4-benzoylamino-2,5-dimethoxyaniline (Koch–Light Laboratories Ltd., Great Britain); diazotised *o*-dianisidine (Koch–Light); *p*-nitrobenzenediazonium fluoborate (Eastman Kodak, U.S.A.); 2,6-dibromoquinone-4-chloroimide (British Drug Houses Ltd., Great Britain); 2,4-dinitrophenylhydrazine; ferric chloride; phosphomolybdic acid; potassium permanganate; bromophenol blue; *p*-dimethylaminobenzaldehyde; and *p*-dimethylaminocinnamaldehyde (Heidenheimer Chemisches Laboratorium, Heidenheim–Brenz, G.F.R.). Furthermore, all compounds were also tested with ninhydrin, *p*-dimethylaminobenzaldehyde in acetic acid anhydride and Dragendorff reagent $KI \cdot BiI_3$ (Merck, G.F.R.). The positive reactions were recorded and indicated by an asterisk in the tables. For the composition of these reagents see the section *Abbreviations used in Tables I–XII*.

The main part of the compounds which are listed in the tables were obtained through commercial sources and used without purification. However, it was discovered that a number of preparations contained several components which generally separated well. The main spot was considered to be representative of the compound appearing on the label.

Guide to Tables I–XII

Tables I–XII present condensed information for approx. 240 organic compounds investigated by the procedure outlined above. The R_F values are recorded in 6 different solvent systems, arranged in a special order, and designated by F, E, A, B, C and D. (For the composition of these solvents, see the list of abbreviations given below.) Under the heading *Detection* (columns 2–13) the colour reactions are recorded for 12 different reagents used for the identification of each compound; the colour produced under UV light is indicated in the first column under this heading. Furthermore, in this investigation, all compounds were also tested with NH, DAB and Bi reagents. When positive reactions were observed, the results were labelled with a, b or c and the explanation is found as a footnote for the corresponding tables. The amount of substance used in these experiments was about 25 μg per spot, which produced different colours when the reagents were applied. These colours are referred to by numbers; the explanation of the code is found in the colour index. For abbreviations and the composition of the reagents, the reader is referred to the following section.

Owing to the lack of space in the tables, the R_F values have been multiplied by 100 and recorded as 12, 56, 88 but should be read: 0.12, 0.56 and 0.88. The colours produced by the reagents are recorded as numbers and the corresponding shades can be found in the colour index. In order to facilitate location of the specific colours in the colour index, a general abbreviated transcript of colours from the numerical code is given in Fig. 1. The — sign generally indicates a negative reaction or an uncertain reaction which was too weak to deserve colour estimation. In few cases the uncertainty in colour shades is expressed by a + sign. Reactions with reagents Mn and Ind are, as a rule, indicated by the signs: —, + or ++. The ++ sign means that a positive reaction was immediately obtained. However, in this investigation, the number 33 often appears for the reagent Ind. This means that certain basic compounds become visible due to the strong absorption of the reagent to the compound, when compared with the background shade. Reagent Bi is only indicated as positive, without differentiating between the shades. In a few cases in which the colours are recorded

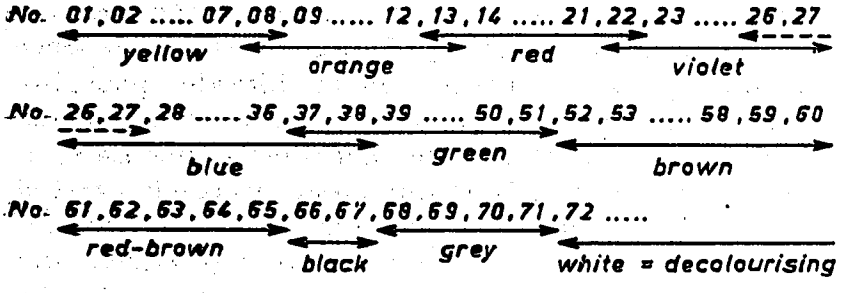

Fig. 1. Colour coding used to record the colour reactions (in abbreviated form) as a complement to the colour index for the tables.

by a number placed on top of another number, *e.g.* $\{^{24}_{57}$, immediately upon spraying a coloured spot (24 in the colour index) appears which, within a few seconds, changes colour (57 in the colour index). Usually most colours are unstable and after some time take on a brownish tone; this is to some extent caused by the chemical influence of other reagents used in the vicinity. This change in colour is neither recorded in the tables nor is there any column for those compounds that, at this low concentration, are visible on the chromatograms because of their own colour.

Abbreviations used in Tables I–XII

Chromatographic solvent systems

A = Methyl isobutyl ketone–formic acid–water (10 parts ketone saturated with 1 part 4 % formic acid)
B = Chloroform–methanol–formic acid–water (10 parts chloroform saturated with a mixture of 1 part methanol and 1 part 4 % formic acid)
C = Benzene–methyl ethyl ketone–formic acid–water (a mixture of 9 parts benzene and 1 part ketone saturated with 1 part 2 % formic acid)
D = Benzene–formic acid–water (10 parts benzene saturated with 1 part 2 % formic acid)
E = Methyl ethyl ketone–diethylamine–water (921 : 2 : 77)
F = Methyl ethyl ketone–acetone–formic acid–water (40 : 2 : 1 : 6)

Reagents used for detection

UV = ultraviolet light
D_1 = diazotised sulphanilic acid (0.3 % solution in dioxane–water, 1 : 2)
D_2 = diazotised 4-benzoylamino-2,5-dimethoxyaniline (0.6 % solution in dioxane–water, 1 : 2)
D_3 = diazotised *o*-dianisidine (0.6 % solution in dioxane–water, 1 : 2)
D_4 = *p*-nitrobenzenediazonium fluoborate (0.4 % solution in dioxane–water, 1 : 2)
DB = 2,6-dibromoquinone-4-chloroimide (0.5 % solution in dioxane–acetone, 4 : 1)
DN = 2,4-dinitrophenylhydrazine (*ca.* 0.1 % solution in 1 *N* HCl)
Fe = ferric chloride (2 % aqueous solution)
Mo = phosphomolybdic acid (2 % aqueous solution)
Mn = potassium permanganate (1 % aqueous solution)
Ind = bromophenol blue (*ca.* 0.05 % solution in ethanol)
EH = Ehrlich reagent (1 % *p*-dimethylaminobenzaldehyde in 1 *N* HCl)
DAC = *p*-dimethylaminocinnamaldehyde (0.1 % solution in 1 *N* HCl)
DAB = *p*-dimethylaminobenzaldehyde (2 % solution in acetic anhydride)
NH = ninhydrin reagent (2 % solution in butanol saturated with water)
Bi = Dragendorff reagent (2 % solution of potassium bismuth tetraiodide in 0.01 *N* HCl)

Colour index for the tables

The colours produced by the action of different reagents on the investigated compounds, presented in Tables I–XII, have been recorded as numbers, according to the following code.

