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

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INTRODUCTION

Further paper-chromatographic data, to those already presented[1,2] for approximately 700 compounds mainly belonging to the series of phenol derivatives, hydroxylated heterocyclic compounds, *e.g.* indole derivatives, metabolites of aromatic amino acids, products of vegetable and mould origin and some compounds of pharmacological interest, are presented in this paper.

As before, six solvent systems were used throughout, but instead of ten standard colour reagents for identification purposes, the number of colour reagents was increased to twelve, including two Ehrlich reagents, in order to cover the colour reactions of a number of amino derivatives of the above-mentioned compounds studied in the present investigation. The systematic presentation of compounds is somewhat more heterogeneous than previously, the main reason being that some of the available sources have been exhausted. The data were obtained and recorded under as uniform working conditions as possible in order to be comparable with the information presented earlier. The main reason for this investigation, in addition to providing the characteristics of each compound in a given system, has been to evaluate more closely the connection between the chemical structure of the compounds and their different colour reactions, and the relationship between the structure and R_F values in different solvent systems. In general the values are in agreement with previous findings, with few exceptions so far, and the recorded data fit the picture fairly well, as regards the 1,2-, 1,3- and 1,4-dihydric phenol derivatives, which give characteristic colour patterns. The chromatographic behaviour of monohydroxylated pyridines, quinolines and more complex ring systems such as phenothiazines and acridines show a typical maximum of R_F values in solvent B, when compared with solvent A; this seems to hold as a general rule. The alkaloids investigated in the previous paper behave similarly in this respect. The R_F values and the colour code provide a method for distinguishing between these heterocyclic types of compounds.

* For previous papers in this series, see refs. 1 and 2.

MATERIALS AND METHODS

For one-dimensional descending chromatography rectangular glass jars of size 20 × 30 × 60 cm were used. Whatman No. 1 filter paper of dimensions 24 × 48 cm was used throughout the experiments and the composition of the six solvent systems is given in the section "Abbreviations used in the Tables".

Spray reagents

The following twelve standard spray reagents were used to detect the compounds:

Diazotized sulphanilic acid (obtained from Th. Schuchart Co., Munich); diazotized 4-benzoylamino-2,5-dimethoxyaniline (Light Chemicals Co., England); diazotized *o*-dianisidine (Light Co.); *p*-nitrobenzenediazonium fluoborate (Eastman Kodak, U.S.A.); 2,6-dibromoquinone-4-chloroimide (British Drug Houses Ltd., England); 2,4-dinitrophenylhydrazine; ferric chloride; phosphomolybdic acid; potassium permanganate; bromophenol blue; *p*-dimethylaminobenzaldehyde; and *p*-dimethylaminocinnamaldehyde (Heidenheimer Chemisches Laboratorium, Heidenheim-Brenz, Germany). For the composition of these reagents, see the section "Abbreviations used in the Tables".

Additional reagents

For detection of indoles the following three reagents (Frinton Laboratories Inc., Vineland, N.J.) were tried out: *p*-N,N-bis(2-chloroethyl)-aminobenzaldehyde (abbreviated as E3), 2-chloro-4-N,N-bis(2-chloroethyl)-aminobenzaldehyde (E4) and 4-N,N-bis(2-chloroethyl)-amino-2-tolualdehyde (E5). The best results with respect to colour development were obtained by dissolving these compounds in conc. HCl. Reagents E3 and E5 behaved similarly giving red-violet or blue-violet colours, although there were a few marked differences with certain compounds. Reagent E4 gives predominantly blue to green shades. A comparison of these reagents with the reagents EH and DAC used earlier is given in Table XVI. However, some caution should be exercised in interpreting the colour differences as these reagents react very rapidly in this medium and in most cases ran through many shades in a few seconds. They should therefore be tested together with a number of known compounds, when applying them to unknown substances. Consequently, the values in Table XVI serve only as guiding values among several other possibilities, it being almost impossible to record them all. Reagent E4 should be prepared immediately before use, in order to avoid background colorization. When these three reagents were dissolved in 1 *N* HCl colour development was too slow.

For detecting aldehydes, a number of aromatic amines were tested. The overall picture is presented in Table XVII. Primary aromatic amines are known to condense in acidic solution with aldehydes forming coloured compounds, *e.g.* Schiff bases[3,4]. The formation of coloured Schiff bases was instantaneous in most cases, and any colour changes with time were recorded as indicated in the footnote to Table XVII. No heating was required to develop the colours. The freshly recrystallized amines were made up as a 1 % solution in 2 *N* HCl prior to spraying.* In the cases of benzidine, *o*-tolidine and *o*-dianisidine, saturated solutions in 2 *N* HCl were used. The following

* Caution in handling of aromatic amines, see ref. 11.

amines were used for the detection and recording of the colour sequences given in Table XVII: *o*-phenylenediamine, *m*-phenylenediamine, *p*-phenylenediamine, 2,4-diaminophenol, *p*-anisidine, *o*-toluidine, *p*-toluidine, α-naphthylamine, β-naphthylamine, benzidine, *o*-tolidine, dimethyl-*p*-phenylenediamine, *p*-aminoacetophenone, 4-amino-antipyrine, 1,2-diaminonaphthalene and 2,7-diaminofluorene. Yellow, orange, red and brown colours were generally obtained. *o*-Phthalaldehyde gave predominantly grey and green colours, except with *o*-toluidine where a transient red-violet colour was observed. Another red-violet coloration was obtained when 1,2-diaminonaphthalene reacted with cinnamaldehyde.

The following compounds were also tested under similar conditions, but are not recorded in the tables. However, some useful information is given below, when marked differences were noted.

Phenylhydrazine. This reagent gave green colours with terephthalaldehyde, cinnamaldehyde, salicylaldehyde, 3-hydroxybenzaldehyde, and 2,4-dihydroxybenzaldehyde and grey colours with piperonal and 4-methoxybenzaldehyde.

N-(1-Naphthyl)-ethylenediamine. This reagent gave a red-violet colour with *o*-phthalaldehyde and was rose pink with cinnamaldehyde and *o*-veratraldehyde.

Azobenzene-phenylhydrazinesulphonic acid. The reaction with 3-indolealdehyde and 2,4-dihydroxybenzaldehyde was green.

1,5-Diaminonaphthalene. There was a rose-pink colour with coniferylaldehyde.

2,3-Diaminonaphthalene. A brown coloration was given with 4-acetoxy-5-methoxy-isophthalaldehyde and 2,5-dihydroxybenzaldehyde.

2,7-Diaminonaphthalene. This reagent was green with *o*-phthalaldehyde, brown with terephthalaldehyde, and carmine with 4-acetoxy-5-methoxy-isophthalaldehyde.

3,3'-Diaminobenzidine. Orange colours were given with 3-indolealdehyde and 2,5-dihydroxybenzaldehyde; with coniferylaldehyde the colour was rose-pink and brown with cinnamaldehyde and 4-acetoxy-5-methoxy-isophthaldehyde.

2,6-Dibromoquinone-4-chloroimide (DB) reagent was found to be useful for the detection of phenylthiohydantoin (PTH) derivatives of amino acids (Table XV), producing red and brown colours, and in some cases it enabled one to distinguish between different PTH-derivatives. The sensitivity of the reagent seemed to be acceptable; furthermore it was easy to prepare, and the spots appeared almost instantaneously.

For detecting hippuric acid derivatives a 2 % solution of recrystallized *p*-diethylaminobenzaldehyde (DAB) in acetic anhydride was used[2,5] and the results are presented in Table XIII. In a few cases weak colorations were observed with compounds other than the hippuric acid derivatives described in this paper. Pale yellow colours were produced by: 3,4,5-trimethoxy-aniline, β-3,4-dimethoxyphenyl-ethylamine, 2,3-diaminopyridine, 2-quinoline-4-carboxylic acid, 3-indoleacetic acid methyl ester, 5-methoxygramine and N-methylanthranilic acid. Compounds such as 5-hydroxy-3-indoleacetamide, N-methyltryptamine, N,N-diethyltryptamine, 5-fluorotryptamine, tryptophol, tryptophan methyl ester and ergotamine, after treatment with DAB reagent, showed yellow colours only under U.V. light.

The general paper-chromatographic procedure followed essentially the same principles as described earlier[1]. The colour reactions were recorded as numbers, following the "Derwent" coloured pencils index of Cumberland Pencil Co., Keswick, England. The descriptions of these colours is found under the heading "Colour index for the Tables".