01 Zinc yellow	19 Madder carmine	37 Oriental blue	55 Vandyke brown
02 Lemon cadmium	20 Crimson lake	38 Kingfisher blue	56 Raw umber
03 Gold	21 Rose madder lake	39 Turquoise blue	57 Brown ochre
04 Primrose yellow	22 Magenta	40 Turquoise green	58 Raw sienna
05 Straw yellow	23 Imperial purple	41 Jade green	59 Golden brown
06 Deep cadmium	24 Red violet lake	42 Juniper green	60 Burnt yellow ochre
07 Naples yellow	25 Dark violet	43 Bottle green	61 Copper beech
08 Middle chrome	26 Light violet	44 Water green	62 Burnt sienna
09 Deep chrome	27 Blue violet lake	45 Mineral green	63 Venetian red
10 Orange chrome	28 Delft blue	46 Emerald green	64 Terra cotta
11 Spectrum orange	29 Ultramarine	47 Grass green	65 Burnt carmine
12 Scarlet lake	30 Smalt blue	48 May green	66 Chocolate
13 Pale vermillion	31 Cobalt blue	49 Sap green	67 Ivory black
14 Deep vermillion	32 Spectrum blue	50 Cedar green	68 Blue grey
15 Geranium lake	33 Light blue	51 Olive green	69 Gunmetal
16 Flesh pink	34 Sky blue	52 Bronze	70 French grey
17 Pink madder lake	35 Prussian blue	53 Sepia	71 Silver grey
18 Rose pink	36 Indigo	54 Burnt umber	72 White (colourless)

TABLE I

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME MONOHYDRIC PHENOLS AND THEIR DERIVATIVES

R_F values × 100						Compounds
F	*E*	*A*	*B*	*C*	*D*	
95	92	91	95	92	87	*p*-Ethylphenol
91	82	89	86	83	52	*p*-Cyanophenol
94	86	93	93	93	91	2,4,6-Tribromophenol
63	69	08	00	00	00	*p*-Nitrophenolsulphate
94	82	92	92	92	77	4-Nitro-*m*-cresol
95	84	91	93	90	85	2-Nitro-*p*-cresol
94	95	94	97	92	91	*o*-Aminothiophenol[a]
95	95	95	97	89	82	*p*-Aminothiophenol[a]
94	92	95	94	88	87	2-Amino-6-nitrophenol
78	93	77	92	70	61	*o*-Anisidine[b]
78	92	73	93	64	57	*m*-Anisidine
91	86	32	27	04	02	3-Hydroxy-4-methylaniline
92	87	46	48	08	03	2-Hydroxy-5-methylaniline
93	93	46	88	28	23	4-Methoxy-2-methylaniline
93	93	90	93	95	92	2-Methoxy-4-nitro-5-methylaniline
29	45	01	04	00	00	3-Hydroxy-2-hydroxymethylpyridine
90	90	87	86	73	00	4-Methyl-6-hydroxycoumarin
92	93	94	95	94	94	9-Hydroxyanthracene
94	92	96	93	94	93	*o*-Methoxybenzaldehyde
91	41	90	67	73	19	Salicylic acid hydrazide[b]
92	57	92	52	85	32	4-Nitrosalicylic acid
93	22	91	48	76	03	Diphenolic acid

[a] Bi reagent, positive.
[b] DAB reagent, pale yellow.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	17	59	64	64	—	—	—	—	++	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
26	—	—	—	—	58	—	—	39	++	—	—	—
56	—	—	—	—	—	—	—	—	—	—	—	—
59	—	—	—	—	51	—	—	—	+	—	—	—
03	—	—	—	—	+	—	—	—	+	—	—	—
53	06	—	+	09	+	—	—	—	++	—	08	65 63
53	57	—	62	03	70	—	—	70	+	—	09	65 63
+	59 62	53	+	+	56	—	—	51	+	—	03	23 65
54	12	+	62	11	38 70	—	—	40	++	—	08	14 64
+	11	65 11	23 65	62	41	—	—	40	+	—	08	22 64
+	64	64 65	65	64 65	25	—	—	51	++	—	08	23 61
—	20 65	63	65 63	62 65	51	—	65	68	++	—	08	22 63
—	—	—	64	03 52	—	—	56	68	+	—	02	22 62
52	—	—	—	—	—	—	—	—	+	—	09	52 25
33	07	63	24	17	38	—	—	—	++	—	—	—
33	08 62	60	65 64	11	—	—	—	—	++	—	—	—
04	—	—	—	—	—	—	—	—	++	—	—	—
+	—	—	—	—	30 68	08	—	+	+	—	—	—
—	—	61	12 65	06	43 68	04	—	68	++	+	04	17
52	—	—	—	—	—	—	58	—	—	++	06	22
—	10	10 62	62 56	08 65	62 35	—	—	68	++	+	—	17

TABLE II

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME 1,2-DIHYDRIC PHENOLS AND THEIR DERIVATIVES

R_F values × 100						Compounds
F	E	A	B	C	D	
60	24	29	22	06	02	2,3-Dihydroxypyridine
34	04	03	01	00	00	3,4-Dihydroxypyridine
93	51	89	47	46	04	4-Nitropyrocatechol[a]
79	21	08	00	00	00	4-Nitropyrocatecholsulphate
84	78	75	67	34	18	3-Hydroxy-4-methoxybenzyl alcohol (isovanillyl alcohol)
54	32	15	00	00	00	3,4-Dihydroxyphenylglycol
92	69	91	67	52	08	3,4-Dihydroxypropiophenone
92	52	93	90	93	90	5-Nitrovanillin
92	83	89	85	80	28	3,4-Dihydroxybenzoic acid ethyl ester
84	04	91	05	03	00	2,3-Dihydroxyphenylacetic acid
89	03	82	66	38	07	3-Hydroxy-4-methoxycinnamic acid
92	08	90	91	85	57	β-Piperonylacrylic acid

[a] NH reagent, pale yellow.