The compounds listed in the tables, except the PTH- and DNP-amino acid derivatives, were mainly obtained from commercial sources.

The hippuric acid derivatives listed in Table XIII were synthesized by the author using the methods of SHEEHAN AND HESS[7] and ITO AND NEILANDS[8], whereby glycine ester is condensed with an aromatic acid, in the presence of dicyclohexyl-carbodiimide, and the ester is then hydrolysed to yield the corresponding aroylglycine.

GUIDE TO TABLES I–XV

Tables I–XIII present the information compiled for approximately 170 organic compounds, investigated by the procedure outlined above. Tables XIV and XV give only the R_F values for 35 amino acid derivatives. The R_F values are recorded in six different solvent systems 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; in addition, the colour produced in ultraviolet light is indicated in the first column under this heading. The amount of substance used in these experiments was 25–50 γ per spot, which produced different colours when the reagents were applied. These colours are referred to by numbers, the explanation of which is found in the colour index. For abbreviations and the composition of the reagents, the reader is referred to the following section.

Table I	Monohydric phenols and their derivatives.
Table II	1,2-Dihydric phenols and their derivatives.
Table III	1,3-Dihydric phenols and their derivatives.
Table IV	1,4-Dihydric phenols and their derivatives.
Table V	Trihydric phenols.
Table VI	Naphthalene and quinoline derivatives.
Table VII	Benzoic and phthalic acid derivatives.
Table VIII	Aromatic non-phenolic and heterocyclic monocarboxylic acids with the COOH group in the side chain, and their derivatives.
Table IX	Aliphatic and heterocyclic amino acid derivatives.
Table X	Biologically active nitrogen compounds, *e.g.* biogenic amines etc.
Table XI	Indole derivatives.
Table XII	Miscellaneous compounds.
Table XIII	Hippuric acid derivatives.
Table XIV	2,4-Dinitrophenyl derivatives of amino acids.
Table XV	Phenylthiohydantoins of amino acids.

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, 0.88 etc. The colours produced by the reagent are recorded as numbers and the corresponding shades can be found in the colour index. The —sign indicates an uncertain reaction, which was too weak to deserve colour estimation. Reactions with the reagents Mn and Ind are only indicated by the signs: —, + or ++. The ++ sign means that a positive reaction was obtained immediately. In the few cases where the colours are recorded by a number placed on top of another number, *e.g.* $\left.\begin{matrix}24\\57\end{matrix}\right\}$, this indicates that immediately upon spraying a violet spot (24 in the colour index) appears which, within a few seconds, turns to light brown (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 not 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.

The various compounds listed in the tables have been arranged as far as possible according to the following principles:

(1) Free phenols together with their methoxy- and alkyl-derivatives; (2) the corresponding amino- and nitro-derivatives; and (3) further derivatives in the order: hydroxymethyl, keto, aldehyde and finally monocarboxylic acid derivatives, derivatives with a carboxylic acid group in the side chain and dicarboxylic acids.

ABBREVIATIONS USED IN TABLES I–XVII

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 of chloroform saturated with a mixture of 1 part methanol and 1 part 4 % formic acid)
C = Benzene–ethyl methyl 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 = Ethyl methyl ketone–diethylamine–water (921 : 2 : 77 parts by volume)
F = Ethyl methyl ketone–acetone–formic acid–water (40 : 2 : 1 : 6 parts by volume)

Reagents used for detection

U.V. = Ultraviolet light
D1 = Diazotized sulphanilic acid (0.3 % solution in dioxane–water 1 : 2)
D2 = Diazotized 4-benzoylamino-2,5-dimethoxyaniline (0.6 % solution in dioxane–water 1 : 2)
D3 = Diazotized *o*-dianisidine (0.6 % solution in dioxane–water 1 : 2)
D4 = *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)

Special reagents used for detection

E3 = *p*-N,N-Bis-(2-chloroethyl)-aminobenzaldehyde (2 % in conc. HCl)
E4 = 2-Chloro-4-N,N-bis-(2-chloroethyl)-aminobenzaldehyde (2 % in conc. HCl)
E5 = 4-N,N-Bis-(2-chloroethyl)-amino-2-tolualdehyde (2 % in conc. HCl)

COLOUR INDEX FOR THE TABLES

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

01	Zinc Yellow	25	Dark Violet	49	Sap Green
02	Lemon Cadmium	26	Light Violet	50	Cedar Green
03	Gold	27	Blue Violet Lake	51	Olive Green
04	Primrose Yellow	28	Delft Blue	52	Bronze
05	Straw Yellow	29	Ultramarine	53	Sepia
06	Deep Cadmium	30	Smalt Blue	54	Burnt Umber
07	Naples Yellow	31	Cobalt Blue	55	Vandyke Brown
08	Middle Chrome	32	Spectrum Blue	56	Raw Umber
09	Deep Chrome	33	Light Blue	57	Brown Ochre
10	Orange Chrome	34	Sky Blue	58	Raw Sienna
11	Spectrum Orange	35	Prussian Blue	59	Golden Brown
12	Scarlet Lake	36	Indigo	60	Burnt Yellow Ochre
13	Pale Vermilion	37	Oriental Blue	61	Copper Beech
14	Deep Vermilion	38	Kingfisher Blue	62	Burnt Sienna
15	Geranium Lake	39	Turquoise Blue	63	Venetian Red
16	Flesh Pink	40	Turquoise Green	64	Terra Cotta
17	Pink Madder Lake	41	Jade Green	65	Burnt Carmine
18	Rose Pink	42	Juniper Green	66	Chocolate
19	Madder Carmine	43	Bottle Green	67	Ivory Black
20	Crimson Lake	44	Water Green	68	Blue Grey
21	Rose Madder Lake	45	Mineral Green	69	Gunmetal
22	Magenta	46	Emerald Green	70	French Grey
23	Imperial Purple	47	Grass Green	71	Silver Grey
24	Red Violet Lake	48	May Green	72	White = colourless

TABLE I

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

R_F values × 100						Compounds	Detection											
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
95	93	95	95	95	94	*o-tert.*-Butylphenol	—	07	08	63 56	08 24	43 35	—	—	43 68	++	—	—	—
95	93	94	95	95	94	2,6-Di-*tert.*-butylphenol	—	17	17	17	09 28	28 25	—	—	43	+	—	—	—
89	85	84	75	64	30	*o*-Acetaminophenol	—	06 10	09 15	19 65	07 23	41 37	—	26 70	69	43 66	—	—	—
87	84	79	20	17	02	*m*-Acetaminophenol	—	06 09	15	19 15	09 15	38 35	—	70	71	++	—	03	+
83	81	72	09	07	00	*p*-Acetaminophenol	—	14	12 64	63 65	62 65	—	—	27	30	++	—	—	60
34	85	13	16	00	00	*p*-Methylaminophenol	56	—	—	+	63	53	—	+	68	++	—	52	21
40	82	10	12	03	00	4-Amino-3-methylphenol	55	—	—	64	07	—	—	—	69	++	—	07	21
92	65	92	56	57	08	2-Amino-4-nitrophenol	55	+	58	55	58	55	—	60	43 68	++	—	06	23
94	89	92	56	57	08	2-Amino-5-nitrophenol	59	63	63	55 65	55	21 63	—	55	55 69	++	—	15 12	25 28
91	82	87	86	78	70	4-Amino-2-nitrophenol	55	56	62	63	64	—	—	—	63 68	++	—	09	19
49	86	27	79	24	17	*p*-Anisidine	70	08	59	65 63	59	—	—	24	50 53	++	—	09	19
72	88	48	93	53	41	*o*-Dianisidine	56	63	—	—	56	50	—	43 68	53	++	—	15 20	23 65
92	90	94	90	92	86	4-Nitro-*o*-anisidine	65	—	—	—	—	—	—	—	—	+	—	12	65 25
92	91	95	91	92	86	5-Nitro-*o*-anisidine	65	—	—	—	—	—	—	—	—	+	—	07	19
93	91	92	92	89	86	2-Nitro-*p*-anisidine	64	—	—	—	—	57	—	—	—	+	—	11	24 25
92	91	87	43	59	22	5-Hydroxyindole	—	64	55 65	24 25	56 63	28	16	55	43 68	++	—	23	43 28
96	53	94	93	89	88	2-Nitro-3-hydroxyaceto-phenone	57	—	—	—	+	—	+	—	—	—	—	—	—
95	91	93	89	88	68	Salicylaldoxime	—	—	—	63	—	03 41	—	54	68	++	—	—	—
92	79	86	76	69	44	Salicylamide	33	07	08	65	10	41	—	23	—	++	—	06	21
95	65	93	88	87	66	5-Bromosalicylic acid	34	—	—	—	—	—	—	24	—	+	++	—	—
93	67	87	76	77	24	5-Nitrosalicylic acid	26	—	—	—	—	—	—	64	—	—	++	—	—
89	92	91	84	87	71	3-Hydroxybenzoic acid methyl ester	—	08	62	63 65	12	43	—	+	—	++	—	—	—
92	06	92	83	73	26	3-Hydroxy-4-nitrobenzoic acid	59	+	08	56	63	48	—	+	08 34	+	+	—	57
94	93	93	74	76	15	3,5-Dichloro-4-hydroxy-benzoic acid	—	—	—	—	08 63	40	—	57	—	++	+	—	—
81	00	48	00	00	00	5-Hydroxyanthranilic acid	34	07	—	24 65	03 64	—	—	—	34	+	+	—	06
93	14	89	88	87	84	*o*-Methoxyphenylacetic acid	—	—	—	—	—	—	—	—	—	—	+	—	—
91	05	87	19	22	01	*m*-Hydroxyphenylacetic acid	—	06 09	14	15 65	08 65	37 25	—	—	71	++	+	—	—
93	10	88	37	41	03	*m*-Hydroxyphenylpropionic acid	—	06 10	12	65	09 65	38 25	—	—	30 71	++	+	—	—
95	16	94	91	90	76	*o*-Methoxycinnamic acid	33	—	—	—	—	—	—	—	—	++	+	—	—
95	14	94	90	84	69	*m*-Methoxycinnamic acid	33	—	—	—	—	—	—	—	—	++	+	—	—
85	04	57	02	02	00	*dl-p*-Hydroxymandelic acid	—	08	12 64	65	06 65	38 25	—	03	71	++	+	—	—
87	62	78	76	39	08	*o*-Methoxymandelic acid	—	—	—	—	—	—	—	58	—	+	++	—	—