TABLE III

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME 1,3-DIHYDRIC, 1,4-DIHYDRIC AND TRIHYDRIC PHENOLS AND THEIR DERIVATIVES

R_F values × 100						Compounds
F	E	A	B	C	D	
95	93	95	73	83	37	4-n-Propylresorcinol
91	05	87	04	04	00	3,5-Dihydroxy-4-methylbenzoic acid
46	06	08	01	00	00	2,5-Dihydroxypyridine
94	92	92	92	92	84	p-(Methylthio)-phenol
33	16	02	00	00	00	Arbutin
79	93	82	93	84	76	2,5-Dimethoxyaniline
94	94	92	96	96	93	9,10-Anthradiol
92	94	92	96	95	94	6,11-Dihydroxynaphthacenequinone
88	87	84	78	64	42	3-Methoxypyrocatechol
86	84	85	90	79	73	3,4,5-Trimethoxybenzyl alcohol
24	08	00	02	00	00	Syringin
87	62	76	15	06	00	Gallic acid methyl ester

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	08 13	56 25	25 35	09 65	29 24	—	28 46	25 33	+	—	—	—
—	08	—	—	14 19	—	—	18 48	33	+	—	—	—
52	08	07	—	52 60	12	—	52	06 69	++	—	07	—
58	—	—	—	—	—	—	—	—	+	—	—	—
27	10	19	65	08	28 64	—	—	68	++	—	—	—
—	62 60	19 64	21 17	59 24	28	—	—	28 68	++	—	—	—
+	60	+	+	64 56	60 69	58	—	52 68	++	—	—	—
53	—	—	—	—	—	06	—	—	+	—	—	—
—	+	63	63	63	50 70	—	51 70	03 68	++	—	—	—
—	60	64	+	07	25	—	—	68	+	+	—	—
33	60	64	62 65	09 23	37	—	—	68	+	+	—	—
33	—	—	—	—	—	—	—	+	+	+	—	—

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	09 62	52 19	65 25	09 12	24	—	+	43 33	++	—	18	39
—	07	62	63 23	06	25	—	—	69	+	+	—	—
26	19 09	49 28	25 28	14	38 41	—	+	33	+	—	—	—
—	57 63	08 64	60 66	62 25	33	—	+	33	++	—	—	—
—	11 17	64 62	25 56	25 23	33 25	—	—	38	++	—	—	—
—	15 63	25 15	25 19	63 23	43 28	—	43 51	68	++	—	09 60	63 23
57	—	—	—	—	—	—	—	—	—	—	—	—
59	—	—	—	—	—	—	—	—	—	—	—	—
57	57	63	59	10	25	—	59 69	52 68	++	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
—	—	—	—	—	—	—	—	—	++	—	—	—
59	60	24 65	65	56	60 52	—	51 66	50 68	++	—	—	—

TABLE IV

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME NAPHTHALENE AND ANTHRACENE DERIVATIVES

R_F values × 100						Compounds
F	E	A	B	C	D	
94	92	92	92	87	68	5-Hydroxy-1-tetralone
92	91	90	93	93	92	5-Hydroxy-1,4-naphthoquinone[a]
92	92	87	72	35	08	1-Amino-5-naphthol
92	87	92	93	94	93	2,4-Dinitro-1-naphthol
92	42	92	93	80	37	2-Hydroxy-3-naphthoic acid hydrazide
94	43	93	28	56	03	2,8-Dihydroxy-3-naphthoic acid
95	95	93	96	93	94	1,8-Dihydroxyanthracene (chrysazol)
92	94	93	95	93	92	1,5-Dihydroxyanthraquinone (anthrarufin)
93	94	89	95	92	92	1,8-Dihydroxyanthraquinone (chrysazin)
92	00	88	29	19	00	1,2,5,8-Tetrahydroxyanthraquinone (quinalizarin)
38	00	00	00	00	00	Carminic acid
93	93	96	94	92	90	1-Aminoanthraquinone
91	90	95	92	87	82	2-Aminoanthraquinone

[a] NH reagent, pale yellow.

TABLE V

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME QUINOLINE DERIVATIVES

R_F values × 100						Compounds
F	E	A	B	C	D	
91	95	93	91	92	86	8-Mercaptoquinoline[a]
90	04	82	78	29	02	4-Hydroxyquinazoline
86	16	84	88	49	00	4-Hydroxy-N-methyl-2-quinoline
83	89	78	86	80	59	1,3-Dihydroxyisoquinoline
72	62	45	48	05	00	4-Hydroxy-6-methoxyquinoline[a]
64	76	02	12	00	00	4-Amino-7-chloroquinoline[a]
87	93	84	95	87	80	8-Aminoquinoline
84	93	87	92	86	83	8-Amino-6-methoxyquinoline[a]
62	05	27	09	03	00	8-Methoxyxanthurenic acid
74	35	44	75	08	04	4-Methoxyxanthurenic acid
92	85	88	95	90	78	4,8-Dimethoxyxanthurenic acid
87	11	89	48	04	00	4-Hydroxy-6-methoxy-3-quinoline-carboxylic acid
82	18	74	65	10	00	4-Hydroxy-7-methoxy-3-quinoline-carboxylic acid
84	17	79	82	21	05	4-Hydroxy-8-methoxyquinoline-carboxylic acid
82	09	61	63	24	03	6-Methoxy-4-quinolinecarboxylic acid (quininic acid)

[a] Bi reagent, positive.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
17	09	60	—	—	08 38	—	—	—	+	—	—	—
53	—	—	—	—	—	62	—	23 71	+	—	—	—
70	—	51	24 70	63	—	—	+	70	+	—	08 60	24
03	—	—	—	—	—	—	72	—	++	—	—	—
57	10 15	45 25	24 25	15	50 53	—	—	50 33	+	—	05	17
03	15 23	56 23	25	63 35	28	—	51	43 68	++	+	—	—
—	—	—	—	—	—	—	—	69	—	—	—	—
57	—	—	—	—	—	—	—	—	—	—	—	—
60	—	—	—	—	+	—	—	26	—	—	—	—
57	—	—	—	—	—	—	—	27	—	—	—	—
17	—	—	—	—	—	—	—	—	—	33	—	—
17	10	+	—	—	—	—	—	—	—	—	17	26
18	—	—	—	—	—	—	—	—	—	—	17	27

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	—	—	64	—	—	45 70	+	—	—	—
34	—	—	—	—	—	—	—	—	—	—	—	—
—	07	63	14	07	25	—	+	—	+	—	08	—
—	06	15 62	19 65	08	23 60	—	—	42 68	++	—	+	—
27	—	—	—	—	—	—	—	39	—	—	—	—
34	—	—	—	—	—	—	—	—	—	—	—	—
40	20 11	12 62	23 53	63	42 23	—	+	03 70	+	—	08	19 65
—	63	28 63	23	63 24	41	+	52	69	++	—	09 62	63 65
33	—	—	—	—	—	—	—	—	—	+	—	—
37	—	—	—	—	—	—	—	—	—	+	—	—
38	—	—	—	—	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	58	—	—	+	—	—
34	—	—	—	—	—	—	57	—	—	+	—	—
27	—	—	—	—	—	—	58	—	—	+	—	—
33	—	—	—	—	—	—	—	—	—	+	—	—

TABLE VI

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME AMINOBENZENE DERIVATIVES

R_F values × 100						Compounds
F	E	A	B	C	D	
69	93	56	92	45	37	Aniline
77	92	75	97	77	73	*N*-Methylaniline
79	93	76	97	78	74	N,N-Dimethylaniline
95	95	89	93	92	91	2,4-Dichloroaniline
91	89	93	91	90	87	*p*-Bromoaniline
93	92	89	91	90	87	*o*-Nitroaniline
94	95	91	92	91	86	*m*-Nitroaniline
93	92	93	88	86	79	*p*-Nitroaniline
79	93	76	92	71	63	*o*-Toluidine[a]
93	92	94	92	93	93	2-Amino-5-chlorotoluene[a]
75	93	62	92	64	52	*m*-Toluidine[c]
66	92	48	88	39	30	*p*-Toluidine
93	96	90	96	95	92	2-Nitro-4-methylaniline
94	90	95	93	95	92	3,5-Dinitro-4-methylaniline
94	95	92	94	93	91	3-Nitro-5-methylaniline
91	92	93	95	96	92	2-Nitro-6-methylaniline
94	92	93	95	90	87	3-Nitro-6-methylaniline
92	94	92	95	90	85	4-Nitro-6-methylaniline
74	83	00	57	13	05	*o*-Phenylenediamine[a, b]
94	92	87	71	90	23	4-Nitro-*o*-phenylenediamine[c]
15	77	00	09	00	00	*m*-Phenylenediamine
28	59	00	05	00	00	*p*-Phenylenediamine[a, c]
96	95	93	95	94	87	2-Aminobenzophenone[b, c]
68	90	54	78	00	00	1,5-Diaminonaphthalene[a]
52	87	23	58	01	00	2,7-Diaminonaphthalene
56	92	19	80	03	02	Benzidine[a, c]
73	90	63	92	42	34	*o*-Tolidine
91	92	90	93	87	84	1-Aminonaphthalene[c]
87	92	87	92	84	78	2-Aminonaphthalene[b, c]
33	87	07	46	00	00	2,7-Diaminofluorene[b]

[a] NH reagent, pale violet.
[b] Bi reagent, positive.
[c] DAB reagent, pale yellow.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	08	—	11	10	—	—	—	—	++	—	07	22
—	09	+	62	09	27 41	—	—	33	+	—	04	15
—	08	—	65	60	39	—	—	70	+	—	—	—
08	—	—	—	—	+	—	—	—	—	—	07	21
—	—	—	—	03	—	—	—	—	+	—	07	22
53	—	—	—	—	—	—	—	—	+	—	—	65
57	—	—	—	—	—	—	—	—	—	—	06	22
53	—	—	—	—	—	—	—	—	+		12 64	65 25
—	10	06	64 65	14	38	—	+	69	++	—	06	17 20
—	—	—	62	03	—	—	—	—	+	—	01	22
—	08	—	65 56	—	23	—	48	71	++	—	06	15 21
—	—	06	13 65	06	—	—	+	71	+	—	07	17 20
59	—	—	—	—	—	—	—	+	—	—	64	24
59	—	—	—	—	—	—	—	—	—	—	03	63
57	—	—	—	—	—	—	—	—	—	—	07	23
57	—	—	—	—	—	—	—	—	—	—	04	26
+	—	—	—	—	—	—	—	—	—	—	01	22
51	—	—	—	—	—	—	—	—	—	—	08	49 54
+	—	—	62	63	+	+	64	68	++	—	09	23
56	—	—	—	—	+	—	—	51	+	—	08	25
—	10	23 11	19 65	10	35	—	—	69	++	—	08	19 23
—	50	51	52	09	49	—	43 25	40 68	++	—	15	23 35
48	—	—	—	08	59	05	—	—	+	—	09	23
26	53 23	23 65	68 53	69	63 23	—	25 69	69	++	—	09	23 65
26	52 62	64	65	63 65	52	—	57	60	++	—	10	23 65
—	51	58	63	51	—	—	43 68	54 68	++	—	14	23 25
—	45 62	48 57	45 62	49	42 70	—	68 54	43	++	—	12	23 25
33	19 15	14 63	24 25	23	41 23	—	26	68	+	—	07	22 23
26	62 09	12	25	19 23	70	—	59	68	++	—	09	22
26	38 57	45 52	45 52	51 59	50	—	52 53	60 68	++	—	15	65 25

TABLE VII

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME AROMATIC AND HETEROCYCLIC AMINO ACID DERIVATIVES

R_F values × 100						Compounds
F	E	A	B	C	D	
35	87	00	02	00	00	*l*-Phenylalaninol
53	86	01	59	00	00	*l*-Phenylalanine methyl ester
81	02	58	03	00	00	N-Formyl-*l*-tyrosine[b]
85	80	76	57	18	04	N-Acetyl-*l*-tyrosine
20	80	00	00	00	00	*l*-Tyrosine methyl ester[b]
35	00	00	00	00	00	*l*-Tyrosinol
49	02	00	00	00	00	*dl*-α-Methyl-*m*-tyrosine
25	00	00	00	00	00	3-Methoxy-*dl*-tyrosine
42	01	00	00	00	00	3-Iodotyrosine
35	00	00	00	00	00	3-Nitrotyrosine
44	00	00	00	00	00	3,5-Dinitrotyrosine
75	10	18	00	00	00	3,5-Diiodothyronine
86	05	79	25	09	01	N-Formyl-*dl*-tryptophan[b]
78	65	54	33	04	01	N-Acetyltryptophanamide
49	01	01	00	00	00	6-Methyltryptophan
39	01	01	00	00	00	*dl*-5-Methoxytryptophan
39	90	02	00	00	00	*dl*-Tryptophanol
58	05	19	00	00	00	5-Hydroxykynurenine
01	00	00	00	00	00	4-Piperidinecarboxylic acid (isonipecotic acid)
04	00	00	00	00	00	N-Methyl-2-piperidinecarboxylic acid[b]
02	00	00	00	00	00	Mimosine
09	02	00	00	00	00	Thioproline (thiazolidine-4-carboxylic acid)[b]
02	00	00	00	00	00	*l*-2-Thiolhistidine

[a] NH reagent. DN reagent gave no reaction with the compounds listed in this table.
[b] DAB reagent, pale yellow.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	NH^a	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	—	07	—	23	—	—	—	33	—	—
—	—	—	—	+	17	18	—	—	—	33	+	—
—	10	+	63	23	—	23	—	39	++	+	—	—
—	12	—	+	07	—	—	—	71	++	+	—	—
—	10	60	62	59	+	24	—	33	++	33	04	64
—	—	—	—	—	—	26	—	27	++	—	—	—
—	10	11	65	64	29 23	23	—	44	++	33	—	—
—	64	+	56	23 52	+	26	—	68	++	33	—	—
—	11	—	—	63	—	23	—	38	++	—	—	—
53	—	—	—	—	—	23	—	71	+	—	—	—
53	—	—	—	—	—	60	—	—	—	—	—	—
—	62	—	—	—	—	23	—	38	+	—	04	—
—	—	—	+	08	52	—	—	—	++	+	23 70	25
27	—	—	+	09	70	—	—	—	++	—	23 27	25
34	60	—	61	60	—	26	—	—	+	33	25 48	25 28
—	—	—	+	10	26	23	—	—	+	—	27	29
+	—	—	—	—	—	27	—	71	++	33	23 58	24 25
40	62	61	54	17	38	57	—	43 38	+	—	09	22
—	—	—	—	—	—	23	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	33	—	—
34	—	—	—	—	—	23	23	69	—	33	—	—
—	—	—	—	72	—	26	—	—	++	+	—	+
—	09	64	17	62	62	23	—	38	++	—	05	17