(continued on p. 481)

TABLE I (*continued*)

R_F values × 100						Compounds	Detection												
F	*E*	*A*	*B*	*C*	*D*		*U.V.*	*D1*	*D2*	*D3*	*D4*	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
90	18	78	68	46	05	*m*-Methoxymandelic acid	—	—	—	—	—	—	—	+	—	+	++	—	—
92	09	76	54	35	04	*p*-Methoxymandelic acid	—	—	—	—	—	—	—	—	—	+	+	—	—
93	91	89	75	63	25	5-Hydroxy-3-indolylacetic acid methyl ester	—	17	+	+	+	—	—	—	—	—	—	34	30 27
91	10	91	71	53	11	5-Methoxy-3-indolylacetic acid	—	03	—	+	07	—	60	26 23	44 71	++	+	26 27	27 30
68	57	20	01	00	00	5-Hydroxy-3-indolyl-acetamide	+	64	23	23 65	64	68	—	70	71	++	—	51	25 28
88	00	83	00	00	00	5-Hydroxyisophthalic acid	—	09	—	63	60	38 25	—	+	—	+	+	—	—
91	76	88	88	88	87	2-Chloro-6-hydroxypyridine	—	—	—	—	—	—	—	57	—	—	—	—	—
72	64	58	68	24	15	2-Mercaptopyridine	63	—	—	62	—	08 63	—	—	51 69	++	—	—	—
45	15	12	14	00	00	4-Mercaptopyridine	03	05	09	11	06	59	—	—	45 68	++	—	—	—

TABLE II

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

R_F values × 100						Compounds	Detection												
F	*E*	*A*	*B*	*C*	*D*		*U.V.*	*D1*	*D2*	*D3*	*D4*	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
94	94	92	86	86	66	4-Isopropylpyrocatechol	—	59 09	24	56 24	64 65	28	+	+	43 68	++	—	17	17
93	69	93	88	83	75	3-Nitropyrocatechol	56	07 19	63	65	63 68	68	—	45	69 09	+	—	03	58
90	04	82	59	52	12	3-Methoxy-4-hydroxyphenyl-acetic acid = homovanillic acid	—	59 17	—	—	63	33	—	—	69	+	+	—	—
91	04	79	59	28	05	3-Hydroxy-4-methoxy-cinnamic acid	30	09 56	15 08	63 65	11 55	38 70	—	57	35 69	++	+	—	—
87	01	76	13	05	00	3,4-Dihydroxyphenyl-propionic acid = hydrocaffeic acid	—	06 64	23 19	23 19	09 65	25	—	53	43 68	++	+	—	—
60	00	25	00	00	00	*dl*-3,4-Dihydroxymandelic acid	—	58 17	17	17	08 65	28	—	43	68	++	+	—	—
77	03	47	04	02	00	3-Hydroxy-4-methoxy-mandelic acid	—	07 10	08 15	11 25	13 65	38 35	—	58	71	+	+	—	—
70	03	48	05	04	00	4-Hydroxy-3-methoxy-mandelic acid	—	63	60	+	24 03	38 28	—	—	69	+	+	—	—

TABLE III

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

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
87	32	67	16	11	00	4-Nitrosoresorcinol	56	08 56	23 62	65	10 65	+	—	—	69	++	—	—	—
64	05	32	17	04	00	2,4-Dinitrosoresorcinol	55	58	58	59	+	52	+	43	43	++	—	—	—
89	80	90	88	87	68	2,4-Dihydroxypropiophenone	34	08 56	56 64	23 65	09 65	06 28	06	23 65	34 71	++	—	—	—
94	20	92	36	49	03	5-Bromo-2,4-dihydroxybenzoic acid	—	06 59	19 63	24 65	12	—	—	24 27	—	++	+	—	—
94	35	94	92	90	71	4-Ethoxy-2-hydroxybenzoic acid	—	56 08	62	64	09	—	—	23	+	+	+	—	—
92	24	89	90	85	74	2,4-Dimethoxybenzoic acid	—	—	—	—	—	—	—	—	—	—	+	—	—
58	06	26	81	04	04	2,6-Dihydroxypyridine	40 56	06 10	65 63	66	60 55	43 28	—	55	52 68	++	—	18 21	05 52
62	00	10	00	00	00	2,6-Dihydroxyisonicotinic acid	40	06 08	14	14 62	06	25	—	—	+	+	+	—	—

TABLE IV

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

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
94	85	92	46	61	12	2-Bromohydroquinone	—	—	56	60	59	—	—	—	41 69	+	—	—	—
87	84	81	93	83	78	2,6-Dimethoxy-1,4-benzoquinone	57	—	—	—	—	—	—	—	—	—	—	—	—
92	88	89	88	85	83	2,5-Dimethoxybenzoic acid	+	—	—	—	—	—	—	—	—	—	++	—	—
73	05	42	08	04	02	3,6-Dihydroxyphthalimide	40	07	11	11	60	56	—	51	69 03	++	—	—	—

TABLE V

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

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
78	08	62	00	02	00	1,2,4-Trihydroxybenzene	56	+	+	56	56	56	55	54	68 55	++	—	+	27 44
89	39	81	56	26	06	3,4-Dihydroxy-5-methoxybenzaldehyde	—	57	59	60	59	43 70	09	70	43 68	++	—	—	—
85	41	64	03	01	00	2,4,6-Trihydroxybenzoic acid	—	10	60 63	23 25	12 65	26 70	—	25 26	39 70	++	++	18 23	30 25
38	03	77	75	28	06	3,5-Dimethoxy-4-hydroxycinnamic acid = sinapic acid	38	15 23	63	25 65	14 62	25	—	21	52 28	++	+	—	—
75	83	52	94	51	35	3,4,5-Trimethoxyaniline	+	58	58	65 64	64	71	—	—	52 70	++	—	07	19
08	00	00	00	00	00	Tetrahydroxy-*p*-benzoquinone	+	—	58	—	—	—	—	—	70	—	—	—	—