TABLE VIII

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME ALIPHATIC AMINO ACID DERIVATIV

R_F values × 100						Compounds
F	*E*	*A*	*B*	*C*	*D*	
17	07	00	00	00	00	Aminohydroxyacetic acid[b]
03	00	00	00	00	00	Glycine
08	00	00	00	00	00	Glycylglycine
06	00	00	00	00	00	Alanine
73	01	24	16	01	00	N-Acetyl-*dl*-alanine
06	00	00	00	00	00	*β*-Alanine[b]
08	00	00	00	00	00	*dl*-*α*-Amino-*n*-butyric acid[b]
24	00	00	00	00	00	*dl*-Norvaline[b]
36	00	00	00	00	00	*dl*-Norleucine
18	00	00	00	00	00	*ε*-Aminocaproic acid
03	00	00	00	00	00	*dl*-Serine
07	00	00	00	00	00	O-Acetylserine
00	00	00	00	00	00	*dl*-O-Phosphoserine
05	03	00	00	00	00	*dl*-Threonine
00	00	00	00	00	00	*dl*-O-Phosphothreonine
07	00	00	00	00	00	Taurine
03	00	00	00	00	00	N-Methyltaurine[b]
10	00	00	00	00	00	S-Methyl-*l*-cysteine[b]
03	00	00	00	00	00	*l*-Cysteinesulphinic acid[b]
02	03	00	00	00	00	*dl*-Homocysteine
22	04	00	05	00	00	*l*-Homocysteinethiolactone
02	00	00	00	00	00	*dl*-Homocystine
73	19	00	00	00	00	*dl*-Penicillamine (*β*-mercaptovaline)[b]
18	01	00	00	00	00	*dl*-Methionine
02	00	00	00	00	00	*dl*-Methionine sulphoxide
07	00	00	00	00	00	*dl*-Methionine sulphone[b]
27	00	00	00	00	00	*dl*-Methionine methylsulphonium chloride
00	00	00	00	00	00	*dl*-Djenkolic acid

[a] NH reagent. DN reagent gave no reaction with the compounds listed in this table.
[b] DAB reagent, pale yellow.

tection

	D_1	D_2	D_3	D_4	*DB*	NH^a	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
	—	—	—	—	—	—	—	—	—	—	05	—
	—	—	—	—	—	24	—	—	—	33	04	—
	—	—	—	—	—	23	—	—	—	33	—	—
	—	—	—	—	—	24	—	—	—	33	04	—
	—	—	—	—	—	—	—	—	—	+	—	—
	—	—	—	—	—	25	17	—	—	33	+	—
	—	—	—	—	—	24	—	—	—	33	04	—
	—	—	—	—	—	23	—	—	—	33	05	—
	—	—	—	—	—	23	—	—	—	33	04	—
	—	—	—	—	—	23	—	—	—	33	—	—
	—	—	—	—	—	24	—	—	+	33	—	—
	—	—	—	—	—	23	—	—	—	33	—	—
	—	—	—	—	—	23	—	—	—	+	—	—
	—	—	—	—	—	24	—	—	+	33	—	—
	—	—	—	—	—	23	—	—	—	+	—	—
	—	—	—	—	—	23	—	—	····	33	—	—
	72	—	—	72	—	—	—	—	—	33	—	12
	—	—	—	—	—	23	—	—	+	33	06	14
	—	—	—	—	—	23 64	—	—	+ +	+ +	—	—
	—	—	—	—	59	23	—	30	+ +	33	04	—
	—	—	—	—	—	23	—	32	+	33	04	—
	—	—	—	—	—	23	—	—	+	33	04	—
	72	—	—	72	06	06	+	45	+ +	+	05	17
	—	—	—	—	—	24	—	—	+	33	—	—
	—	—	—	—	—	23	—	—	+ +	—	—	—
	—	—	—	—	—	23	—	—	—	33	—	—
	—	—	—	—	—	23	—	—	+ +	—	04	—
	—	—	—	—	—	23	—	—	+	33	05	17

TABLE IX

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME BIOLOGICALLY ACTIVE NITROGEN COMPOUNDS

R_F values × 100						Compounds
F	*E*	*A*	*B*	*C*	*D*	
48	88	02	01	00	00	Benzylamine
50	79	01	01	00	00	*p*-Methoxybenzylamine[b]
68	92	03	32	00	00	*β*-Phenyl-*β*-propylthioethylamine[b,c]
40	53	00	00	00	00	N-Isopropylnoradrenaline
59	84	01	05	00	00	3-Methoxy-4,5-methylenedioxyamphetamine
54	87	03	05	00	00	Isomescaline[b]
78	79	03	16	00	00	N-Methylmescaline[c]
25	03	00	00	00	00	Acetylcholine[c]
05	22	00	00	00	00	Celocurine[c]
04	00	00	00	00	00	Ethanolamine[b]
00	00	00	00	00	00	O-Phosphoethanolamine
10	08	00	00	00	00	2,2'-Aminomethyl-1,3-propanediol[b]
12	92	00	00	00	00	2-Diethylaminoethanethiol[c]
47	10	01	00	00	00	2-Dimethylaminomethylpyrrole[b]
37	22	07	01	00	00	Cyanuric acid
21	00	00	00	00	00	Creatin
92	89	88	95	94	83	1-Hydroxypyridine-2-thione[c]
39	19	07	12	01	00	N-Methyl-2-pyridone-5-carboxamide
06	01	00	00	00	00	Uramil (5-aminobarbituric acid)
05	03	00	00	00	00	Dilituric acid (5-nitrobarbituric acid)
59	51	27	27	04	02	Acetylurea
66	51	24	28	04	02	1,3-Dimethylurea
27	12	03	00	00	00	Hydroxyurea
05	01	00	00	00	00	*p*-Urazine (diurea)[c]

[a] NH reagent. DN reagent gave no reaction with the compounds listed in this table.
[b] DAB reagent, pale yellow.
[c] Bi reagent, positive.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*NH*[a]	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
–	–	–	08	06	–	53	–	–	+	33	–	–
–	–	–	+	–	–	53	–	–	+	33	05	–
–	–	–	–	–	60	60	–	–	+	–	+	+
–	62	63	23	08 65	23 52	27	–	28 68	+ +	33	–	–
+	–	–	–	–	–	24	–	–	–	33	–	–
–	–	–	–	–	–	25	–	–	–	33	–	26
–	–	–	–	–	+	–	–	–	+	33	–	–
–	–	–	–	–	–	–	–	–	–	33	–	–
+	–	–	–	–	58	–	–	–	–	–	–	–
–	–	–	–	–	+	23	–	–	–	33	05	17
–	–	–	–	–	–	23	–	–	–	–	04	–
–	–	–	–	–	–	–	57	–	–	–	–	–
–	–	–	–	–	51	–	–	15	+ +	–	–	–
+	63	62	64	08	70	17	–		–	33	17 22	69
–	–	–	–	–	–	–	–	–	–	–	06	17
–	–	–	–	–	–	27	–	–	–	–	–	–
–	–	–	–	–	07	–	–	–	+	–	–	–
–	–	–	–	–	–	–	08	–	+	–	+	17
+	–	–	–	–	–	–	–	29	–	–	05	59
57	–	–	–	–	–	–	–	–	–	+	–	–
–	–	–	–	–	–	–	–	–	–	–	04	22
–	–	–	–	–	–	–	–	–	–	33	05	22
–	–	72	72	–	+	–	27	30	– –	–	07	22
–	–	60	43 51	–	–	–	–	39	–	–	–	–

TABLE X

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME INDOLE DERIVATIVES

R_F values × 100						Compounds
F	*E*	*A*	*B*	*C*	*D*	
95	96	93	94	94	93	5-Bromoindole
93	93	93	94	93	94	6-Methoxyindole[a, b]
94	95	90	94	92	84	5-Nitroindole[b]
92	85	87	89	78	42	5-Methylisatin
93	91	88	78	82	44	5-Hydroxy-2-methylindole[a, b]
90	88	80	58	62	39	2-Methyl-3-ethyl-5-aminoindole
84	92	36	92	18	12	2-Methyl-3-ethyl-5-dimethylamino-indole[c]
95	94	53	92	73	62	N-1-Methyl-2-methyl-3-ethyl-5-dimethylaminoindole
91	85	87	93	90	75	3-Indoleacetaldehyde
93	90	92	93	88	62	7-Methyl-3-indolealdehyde
93	16	90	76	71	28	Indole-2-carboxylic acid
93	93	92	94	92	91	5-Methoxyindole-2-carboxylic acid ethyl ester
86	79	77	79	37	16	2-Methyl-5-methoxy-3-indolylacetamide
92	90	89	47	82	04	3-Indolylacetic acid hydrazide[b]
85	82	79	88	68	55	Indolon-2
91	92	89	64	74	34	4-Hydroxyindole[a, b]
91	92	91	75	91	67	4-Hydroxyskatole
94	90	91	68	82	47	6-Hydroxyskatole
86	05	79	25	09	01	N-Formyl-*dl*-tryptophan[b]
78	65	54	33	04	01	N-Acetyltryptophanamide
49	01	01	00	00	00	6-Methyltryptophan[a]
39	01	01	01	00	00	*dl*-5-Methoxytryptophan[a]
39	90	02	00	00	00	*dl*-Tryptophanol[a]
59	85	02	00	00	00	α-Methyltryptamine
66	86	03	00	00	00	5-Methyltryptamine
67	91	04	00	00	00	7-Methyltryptamine[a]
72	92	05	00	00	00	2-Methyl-5-nitrotryptamine[a, b]
58	85	03	00	00	00	6-Methoxytryptamine[a, b]
52	64	02	00	00	00	N-ω-Methyl-5-hydroxytryptamine
81	92	06	28	03	02	2-Methyl-N,N-dimethyltryptamine
59	02	04	00	00	00	*d*-Lysergic acid
82	89	09	93	07	02	*d*-Bromolysergic acid diethylamide[c]
55	86	07	31	01	00	Norharman[c]
64	77	02	09	00	00	Harmol[a]
57	88	05	04	00	00	Tetrahydroharman[c]
51	21	03	00	00	00	6-Hydroxytetrahydroharman
55	84	04	06	00	00	6-Methoxytetrahydroharman[b]

[a] NH reagent, pale pink. [b] DAB reagent, pale brown. [c] Bi reagent, positive.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DX*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	—	—	—	—	—	+	+	—	23 22	32 68
57	60	—	+	64	25	—	43	68	++	—	25 64	52 35
60	—	—	—	—	+	—	—	—	+	—	62 23	40 28
60	—	—	—	—	—	—	—	—	—	—	59	+
04	07	56	23 56	10 64	27 69	18	27 51	39 69	++	—	22	43 53
57	62	64	68	63	70	—	+	50 37	+	—	07 59	62 23
03	64	52 60	52 63	23 52	38 69	—	+	23 28	++	33	40	62 23
27	64	63	24	23 56	38 42	—	—	23 28	++	33	40	23
—	—	—	—	—	—	+	60	39	+	—	23	25
34	—	—	—	—	62	17	—	·	—	—	18	—
33	+	53 25	25	+	—	—	—	—	+	+	17 23	34 27
33	—	—	—	—	62	—	—	—	—	—	—	—
59	+	07	60	08	28	—	—	—	+	—	40	52 24
—	+	+	62	08	—	—	—	30	++	—	06 58	22 23
—	—	—	—	—	62	—	—	68	+	—	06	59
—	58 17	59 15	23 25	64 25	69 28	70	43 68	33 27	++	—	29 38	35 65
—	60	52	56	62	33	—	51	39	++	—	29 71	33 27
+-	12 63	12 64	54 66	63 65	35	51	60 03	45 42	++	—	35	43 28
—	—	—	—	08	52	—	—	—	· +	+	23 70	25
27	—	—	59	09	70	—	—	—	++	—	23 27	25
34	60	—	61	60	—	—	—	—	+	33	25 48	25 28
—	—	—	—	10	26	—	—	—	+	—	27	27 29
+	—	—	—	—	—	—	—	71	++	33	23 58	24 25
—	—	—	—	—	—	—	—	44	+	—	22 30	23
—	—	—	—	+	+	—	—	40	+	—	23	25
—	—	—	—	+	—	—	—	—	+	—	23	30
56	—	—	—	—	—	—	—	—	+	—	03	22
39	—	—	23	15	71	40	08	51 44	+ -	—	30 38	27 29
—	59	61	65	60	69	—	—	70	—	—	25 70	33 23
—	—	—	—	08	—	57	—	44	—	—	57 70	26 70
27	—	—	—	17	—	—	—	40	+	—	24 48	25 68
57	—	—	—	—	—	—	—	71	+	—	—	—
33	—	—	—	—	—	—	—	—	—	33	—	—
33	—	—	—	—	57	—	—	69	++	33	+	—
39	—	—	—	08	52	—	—	39	++	—	—	—
48	60	+	—	64	70	—	—	69	+	33	70	27
03	+	+	56	59	71	—	—	40	+	33	—	70

TABLE XI

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME ALKALOIDS AND OTHER NATURA PRODUCTS