TABLE VI

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

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
95	94	83	77	06	04	1-Amino-2-naphthol	57	60	56	56	56	—	23	56	53	++	—	06	64
88	91	80	55	36	09	2-Amino-5-naphthol	33	12, 23	23, 65	65	65, 64	63, 28	—	56	68	++	—	08	30, 24
93	09	90	53	37	01	2-Hydroxy-6-naphthoic acid	33	13	24, 65	23, 65	12, 15	43, 63	—	60	30	++	+	—	—
94	36	92	33	36	01	3,5-Dihydroxy-2-naphthoic acid	06	15, 23	24	25, 28	64, 68	63, 29	—	28, 69	43, 68	++	+	05	—
57	85	29	56	07	01	5-Hydroxyisoquinoline	34	12, 15	20	23, 65	64, 55	35, 28	—	71	68	++	—	—	—
84	68	68	25	06	00	1,5-Dihydroxyisoquinoline	34	17, 15	63, 25	25, 28	15, 65	39	—	26	68	++	—	—	—
79	00	37	08	05	00	2-Hydroxyquinoline-4-carboxylic acid	34	—	—	—	—	—	—	—	—	—	+	—	—

TABLE VII

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME BENZOIC ACID DERIVATIVES

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
95	90	85	84	88	83	*m*-Nitrobenzyl alcohol	27	—	—	—	—	—	—	—	—	+	—	+	—
94	93	93	93	93	93	*o*-Aminoacetophenone	39	—	—	—	—	—	+	—	34	+	—	08	24
90	89	81	91	74	66	*m*-Aminoacetophenone	57	08	—	64	62	—	07	—	+	+	—	06	19
93	92	85	92	78	70	*m*-Aminobenzaldehyde	—	—	—	—	—	—	06	—	—	+	—	06	22
96	94	93	95	93	92	*o*-Nitrobenzaldehyde	+	—	—	—	—	—	06	—	—	+	—	04	23
94	58	92	88	87	86	Thiobenzoic acid	—	+	60	+	+	64	—	65, 72	69	++	+	—	—
91	39	82	84	84	65	2,6-Dichlorobenzoic acid	—	—	—	—	—	—	—	—	—	—	++	—	—
89	06	80	49	24	05	*p*-Hydrazinobenzoic acid	39	—	59	63, 56	—	—	—	—	—	+	—	05	17
94	31	86	93	86	84	N-Methylanthranilic acid	33	06	—	12, 62	08	38	—	26, 51	45, 50	++	+	07, 03	21, 15
94	14	92	87	78	50	2-Amino-4-methylbenzoic acid	33	08	—	64	12	45	—	—	+	+	—	07	24
94	18	91	84	78	52	2-Amino-5-methylbenzoic acid	33	06	—	62	08	60	—	58	30	++	+	09	19, 65
90	04	80	66	33	07	3-Amino-4-methylbenzoic acid	34	08	—	64	12	43	—	—	—	+	—	05	22
91	06	88	68	46	15	4-Amino-3-methylbenzoic acid	—	08	—	64	10	—	—	—	—	+	—	06	23
93	15	89	92	85	58	3,5-Dimethylanthranilic acid	39	—	—	64	06	—	—	—	+	+	—	—	+
88	86	33	06	03	00	3,4-Diaminobenzoic acid	38	+	58	23, 56	59	56	—	63, 56	69	++	—	09	23, 25
92	48	89	67	79	24	2,5-Dinitrobenzoic acid	26	—	—	—	—	—	—	—	—	—	++	58	—
85	02	83	45	07	00	3-Nitrophthalic acid	26	—	—	—	—	—	—	—	—	—	+	—	—

TABLE VIII

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME AROMATIC NON-PHENOLIC AND HETEROCYCLIC MONOCARBOXYLIC ACIDS WITH THE COOH GROUP IN THE SIDE CHAIN, AND THEIR DERIVATIVES

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
94	19	93	90	88	82	4-Chlorocinnamic acid	—	—	—	—	—	—	—	—	—	+	+	—	—
93	42	91	75	67	12	4-Bromomandelic acid	—	—	—	—	—	—	—	—	—	+	+	—	—
88	06	90	79	61	18	3-Methyl-3-phenylglutaric acid	—	—	—	—	—	—	—	—	—	—	+	—	—
34	01	07	34	00	00	3-Pyridylacetic acid	—	—	—	—	—	—	—	—	—	—	+	—	—
00	03	00	00	00	00	4,5-Imidazole-dicarboxylic acid	—	—	—	—	+	—	—	—	—	—	+	—	—
05	00	00	00	00	00	Imidazole-4-acetic acid	34	15 60	62	64	08 65	—	—	—	—	—	+	—	—
06	00	00	00	00	00	1-Methylimidazole-4-acetic acid	—	—	+	56	+	—	—	—	—	—	+	—	—
08	00	00	00	00	00	Dihydrourocanic acid	—	15	60	65	07	—	—	—	—	—	+	—	—
87	73	82	44	34	09	Pyrrole-2-carboxylic acid	—	07 11	64	23	12 64	—	—	62	—	+	+	22 23	39 69
93	94	84	88	87	84	3-Indolylacetic acid methyl ester	+	—	60	60	08	—	—	—	—	+ +	—	18 69	24 25
88	08	86	18	24	05	3-Indolylglyoxylic acid	—	—	—	56	—	+	03	64	03 72	—	+ +	—	—
91	07	00	23	28	04	3-Indolylpyruvic acid	03	58	62	63	+	63	+	24	69	+	+	17	23 65

TABLE IX

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

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
08	00	00	00	00	00	DL-γ-Aminobutyric acid	—	—	—	—	—	—	—	—	—	—	30	—	—
02	00	00	00	00	00	DL-Methionine sulfoxide	—	—	—	—	—	—	—	—	—	—	33	05	—
20	01	00	00	00	00	DL-Ethionine	—	—	—	—	—	—	—	—	—	+ +	—	02	—
00	00	00	00	00	00	Cysteic acid	—	—	—	—	—	—	—	—	—	—	+	04	—
87	55	67	60	10	01	N^a-Acetylkynurenine	39	—	—	—	—	—	—	—	70	+	+	08	23
38	00	03	01	00	00	Hydantoin-5-acetic acid	—	—	—	—	—	—	—	—	—	—	30	—	—
05	00	00	00	00	00	N-Acetyl-L-histidine	—	15 08	62	68 56	07 24	—	—	—	—	—	—	—	—
59	00	07	04	00	00	N-Acetyl-L-glutamic acid	—	—	—	—	—	—	—	—	—	—	+	—	—

TABLE X

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

R_F values × 100						Compounds	Detection											
F	E	A	B	C	D		U.V.	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
85	82	73	52	21	05	N-Acetyltyramine	—	07	60	64	23 08	—	—	—	68	+	—	—	—
06	15	00	00	00	00	N-Acetylhistamine	—	15 08	62	64 56	07 24	—	—	—	—	—	—	—	—
00	02	00	00	00	00	1-Methylhistamine	34	—	57	56	+	—	—	—	—	—	—	—	—
52	82	04	25	00	00	*dl*-Desoxyephedrine*	—	72	—	—	—	—	—	—	—	—	—	—	—
87	94	63	94	47	30	Cinnamylephedrine*	—	—	—	—	—	—	—	—	—	+	—	—	—
42	85	07	11	05	02	β-(*p*-Methoxyphenyl)-ethylamine	34	58	60	07 64	08 63	—	—	58	58 71	+	33	20	60 14
12	69	00	00	00	00	β-(3-Methoxy-4-hydroxy-phenyl)-ethylamine	—	17	59	59	58 25	—	—	—	68	++	—	—	—
23	05	02	03	00	00	β-(3,4-Dimethoxyphenyl)-ethylamine	—	06	—	58 60	06 60	23 56	—	57	47	+	—	05	57 08
21	07	00	00	00	00	Noradrenalone	—	57 18	57 18	60	57	52	+	43	68	++	—	05	62
27	04	00	00	00	00	*dl*-Metanephrine	—	59 09	12	60 64	06 23	35	—	71	30	++	—	—	—
15	71	00	00	00	00	*dl*-Normetanephrine	—	59	10	56 23	58 23	35	—	—	30	+	—	—	—
36	14	00	00	00	00	2,3-Diaminopyridine	33	08 59	56	56	19 63	23	—	60	69	+	—	06	23
11	02	00	00	00	00	3-Hydroxy-N-methyl-piperidine	—	—	—	—	—	—	—	—	—	+	—	—	17
49	33	13	02	00	00	Thiourea	—	—	—	60	58	24	—	—	71	++	—	05	—
35	25	08	03	00	00	Biuret = carbamylurea	—	—	—	—	—	—	—	—	—	—	—	05	17

* Detected by $KI \cdot BiI_3$ reagent, see ref. 2.