R_F values × 100						Compounds
F	E	A	B	C	D	
84	94	40	92	02	01	Aconitine[a]
79	90	35	67	08	02	Ajmaline
55	89	02	09	00	00	Boldine
66	58	47	91	61	55	Caffeine[a]
77	90	13	62	41	14	Cocaine[a]
39	82	01	22	00	00	Codeine[a]
84	95	14	19	00	00	Corynanthine[a]
61	90	04	78	02	01	Heroin[a]
80	94	28	66	05	00	Ibogaine[a]
68	92	10	25	04	00	Laudanosine[a]
23	84	00	19	00	00	Nicotine[a]
64	90	38	96	42	37	Papaverine[a]
55	90	05	87	01	00	Quinine[a]
60	91	08	82	00	00	Quinidine[a]
07	26	00	00	00	00	3-Quinuclidinol[a]
86	02	77	09	03	00	Brazilin[a]
74	00	28	27	03	00	(+)-Biotin
93	22	89	04	06	00	Cyanidin chloride
09	02	00	00	00	00	*d*-Cycloserine (*d*-4-amino-3-isoxalidone)[1]
78	00	54	59	08	01	*dl*-Desthiobiotin (5-methyl-2-imid-azolidone-4-caproic acid)[c]
48	23	04	55	00	00	Pyocyanine chloride[a]
94	90	94	92	89	88	Vulpinic acid[a]

[a] Bi reagent, positive.
[b] NH reagent, violet.
[c] DAB reagent, pale yellow.

TABLE XII

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF MISCELLANEOUS COMPOUNDS, MAINL DRUGS

R_F values × 100						Compounds
F	E	A	B	C	D	
27	78	02	08	00	00	2-Aminopyridine[a, b]
24	69	02	15	00	00	3-Aminopyridine[a]
84	82	67	08	04	00	Sulphanilamide[b]
95	92	90	96	91	81	N',N'-Diethylsulphanilamide[b]
95	94	91	92	93	89	Phenothiazine
62	87	10	83	04	02	Perphenazine[a]
77	95	62	92	39	24	Methopromazine[a]
68	30	03	58	00	00	Levomepromazine-5-sulphoxide[a]
55	93	35	95	35	31	Aminopyrine (pyramidon)[a, b]
68	72	42	93	20	09	Aminophenylpyridone[a]

[a] Bi reagent, positive.
[b] DAB reagent, pale yellow.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	—	—	+	—	—	—	—	—	—	—
—	22	—	62	62	—	—	17	—	+	33	40	—
—	23 52	52 54	24 35	60	23 69	—	—	68	+	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
—	—	—	—	—	59	—	—	—	+	—	—	—
40	—	—	—	—	+	—	—	—	+	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
04	—	—	—	—	70	—	—	40	+	33	44	27
33	—	—	—	—	—	—	—	—	+	33	—	—
—	—	—	+	09	70	—	—	—	+	-	24	70
—	—	—	—	—	+	+	—	—	+	—	—	—
38	—	—	—	—	—	—	—	—	+	—	—	—
33	+	—	—	+	—	—	-	—	+	33	—	—
—	—	—	—	—	—		—	—	—	33	—	—
06	—	—	—	—	+	17	—	68	+	—	17	—
—	—	—	—	—	—	—	—	—	+	33	05	15 20
03	59	55 65	63	63	52	—	51	59 43	++	—	58	—
—	—	—	—	—	+	—	—	39	++	33	06	17
—	—	—	—	—	—	—	—	—	—	33	05	22 20
17	59	62	23	62	—	—	—	41	+	—	—	—
02	—	—	—	—	—	—	—	—	+	—	—	—

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
34	—	—	—	—	70	—	—	—	—	33	05	22
—	—	—	—	—	—	—	—	44	+	33	07	22
—	—	—	64	06	+	—	—	—	+	—	09	19
—	—	—	—	—	60	—	—	—	—	—	08	65
34	—	—	—	—	45	+	62	38	+	—	17 40	17 23
18	—	—	—	—	+	+	17	62 39	+	33	—	—
—	—	—	—	+	70	+	24 25	28 68	++	33	26 23	23 25
—	—	—	—	—	—	—	57	+	+	—	29	27 70
+	—	—	—	—	—	—	26	68	++	—	+	17
—	—	—	08	08	62	—	58	—	+	—	08	20

RESULTS AND DISCUSSION

Due to the inclusion of a large number of amino derivatives, the two Ehrlich reagents (EH and DAC), ninhydrin (NH), Dragendorff (Bi) and DAB reagents, the latter usually recommended for hippuric acid derivatives, were used throughout. This screening was done in order to reveal some of the possible unexpected colour reactions. Generally all amino-substituted compounds, especially when the amino substitution was in the ring structure, gave a yellow colour with EH and red or magenta with DAC. The same was also the case for several acid hydrazides investigated: salicylic acid hydrazide (Table I) and 2-hydroxy-3-naphthoic acid hydrazide (Table IV). For the compounds which did not contain an amino group or in which obvious discrepancies were prevailing, positive EH reactions were obtained with *n*-propylresorcinol (Table I), 2-thiolhistidine and tyrosine methyl ester but not with phenylalanine methyl ester (Table VII). Most of the indoles (Table X) showed a red-violet type colour reaction with EH reagent; however, strong blue colours were obtained with 4-hydroxyindole, 4-hydroxy- and 6-hydroxyskatole. Few yellow colours with 2-methyl-3-ethyl-5-aminoindole, 3-indolylacetic acid hydrazide and 2-methyl-5-nitrotryptamine were registered. 6-Hydroxytetrahydraharman gave a grey colour. From Table XI it is seen that green colours were observed with ibogaine and ajmaline, and some of the phenothiazine derivatives also reacted (Table XII). Strong magenta colours were recorded for biotin and desthiobiotin with DAC, similar to the other urea derivatives (Table IX). This might prove to be useful for an analytical procedure for these above-mentioned compounds.

Besides the regular ninhydrin-positive response (red violet) from the amino acid series and several compounds from biogenic amines, yellow colours were noticed with 4-nitropyrocatechol (Table II), 5-hydroxy-1,4-naphthoquinone (Table IV) and *β*-mercaptovaline (Table VIII). Weak pink colourations were obtained with *o*-toluidine, *o*-phenylenediamine (Table VI) and 5-hydroxy-2-methylindole and harmol (Table X).

A positive DAB yellow reaction, weaker than earlier observed for the hippuric acid derivatives, was recorded for *o*-anisidine, salicylic acid hydrazide (Table I), *m*-toluidine, 2-aminobenzophenone, benzidine, 1- and 2-aminonaphthalene (Table VI), N-formyltyrosine, N-formyltryptophan, N-methyl-2-piperidinecarboxylic acid (Table VII), N-methyltaurine, *β*-mercaptovaline (Table VIII), 2-dimethylaminomethylpyrrole (Table IX), desthiobiotin (Table XI) and 2-aminopyridine, sulphanilamide and aminopyrine (Table XII).