TABLE XI

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME INDOLE DERIVATIVES

R_F values × 100						Compounds	Detection											
F	E	A	B	C	D		U.V.	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
37	35	00	00	00	00	Indican	—	—	—	55	08	25	—	—	—	+	—	63	26 23
92	91	87	43	59	22	5-Hydroxyindole	—	64	55 05	24 25	56 63	28	16	55	43 68	++	—	23	43 28
59	08	07	07	00	00	Gramine-N-oxide	—	—	—	64	07	+	17	58	59 71	+	33	58	22 24
48	86	02	01	00	00	5-Methoxygramine	—	—	—	—	08	23	—	—	—	+	33	04	26 71
90	88	87	85	67	45	N-Acetyltryptamine	33	57	—	—	10	—	06	—	30	++	—	08 24	24 25
51	80	03	00	00	00	N-Methyltryptamine	—	—	—	13	08	+	—	—	+	+	33	17 71	23 25
48	92	05	05	02	01	N,N-Dimethyltryptamine	—	—	—	58	58	—	—	58	52	++	—	58 23	23

(continued on p. 486)

TABLE XI (*continued*)

R_F values × 100						Compounds	Detection											
F	E	A	B	C	D		U.V.	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
68	93	04	06	00	00	N,N-Diethyltryptamine	—	—	—	+	08	+	—	—	70	+	33	17, 23	23
67	87	04	00	00	00	5-Fluorotryptamine	—	—	—	—	—	—	—	58	—	—	—	17	24, 27
08	00	00	00	00	00	Psylocybin	—	—	—	—	—	—	—	—	70	—	—	16, 26	27
52	83	02	00	00	00	5-Methoxytryptamine	—	06	—	60	08	—	—	—	70	++	—	26	27, 25
83	82	60	18	01	00	N-Acetyl-5-hydroxy-tryptamine	—	17, 60	24	25	18	+	—	—	30	+	—	69	30, 43
90	88	78	84	48	28	N-Acetyl-5-methoxy-tryptamine = melantonin	—	06	—	—	08	—	—	—	70	++	—	26	25, 28
94	93	93	93	92	92	Indole-3-carbinol	57	—	—	64	09	—	58	17	57, 70	++	—	58, 17	24
90	88	90	81	72	59	Tryptophol	34	58	—	—	08	24	70	—	71	++	—	07, 63	23, 25
51	84	01	01	00	00	L-Tryptophan methyl ester	—	—	—	—	08	23	—	—	—	++	33	03	23, 25
93	19	92	72	68	15	5-Chloroindole-2-carboxylic acid	34	06	24	25	09, 63	—	—	57	—	—	+	—	26
91	09	90	63	51	15	Indole-5-carboxylic acid	30	06	59	64	10	—	—	57	+	++	+	23	30
93	94	84	88	87	84	3-Indolylacetic acid methyl ester	+	—	60	60	08	—	—	—	—	++	—	18, 69	24, 25
85	77	65	50	19	07	3-Indolylacetamide	—	06	—	58	09	—	—	—	44, 71	++	—	26, 69	23, 25
88	08	86	18	24	05	3-Indolylglyoxylic acid	—	—	—	56	—	+	03	64	03, 72	—	++	—	—
94	93	92	93	92	88	3-Indolylglyoxylamide	—	—	—	—	—	—	07	—	—	+	—	—	17
95	93	93	94	92	92	3-Indolylglyoxyldimethyl-amide	—	—	—	—	—	—	07	—	—	+	33	—	+
91	07	60	23	28	04	3-Indolylpyruvic acid	03	58	62	63	+	63	—	24	69	+	+	17	23, 65
94	08	91	66	50	14	3-Indolylacrylic acid	34	63, 23	—	24, 64	64	23	58	63	70	++	+	40	23
93	91	89	75	63	25	5-Hydroxy-3-indolylacetic acid methyl ester	—	17	+	+	+	—	—	—	—	—	—	34	30, 27
68	57	20	01	00	00	5-Hydroxy-3-indolyl-acetamide	+	64	23	23, 65	64	68	—	70	71	++	—	51	25, 28
91	10	91	71	53	11	5-Methoxy-3-indolylacetic acid	—	03	—	+	07	—	60	26, 23	44, 71	++	+	26, 27	27, 3[illegible]
86	74	63	54	15	05	5-Methoxy-3-indolyl-acetamide	—	—	—	—	07	—	—	—	71	+	—	26, 27	27, 24
42	45	00	00	00	00	Harmalol	39	17, 64	64	71, 67	64	43, 71	—	—	71	—	—	—	—
52	88	02	12	00	00	Harmine	32	—	—	—	—	—	—	57	03, 71	—	—	—	+
62	88	05	15	00	00	Harmane	33	—	—	—	—	—	—	—	—	—	—	—	—
80	91	16	47	00	00	Ergotamine	34	—	—	—	—	+	—	—	—	+	—	26, 70	+

TABLE XII

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF MISCELLANEOUS COMPOUNDS

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
17	65	00	01	00	00	4-Aminopyridine	—	—	—	60	59	—	—	59	07, 72	—	33	06	17, 23
28	77	04	23	00	00	2-Amino-4-picoline	34	—	—	62	—	—	—	57	08, 72	—	33	—	22
38	80	04	22	00	00	2-Amino-6-picoline	34	06	58	62	09	+	—	58	09, 72	—	33	+	17
95	03	00	94	00	00	Bilirubin	58	64	41, 25	44, 68	—	48	—	—	71	+	—	+	07
95	00	00	95	00	00	Biliverdin	34	—	—	—	—	71	—	—	71	—	—	—	—
10	00	00	00	00	00	Folic acid	44	—	—	60	—	—	—	—	—	+	+	—	17
92	90	87	74	63	11	7-Hydroxycoumarin	39	11	18, 23	23, 63	59	—	—	—	44, 71	+ +	—	—	—
64	63	60	23	14	00	Isoniazid = isonicotinoyl-hydrazine	05	—	59	65	03, 64	55	—	60	45, 68	+ +	33	58	64
70	91	17	29	46	00	Dromoran	—	09	—	—	—	—	—	—	71	+	—	—	—
81	64	42	88	22	15	Chlorpromazine	39	—	—	—	60	52	—	17	18, 49	+ +	—	—	—
88	80	73	92	53	33	Phenothiazine-5-oxide	—	—	—	—	—	—	42	41	41, 30	+	—	59, 39	57, 56
76	90	70	95	79	73	Acridine	07	58	—	—	+	—	—	03	03, 71	—	—	58	—
79	42	03	00	00	00	2-Ethoxy-6,9-diamino-acridine = rivanol	48	09	64	63	62	—	—	—	—	+ +	—	12	65
47	92	01	07	00	00	Atebrin = quinacrine	03	—	—	—	64	—	—	—	—	+	—	—	60

TABLE XIII

PAPER-CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME HIPPURIC ACID DERIVATIVES

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		U.V.	D1	D2	D3	D4	DB	DAB*	Fe	Mo	Mn	Ind	EH	DAC
86	06	76	83	34	05	*o*-Methoxyphenaceturic acid	—	—	—	—	—	—	05	—	—	—	+	—	—
85	05	76	10	05	00	2,3-Dihydroxyhippuric acid	30	08	62	62	10	28	13	28, 70	68	+ +	+	—	—
84	04	62	02	01	00	2,5-Dihydroxyhippuric acid	33	—	—	—	—	—	12, 13	70	30	+	+	—	—
76	02	43	09	01	00	Vanilloylglycine	—	08	—	65	08, 65	28	10	71	70	+ +	+	—	—
69	02	24	00	00	00	Caffeoylglycine	33	—	63	58	08, 63	+	17	03, 51	69	+ +	+	—	—
76	02	45	13	01	00	Feruoylglycine	33	06, 24	23, 13	23, 43	13	25	17, 15	—	69	+ +	+	—	—
85	05	65	34	08	01	Anthranylglycine	33	—	—	56	08	—	13, 17	—	—	+ +	+	07	22
92	89	87	92	77	67	*o*-Aminohippuric acid methyl ester	33	—	—	—	06	—	06	—	—	+	—	06	22
91	08	84	18	01	00	3-Hydroxyanthranylglycine	38	—	—	17	58	43	06	56	43, 69	+ +	+	05	22
85	05	73	26	07	00	Indole-3-acetylglycine	—	—	—	—	07	—	—	—	—	+ +	+	28	27
77	03	28	01	00	00	5-Hydroxyindole-3-acetyl-glycine	—	63	24	25	63, 65	71, 27	—	59	70	+ +	+	71, 28	30, 33
91	06	74	14	03	00	Indole-3-acrylglycine	34	06	63	23	58	—	17	—	70	+ +	+	13	23
90	07	87	82	58	10	Quinaldylglycine	34	—	—	—	—	—	22	—	—	+	+	—	—

* DAB = hippuric acid reagent. DN reagent gave no reaction with the compounds listed in this table.

TABLE XIV

PAPER-CHROMATOGRAPHIC SEPARATION OF SOME 2,4-DINITROPHENYLAMINO ACIDS

R_F values × 100						Compounds	Detection
F	E	A	B	C	D		U.V.
94	40	92	73	83	35	DNP-alanine	+
93	18	90	85	82	33	DNP-β-alanine	+
92	29	93	92	91	46	DNP-β-aminobutyric acid	+
56	02	00	00	00	00	DNP-arginine	+
88	09	72	03	02	00	DNP-asparagine	+
94	01	89	10	24	00	DNP-aspartic acid	+
87	14	53	02	00	00	DNP-citrulline	+
90	12	68	05	04	00	DNP-glutamine	+
91	26	91	48	73	08	DNP-glycine	+
87	58	00	00	00	00	DNP-histidine	+
91	65	92	92	91	55	DNP-isoleucine	+
92	56	92	92	90	43	DNP-methionine	+
92	68	92	92	91	56	DNP-norleucine	+
92	57	92	92	91	51	DNP-norvaline	+
94	78	93	84	72	05	DNP-ornithine	+
94	68	93	92	91	49	DNP-phenylalanine	+
92	24	88	06	24	01	DNP-serine	+
93	85	93	92	90	33	DNP-tyrosine	+

TABLE XV

PAPER-CHROMATOGRAPHIC SEPARATION OF PHENYLTHIOHYDANTOIN DERIVATIVES OF SOME AMINO ACIDS

R_F values × 100						Compounds	Detection
F	E	A	B	C	D		DB
91	91	90	90	88	83	PTH-alanine	18
92	17	92	81	50	10	PTH-β-aminobutyric acid	18
65	14	03	00	00	00	PTH-arginine	18
85	84	66	44	11	00	PTH-asparagine	18
91	10	88	63	38	02	PTH-aspartic acid	18
83	32	56	37	05	00	PTH-citrulline	18
84	80	68	54	11	01	PTH-glutamine	18
92	92	91	59	35	10	PTH-glycine	62 / 18
92	92	91	85	82	64	PTH-hydroxyproline	64
93	92	92	93	93	89	PTH-isoleucine	58
93	92	92	92	90	90	PTH-leucine	58
92	91	92	92	93	88	PTH-methionine	58
92	55	91	85	33	02	PTH-ornithine	18
94	93	92	92	80	16	PTH-phenylalanine	62 / 18
92	90	90	81	59	10	PTH-proline	58 / 18
90	90	88	57	36	07	PTH-serine	64
91	93	92	84	80	29	PTH-tyrosine	62

TABLE XVI

COMPARISON OF COLOUR REACTIONS FOR SOME INDOLE DERIVATIVES WITH DIFFERENT MODIFIED EHRLICH REAGENTS

Compounds	Detection					Exposure in air
	EH	DAC	E3	E4	E5	
	in 1 N HCl		in conc. HCl			
Indole*	24	28 33	19 23	65	19	62
2-Methylindole*	22	35 25	15 22	19	19	62
5-Methylindole*	22	23 28	+	+	59	+
Indoxyl acetate*	57	23	23	69	23 56	23
Indican = indoxyl-3-sulphate ester	63	26 23	60	51	52 23	34
5-Hydroxyindole	23	43 28	23	23	23	56
5-Methoxyindole*	24 20	38 28	23	23	23	62
5-Benzyloxyindole*	18 22	40 25	19	23	23	63
Isatin*	—	62	—	+	—	—
Indole-3-carbinol	58 17	24	62	56	+	60
Tryptophol = indole-3-ethanol	07 63	23 25	65 51	65	65 35	58
3-Indolealdehyde*	26	22 63	+	46	57	+
5-Methoxy-3-indolealdehyde*	26	26	57	46	57	+
α-(3-Indolyl)-acetic acid*	60 70	22	63	69	63	58
3-Indolylacetic acid methyl ester	18 69	24 25	65 51	65 68	24 65	58
3-Indolylacetamide	26 69	23 25	23	42	23	—
5-Hydroxy-3-indolylacetic acid*	68	24	69	43	70	63
5-Methoxy-3-indolylacetic acid	26 27	27 30	25 28	28	25 28	57
5-Hydroxy-3-indolylacetamide	51	25 28	69 43	42	69	58
5-Methoxy-3-indolylacetamide	26 27	27 24	25 28	23 38	25 28	+
5-Chloro-2-carboxy-indole	—	26	22 24	41	17 26	—
5-Methoxy-2-carboxy-indole*	26 25	70	25 69	28 70	23 27	+
Indole-5-carboxylic acid	23	30	22	23	22 23	+
3-Indolylglyoxylic acid	—	—	—	46	60	+
β-(3-Indolyl)-propionic acid*	23 71	23	23 46	28	23 71	+
γ-(3-Indolyl)-butyric acid*	23 70	23	24	41	24 71	—
DL-2-(3-Indolyl)-lactic acid*	26	25	23 39	23 41	23 27	—
3-Indolylpyruvic acid	17	23 65	—	46	60	+
3-Indolylacrylic acid	40	23	62	46	62	18

(continued on p. 490)

TABLE XVI (*continued*)

Compounds	Detection					Exposure in air
	EH	DAC	E3	E4	E5	
	in 1 N HCl		in conc. HCl			
L-Tryptophan*	57	23	23 40	28	23	—
DL-Acetyltryptophan*	60 26	25 22	23 38	25 28	23 37	—
L-Tryptophan methyl ester	03	23 25	23 41	25 47	23 44	—
DL-4-Methyltryptophan*	—	23	23	28	23	—
DL-4-Hydroxytryptophan*	71	26 52	68	46	68	+
DL-5-Methyltryptophan*	—	23 25	—	44	—	—
DL-5-Hydroxytryptophan*	—	26	28 41	38	25 28	+
Gramine*	57	22	62	46	62	—
Gramine-N-oxide	58	22 24	60	+	60	+
5-Methoxygramine	04	26 71	—	46	—	—
Tryptamine*	70 51	23	23 51	28	23	+
N-Acetyltryptamine	08 24	24 25	23 51	28	23	+
5-Fluorotryptamine	17	24 27	23	+	62	—
5-Hydroxytryptamine = serotonine*	71	26	25 51	41	25	+
5-Methoxytryptamine	26	27 25	28 41	28	23 25	04
N-Acetyl-5-hydroxytryptamine	69	30 43	23	42	23	—
N-Acetyl-5-methoxytryptamine = melantonin	26	25 28	28 38	28	23 25	60
N-Methyltryptamine	17 71	23 25	23 38	25	23 30	—
N,N-Dimethyltryptamine	58 23	23	23 38	25	23 30	58
N,N-Diethyltryptamine	17 23	23	23 38	25	23 30	+
Bufotenine = 5-hydroxy-N,N-dimethyl-tryptamine*	27 71	30 25	26	46	26	—
Psylocybin	16 26	27	23 40	43 38	18	—
Indole-3-acetylglycine	28	27	23	42	23	—
Indole-3-acrylglycine	13	23	60	46	60	—
5-Hydroxyindole-3-acetylglycine	71 28	30 33	69	46	70	58

For compounds marked with an asterisk (*) other chromatographic data can be found in ref. 2, while for the others further data are given in Tables XI and XIII of this paper.