Concerning the Dragendorff reagent which is generally used for tracing alkaloids, few other compounds outside of this group also gave a positive reaction: *o*-amino- and *p*-aminothiophenol (Table I), 8-mercaptoquinoline, 8-amino-6-methoxyquinoline (Table V), *o*-phenylenediamine, 2-aminobenzophenone, 2,7-diaminofluorene (Table VI), 1-hydroxypyridine-2-thione, *p*-urazine (Table IX), 2-methyl-3-ethyl-5-dimethylaminoindole (Table X), brazilin, pyocyanine chloride (Table IX) and 2-amino- and 3-aminopyridine, phenothiazine derivatives and aminopyrine (all in Table XII).

A detailed discussion of other colour reactions is not possible, since this series of compounds was very heterogeneous and the results should be compounded with the earlier data. For the DR reagent, it could be mentioned that comparatively unusual grey and brown colours were mainly obtained with the compounds with a free mercapto (thiol) group: for example, thiophenols (Table I), 2-thiolhistidine (Table VII),

β-mercaptovaline (Table V.II) and β-phenyl-β-propylthioethylamine (Table IX). More uncommon green colours were obtained with *m*-anisidine, salicylic acid hydrazide (Table I), 2,5-dihydroxypyridine, 2,5-dimethoxyaniline (Table III) and *o*-tolidine (Table VI). In some instances, yellow and red colours were noted with 1-hydroxypyridine-2-thione and phenylalanine methyl ester (Table VII), respectively.

For an evaluation of the structural interrelationship and the mobility of these compounds in these solvents, the general features from the earlier series were confirmed with more examples for certain types of compounds. It was mentioned in the introduction that the aim was to reveal more irregular mobility patterns which might aid in the final identification of compounds.

Firstly, the interesting irregular double-peak mobility pattern for alkaloids is discussed. When the R_F values for single compounds were presented in diagrams according to the definition for the regular pattern[5], a gradual decrease in R_F values is expected from solvents F to D (F > E > A > B > C > D) for neutral compounds. This continuous decrease has been associated with the decrease in hydrophilic character in these solvents. Alkaloids, which are basic compounds in general, exhibit not only the first criteria set for simple basic compounds, *e.g.* the R_F values will be elevated in solvent E compared to the values obtained in solvents F and A. They also show another peak (R_F maximum) in solvent B, compared to the R_F values in solvents A and C. This sequence in the mobility F < E > A < B > C > D seemed to be sufficiently characteristic for alkaloids in these solvent systems. Since the alkaloids are in turn a very heterogeneous group of compounds containing quinoline, isoquinoline, pyridine, indole and phenanthrene nuclei, for example, one would expect that there must be differences in the mobility influenced by the variation in the parent ring in a complex molecule. Nevertheless, the main characteristics (double peak in R_F value pattern) still hold despite the nature of the parent ring system involved. The more distinct differences in the mobility caused by the variation in the parent ring are best seen in solvent F in which some groups of alkaloids have their R_F values systematically lower than 0.50; for the others the R_F values are predominantly above R_F 0.50. A similar situation also occurs for solvent A, but the margin for the R_F value changes seems to be from medium to low R_F values. The R_F values for solvents C and D are usually close to zero, with few exceptions higher (papaverine, cocaine) but not higher than R_F 0.50 in solvents C and D. In solvents E and B, in which the double peak was accentuated, the R_F values are over 0.60 (in E) and for solvent B there is a broad region distinguishing the individual compounds (R_F changes from 0.10 to 0.95).

This seemingly analogous double peak also occurred when amino- and diaminobenzene, diaminonaphthalene and aminomethoxybenzene derivatives were run in these solvents. The main difference between these types of purely synthetic compounds and natural products of alkaloid series was that for the majority of aminobenzenes the R_F values practically in all solvents were over R_F 0.50.

When the R_F values of aminobenzenes were less than 0.50 in solvent A and the values were very low in solvents B, C and D, a few directly overlapping patterns with alkaloid-type compounds were observed. The patterns of the phenylenediamine derivatives resembled those obtained with the compounds from harman series and some biogenic amines (simple alkaloids).

Remembering that all the aminobenzene derivatives always give strong EH

and DAC reactions (contrary to the alkaloid-type compounds) and are mainly synthetic products (few drugs: methopromazine, perphenazine, pyramidon, Table XII, also with similar patterns and Ehrlich positive), they are not normally considered to interfere with the search and characterisation of alkaloids by using the mobility criteria alone.

For identification useful irregularities in the mobility were also observed for biotin and desthiobiotin. Both these compounds show an elevation of their R_F values in solvent B (compared to the values in solvents A and C). Mobility patterns in solvents F, E and A are in accordance with a typical acidic compound (presence of free carboxyl group).

The regular mobility pattern for neutral compounds in combination with the irregularity between solvents B and C ($B < C > D$) is another valuable criterion for sorting out hydroxylated indoles. This remarkable shift of the R_F values found earlier[3] for 5-hydroxyindole is also valid, for example, for 4-hydroxyindole and 4-hydroxy-, 5-hydroxy- and 6-hydroxy-3-methylindole. The selection based on the mobility could then be confirmed by a positive Ehrlich reaction for indoles. It is good to remember in this connection that a similar shift even occurs with 1,3- and 1,4-dihydroxyphenols and with all naphthalenediols, except for 2,3-diol[1,2,4]. 3-Indolylacetic acid hydrazide also gives a similar shift (Table X), but the R_F value in solvent D is very low and the reaction with Ehrlich is yellow.

Aliphatic amino acids are not expected to move in these solvents, except in solvent F, in which the region for 2-carbon amino acid (glycine) and 6-carbon amino acid (norleucine) is between the R_F values 0.03–0.36 (Table VIII).

For the compounds of the monohydroxypyridine, monohydroxyquinoline and isoquinoline series, investigated earlier, an elevation of their R_F values in solvent B (irregularity $A < B > C$) was observed. With three examples from Tables II and III, it is shown that dihydroxypyridines loose this characteristical R_F value shift. On the other hand, 1,3-dihydroxyisoquinoline (Table V) retains this qualitative difference. On the basis of these findings, it could be predicted that trihydroxyisoquinoline very probably also looses this shift; no examples are available.

The complicated interactions with the separated compounds and the solvent systems are best illustrated by three compounds from Table V. These three homologues (6-methoxy-, 7-methoxy- and 8-methoxy-4-hydroxy-3-quinolinecarboxylic acid) show, at the beginning with the 6-methoxy derivative, a regular pattern for an acidic compound with relative medium R_F value in solvent B. For the 7-methoxy derivative, the regular pattern is the same, but the R_F value in solvent B is considerably elevated. For the third compound, the 8-methoxy derivative, the mobility pattern is an irregular one with a further increase of the R_F value in solvent B, giving an $A < B > C$ irregularity. This example clearly shows the difficulties in interpreting the influence of the positional effect of the substitution on the mobility, and here only one (comparatively neutral substituent) of the three substituents is shifted from one position to another.

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