TABLE XVII

SOME COLOUR REACTIONS FOR ALDEHYDES DETECTED WITH DIFFERENT AMINES

Compounds	*o*-Phenylenediamine	*m*-Phenylenediamine	*p*-Phenylenediamine	2,4-Diaminophenol	*p*-Anisidine	*o*-Dianisidine	*o*-Toluidine	*p*-Toluidine	α-Naphthylamine	β-Naphthylamine	Benzidine	*o*-Tolidine	Dimethyl-*p*-phenylenediamine	*p*-Aminoacetophenone	4-Aminoantipyrine	1,2-Diaminonaphthalene	2,7-Diaminofluorene
Benzaldehyde*	—	—	—	—	+	—	—	—	—	05	—	—	—	—	60	18	04
p-Tolualdehyde*	—	—	—	—	+	—	—	—	—	05	05	—	—	—	+	18	04
Cumaldehyde*	—	—	—	—	05	—	—	—	—	05	05	—	—	—	+	—	04
o-Chlorobenzaldehyde	+ 18	—	—	05 —	05	05 —	—	— 05	—	— 05	— 05	— 05	—	—	06 —	—	—
m-Chlorobenzaldehyde*	—	—	—	—	+	—	—	—	—	—	05	—	—	—	+	18	—
p-Chlorobenzaldehyde**	—	—	—	—	05	—	—	—	—	05	05	—	—	—	+	18	04
m-Nitrobenzaldehyde*	—	—	+	—	05	—	—	—	—	05	—	— 05	— 64	—	+	—	04
o-Phthalaldehyde*	—	70	52	27	51	70	23	51	63	51	52	18	52	59	50	—	51
Terephthalaldehyde**	—	—	53 —	69 —	53 06	—	51 —	53 —	53 —	52 07	70 —	70 —	51 —	—	52 09	70 06	70 58
			58	05	08	05	05	05	05	08	08	07	23				65
			63			62			—		62						
o-Carboxybenzaldehyde**	—	—	—	—	— 05 —	—	—	—	—	—	— 05 —	—	—	—	—	—	+
Cinnamaldehyde*	09 60	06 60	10 62	08 —	07	11 12	05	06	08	07	09	08	13 63	06 —	08	23	09 12
3-Indolealdehyde*	05 —	06	07 —	05 —	05	05 07	05 —	05	— 05	05 03	06	05	05 09	06 —	06 60	08	05 08
Salicylaldehyde*	06 —	06 —	07 —	06 —	06	09 56	05	05 —	07 —	05 —	06 —	07 —	+ —	05 15	06 —	08 64	06

(continued on p. 492)

TABLE XVII (*continued*)

Compounds	o-Phenylenediamine	m-Phenylenediamine	p-Phenylenediamine	2,4-Diaminophenol	p-Anisidine	o-Dianisidine	o-Toluidine	p-Toluidine	α-Naphthylamine	β-Naphthylamine	Benzidine	o-Tolidine	Dimethyl-p-phenylenediamine	p-Aminoacetophenone	4-Aminoantipyrine	1,2-Diaminonaphthalene	2,7-Diaminofluorene
3-Methylsalicylaldehyde*	—	—	+	05	05	06	05	—	—	05	05	05	—	05	58	—	06
			—	—	—	—	—			—	—	—		12			
5-Formylsalicylic acid**	—	—	—	—	—	+	—	—	—	05	05	+	—	05	58	—	06
					05	—				—	07	05	17	—	—		
3-Hydroxybenzaldehyde*	—	—	—	—	04	05	05	—	—	05	06	—	+	04	06	18	06
			62			—	—			—			14	62	—	—	08
4-Hydroxybenzaldehyde*	06	06	07	07	05	08	06	06	05	06	06	05	58	05	06	—	—
	—	—	63				—		—				14	62	—		07
4-Methoxybenzaldehyde*	04	—	08	07	06	08	05	05	05	06	07	05	—	05	06	62	08
	—		64	—			—		—		—		14	62	—	—	
5-Methoxy-3-indolealdehyde**	05	06	08	07	—	05	05	05	—	05	06	05	06	06	06	—	06
	—		62		05	08							62	—	—	08	08
Protocatechualdehyde*	05	06	08	07	06	08	05	05	05	06	07	05	06	05	06	—	07
	—		62						—		08		13	62	—		08
Piperonal*	04	04	07	06	06	05	05	06	04	—	07	05	05	05	06	—	08
	—	—	62	—		—	—		—		08		14	62			
Veratraldehyde*	04	04	09	06	06	05	05	05	05	04	08	05	05	05	06	—	08
	—	—	62	—		07	—		—	—			14	60			09
o-Veratraldehyde*	05	05	09	06	06	05	04	05	05	04	08	06	06	05	06	—	08
	—	—	64	—		07	—		—	—	09		14	60			09
2-Acetoxy-3-methoxybenzaldehyde*	04	—	+	—	04	05	04	04	04	—	05	05	—	—	—	—	06
	—		62			07	—		—		07	07	13	13			08
Coniferyl aldehyde*	58	58	08	08	58	13	06	07	08	08	09	09	07	08	58	62	09
	—	—	62	—	—				—	—			17	—	—	—	
o-Vanillin*	06	06	08	08	06	09	06	06	06	06	08	09	07	07	06	—	09
	—	—	64	62		62	07		—	—		12	14	19			
Isovanillin*	06	06	08	07	06	08	06	06	06	06	07	07	06	06	06	—	07
	—	—	64	62					—	—			14	64			08
Vanillin*	06	06	09	08	06	05	05	06	06	07	08	05	05	04	07	—	08
			62	62		07			—				13	60			09
3-Ethoxy-4-hydroxybenzaldehyde**	06	06	09	08	06	05	05	06	06	08	08	05	05	05	08	—	08
			62	62		07			—				13	60			

5,6-Dimethoxy-2-formylbenzoic acid**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+
					05												
4-Acetoxy-5-methoxyisophthalaldehyde*	08	58	64	09	07	09	07	06	07	08	11	09	06	08	06	62	10
	17	09	65	64	09	19		09	09	11	14	11	63	62	08		19
5-Methoxy-6-hydroxyisophthalaldehydic acid*	+	—	62	58	05	08	05	05	—	07	09	06	—	58	58	—	58
			—	—	07	63							62	—	—		13
4-Methoxy-5-hydroxyisophthalaldehydic acid*	—	—	—	—	05	05	—	—	—	—	06	06	—	05	58	—	05
						07	05	05		05	—			—	—		60
2,4-Dihydroxybenzaldehyde*	06	03	08	07	06	08	05	05	06	07	07	08	58	06	03	—	07
		08	62	09		09							12	14		08	09
4-Methyl-2,6-dihydroxybenzaldehyde*	—	—	+	+	—	05	—	—	04	04	05	05	—	04	58	—	05
			—	—					—	—	—		17	13	—		—
Orsellinic aldehyde**	05	05	08	06	05	06	05	05	05	05	06	06	58	05	06	—	06
	06	06	09			09			03				09	15		07	08
2,4-Dimethoxybenzaldehyde*	05	05	09	07	06	10	05	05	05	05	08	09	58	05	07	—	09
			—	—					03			10	13	08		07	
2,5-Dihydroxybenzaldehyde**	07	59	10	08	07	11	08	07	07	08	09	09	60	09	07	—	09
			63	64					62	62	14	12	64	19			10
2,5-Dimethoxybenzaldehyde**	06	06	10	08	07	12	07	07	07	08	09	09	07	08	07	63	12
	—		63	62					—	62	13	12	64			—	
Phloroglucinaldehyde*	05	05	07	06	05	07	05	05	05	05	06	06	58	06	07	08	06
			—	—					03				08	62	03		
2,4,6-Trimethoxybenzaldehyde*	—	—	05	06	05	07	05	05	04	04	06	06	—	04	58	—	05
			—	—					—	—			17		07		06
3,4-Dihydroxy-5-methoxybenzaldehyde	06	03	14	07	06	09	02	06	—	07	09	06	06	06	06	—	08
	—		64						03				62				09
Trimethoxygallaldehyde*	—	—	08	—	04	05	—	—	—	04	08	—	—	—	—	—	06
			62			—				—			62	05			08
p-Dimethylaminobenzaldehyde	09	09	15	12	09	11	06	08	08	10	15	11	08	09	08	08	12
						14			60				62			09	14
						20											
p-Dimethylaminocinnamaldehyde	19	19	65	19	14	23	14	14	22	19	19	22	15	65	12	23	19
	64		25		19	65	19	19	19		65	23	23				65
2-Chloro-4-dimethylaminobenzaldehyde	09	09	12	10	09	09	06	08	08	10	12	11	08	09	08	09	12
			15			14			60				63	64			

The first number indicates instantaneous colour development, or during one minute. The second number shows the colour change after one hour. The third number, when present, gives the change during 24 hours. If only one or two numbers are present, no change has been noticed. The sign — indicates no detection or that the colour becomes identical with that of the background. Further identification data for the compounds marked by * and ** can be found in ref. 1 and ref. 2 respectively, while for the other compounds such data is given in this paper.

RESULTS AND DISCUSSION

As mentioned earlier, the number of standard reagents has been increased to twelve, including two Ehrlich reagents. This provides information as to the possible interference from compounds which are not usually expected to react with Ehrlich reagent and yet give some unusual colour reactions. In Table II we find two of the pyrocatechols, which show a light but definite reaction. Further exceptions are: 2,6-dihydroxypyridine (Table III), 1,2,4-trihydroxybenzene and 2,4,6-trihydroxybenzoic acid (Table V), and isoniazid and phenothiazine-N-oxide (Table XII). 2,4,6-Trihydroxybenzoic acid gives shades very similar to indole compounds, *e.g.* red-violet and blue; the others, however, have different red and brown colours. The colour formation of Ehrlich reagent with phloroglucinol has been observed earlier by STEELNIK[9]; some resorcinol derivatives with free 4 or 6 position give purple Ehrlich reaction[10].

The colour patterns with diazonium reagents for 1,2-, 1,3- and 1,4-dihydric phenol derivatives (Tables II–IV) do not appear as clearly as they did earlier. But since only a few of these compounds have been listed this time, results obtained previously should be taken into consideration when evaluating these patterns. The general picture still holds, even for these compounds, indicating three distinct colour patterns. Hydroxylated naphthalenes and also 5-hydroxyisoquinoline (Table VI), show typical strong coloured patterns with diazonium reagents, as observed before. From Table XIII it seems that glycine-conjugates with nitrogen-containing heterocyclic carboxylic acids have a tendency to give strong red to magenta colours with hippuric acid reagent. The example quoted here is quinaldic acid glycine-conjugate; earlier it was nicotinuric acid.

In Table XV the DB reagent has been used to detect amino acid phenylthiohydantoins. The colours produced varied between pink and brown. It has been noticed that certain thio-derivatives, such as thiosalicylic acid, thiobenzoic acid and mercaptopyridines, give similar types of colours including yellow, orange and brown shades, thus a characteristic difference between hydroxylated aromatic and heterocyclic compounds is established. The latter compounds have variations only in red-violet, violet, blue and green shades.

Data obtained by spraying indole derivatives with five modifications of Ehrlich reagent are collected in Table XVI. It is obvious that these values can only serve as guide, because a variety of colours were observed in several cases. The main feature seems to be that purple and red-violet colours are produced with indole derivatives substituted in the 2- or 3-position (the 5-position being free). Blue shades appear to be more dominant with 5-hydroxylation or methoxylation. However, there is some overlapping in the case of N-alkylated tryptamines which also give blue colours. Indican, indole-3-carbinol, gramine, gramine-N-oxide and indole-3-acrylglycine tend to show brown spots. A column was also included in Table XVI in order to demonstrate that untreated spots became visible after one week and were coloured owing to the oxidation of the compounds in the air.

In Table XVII the results of colour reactions with aromatic amines were recorded for 47 aromatic aldehydes. The colour development at room temperature was in most cases instantaneous. However, on standing, a slow increase in the intensity of the colours was observed. Yellow to orange colours dominated the overall picture, but in a few compounds red and violet colours occurred. The best reagents were: *p*-phenylenediamine, *p*-anisidine, *o*-dianisidine, *β*-naphthylamine, benzidine, dimethyl-*p*-phe-

nylenediamine, *p*-aminoacetophenone and 2,7-diaminofluorene. Among the aldehydes tested *o*-phthalaldehyde showed green and grey colours; other compounds which were relatively more reactive were cinnamaldehyde, 2,5-dihydroxybenzaldehyde and 4-acetoxy-5-methoxyisophthalaldehyde. The last three aldehydes in Table XVII are constituents of Ehrlich reagents, consequently the code is also useful for detecting aromatic amines by spraying with Ehrlich reagents EH, DAC and 2-Chloro-EH (as abbreviated in this paper).

As regards the distribution of the R_F values in the solvent systems, it is worth noting the interesting behaviour of the pyridine derivatives. Following the R_F values in the order as presented in the tables we find two maximum values for these derivatives, one for solvent E (or F) and another for solvent B according to the distribution criterion R_F in E $>$ R_F in A $<$ R_F in B. This double peak is more clearly demonstrated if the values are visualised in a diagram. This regularity has been observed earlier[2] and has occurred when monohydroxy derivatives of nitrogen-containing heterocyclic compounds were involved. Pyridines, quinolines, isoquinolines, but not indoles and imidazoles, seem to belong to this group; of the more complex ring structures, alkaloids behaved in the same way. In this paper 2- and 4-mercaptopyridines (Table I), 2,6-dihydroxypyridine (Table III), 5-hydroxyisoquinoline (Table VI), 3-pyridylacetic acid (Table VIII), 2-amino-4-methylpyridine and 2-amino-6-methylpyridine (Table XII), follow this rule, together with other heterocyclic compounds like chlorpromazine, phenothiazine-5-oxide, acridine and atebrin (Table XII) and harmine, harmane and ergotamine (Table XI). Of the approximately 60 simple indole derivatives investigated by this procedure, only N-acetyl-5-methoxytryptamine shows that a similar distribution of the R_F values in the solvents is due to the R_F-elevating effect of methylation in solvent B of the parent compound N-acetylhydroxytryptamine. The similar effect is also noted in the case of *p*-anisidine and *o*-dianisidine which are the exceptions, since the non-methoxylated corresponding amino phenols behave normally, gradually lowering the R_F values towards the solvents A, B, C and D. 3-Indolepyruvic acid, which shows another maximum R_F value in C is not considered to interfere in this reasoning, it even satisfies the distribution of R_F values according to E $>$ A $<$ B. It is also interesting to note an addition to the group of compounds which show a jump of R_F values between solvent B and solvent C (R_F in B $<$ R_F in C), *viz.* 5-hydroxyindole, which was found to behave like some of the resorcinol and hydroquinone derivatives.

In general the R_F values were in accordance with previous observations giving low values for free phenolic carboxylic acids in solvent E and high values in F and A slowly decreasing the values in solvents B, C and D. Amino derivatives of phenols showed relatively high values in solvents F, E and A; thereafter much lower values in B, C and D. Aromatic amines have, as a rule, low to medium values in solvent F, followed by a maximum value in solvent E, continuing rapidly to low values in A, B, C and D. N-Acetylation has an elevating effect on all values, except those from solvent E. Imidazole derivatives are practically immobile in these solvents.

In Tables XIV and XV the R_F values are recorded for some 2,4-dinitrophenyl and phenylthiohydantoin derivatives of amino acids. Although the solvents are not very suitable for the separation of these compounds, causing the spots to diffuse, they might be used in some cases. The R_F values should be regarded as approximate and merely as indicators of their relative rates of movement.

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SUMMARY

Paper-chromatographic data are presented for an additional approx. 170 compounds in six solvent systems together with their colour reactions with twelve standard reagents. The main types of compounds presented in 15 tables are: mono-, di- and trihydric phenol derivatives, naphthalene derivatives, biogenic amines, indole derivatives and hippuric acids. Thirteen hippuric acid derivatives were synthetized for this purpose. The relationship between the colour reactions and the chemical structure of the compounds on the one hand, and the connection between the R_F values and chemical structure on the other hand, is discussed. Special attention was devoted to the detection and characterisation of aromatic aldehydes and indole derivatives, whereby an additional number of reagents was used. For indoles the following reagents were found to be useful in distinguishing between different compounds with the same R_F value: *p*-N,N-bis(2-chloroethyl)-aminobenzaldehyde, 2-chloro-4-N,N-bis(2-chloroethyl)-aminobenzaldehyde and 4-N,N-bis(2-chloroethyl)-amino-2-tolualdehyde. For detection of PTH-amino acid derivatives DB reagent (2,6-dibromoquinone-4-chloroimide) was found suitable, producing red and brown colours.

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