Journal of Chromatography, 88 (1974) 119–147

CHROM. 6981

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF PHENOL DERIVATIVES AND RELATED COMPOUNDS OF BIOCHEMICAL INTEREST USING A REFERENCE SYSTEM

FIFTH SUPPLEMENT

L. REIO*

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

(Received July 16th, 1973)

SUMMARY

Following previous supplements in this series, a further 180 compounds were analyzed by paper chromatography in six solvent systems. The data originating from 15 standard colour reagents are also recorded and presented. The following types of compounds are described: flavonoid derivatives, aliphatic and aromatic aldoximes and ketoximes, substituted benzamide derivatives, aminobenzene derivatives and heterocyclic amines, N-nitroso compounds, N-oxides of tertiary bases, pyrimidine and indole derivatives and sulphonamide drugs. The paper chromatographic mobility patterns were characteristic, especially for 1,3- and 1,4-hydroxybenzaldoximes and ketoximes of the hydroxyacetophenone type. All the heterocyclic bases investigated showed mobility patterns similar to those of the alkaloids described in earlier supplements. Mobility patterns similar to the alkaloids were produced by some of the mercaptopyrimidines. In general amino-, diamino-, hydroxy and dihydroxy derivatives of pyrimidines showed low mobility in these solvents. Several flavonoid compounds were found to be Ehrlich positive and a magenta colour was produced by L-epicatechin. Most of the N-unsubstituted benzamides were detected with *p*-dimethylaminocinnamaldehyde, and most of the N-nitroso derivatives of secondary amines and N-oxides of heterocyclic tertiary amines were revealed by this reagent.

INTRODUCTION

In earlier studies in this series[1], attention was concentrated on developing as specific as possible patterns of mobility for a variety of compounds of biological importance. The R_F values for each of these compounds were determined for six different paper chromatographic solvent systems. When presenting the latter data, the solvents were arranged in order of decreasing polarity, so that for identification several types of characteristic mobility patterns for these compounds were more easily recognized. This system of mobility patterns was interconnected with data

* Present address: The Food Administration, P.O. Box, S-104 01 Stockholm 60, Sweden.

from 15 selected detection reagents, which allowed the identification of functional groups in the molecule and ultimately provided basic information for rapid classifications of these compounds, and their identification.

Of the large number of compounds, such as plant phenolic products[1], biogenic amines[2,3], alkaloids[4,5] and certain indole derivatives[2–4], a great many were found to have irregular mobility (defined in the section Discussion) and within a group this remarkable mobility persists, so far with few exceptions, even when larger numbers of compounds were investigated. These valuable identification criteria were discussed in a recent review[6].

This latest supplement gives data for important new types of compounds, *e.g.* flavonoids, N-nitroso derivatives, N-oxides and benzamides, not previously treated in detail. The data for oxime and pyrimidine derivatives follow on from a previous supplement[5].

As well as taxonomical problems with higher plants that can be resolved by studies of flavonoid contents, always of interest[7], there has recently been concern about the occurrence of these type of compounds, *e.g.* anthocyanines, in foods[8]. The data presented in Table I show a complication in detecting these compounds by colour reactions. The N-nitroso compounds of secondary amines have long been known as potential carcinogens with documented mutagenic action[9], and their distribution[10] and possible formation *in vivo* is currently under investigation[11,12]. In this study chromatographic data are also presented for N-nitrososarcosine, which is formed from creatine by nitrite[11]. It may be of future interest that a compound such as *p*-methylnitrosobenzaldehyde is naturally occurring and is a metabolic product of *Clitocybe suavolens*[13].

Very few aromatic N-oxides of tertiary amines are found as natural products. One of the first reports was the presence of geneserin mono-N-oxide in calabar bean[14]. This was followed by the isolation of iodinin[15] (1,6-dihydroxyphenazine-5,10-dioxide) from *Chromobacterium iodinum*, an antibiotic aspergillic acid[16] (6-*sec.*-butyl-2-hydroxy-3-isobutylpyrazine-1-oxide) from *Aspergillus flavus* and pulcherrimine[17] (iron(III) complex of pulcherrimic acid, the N,N′-dioxide of 2,5-dihydroxypyrazine derivative) from *Candida albicans*. Recently a metabolic conversion of arecoline (1,2,5,6-tetrahydro-1-methylnicotinic acid methyl ester), from betel nut, to arecoline-1-oxide in the rat was reported[18]. On the other hand, quite a number of 4-nitroquinoline-1-oxides and related oxides are potent carcinogens[19], which makes it desirable to obtain more separation and detection data for these types of compounds. Among the oxime compounds investigated, formamide oxime was reported to inhibit RNA and DNA synthesis in *Escherichia coli*, rat liver and rat cerebral mitochondria[20]. From the numerous pyrimidine derivatives listed in the Tables, several are antimetabolites and some are intermediates in pteridine synthesis. Deaminoleucopteridine synthesis requires 4,5-diamino-2,6-dihydroxypyrimidine[21]. Model studies on prebiotic synthesis of nucleic acid bases, run over several intermediates, is indicated by the formation of 5-hydroxymethyluracil, a precursor to thymine[22].

MATERIALS AND METHODS

For one-dimensional descending chromatography, rectangular glass jars of

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 compositions of the six solvent systems used are given in *Abbreviations used in Tables I–X.* (See p. 144.)

Spray reagents

The following twelve standard spray reagents were used to detect the compounds: diazotized sulphanilic acid (Schuchart, Munich, G.F.R.); diazotized 4-benzylamino-2,5-dimethoxyaniline (Koch-Light Labs., Colnbrook, Great Britain); diazotized *o*-dianisidine (Koch-Light); *p*-nitrobenzenediazonium fluoroborate (Eastman-Kodak, Rochester, N.Y., U.S.A.); 2,6-dibromoquinone-4-chloroimide BDH, Poole, Great Britain); 2,4-dinitrophenylhydrazine, ferric chloride, phosphomolybdic acid, potassium permanganate, bromophenol blue, *p*-dimethylaminobenzaldehyde and *p*-dimethylaminocinnamaldehyde (Heidenheimer Lab., Heidenheim-Brenz, G.F.R.). All compounds were also tested with ninhydrin, *p*-dimethylaminobenzaldehyde in acetic anhydride and Dragendorff reagent, $KI \cdot BiI_3$ (E. Merck, Darmstadt, G.F.R.). For the concentrations of these reagents see *Abbreviations used in Tables I–X.*

Most of the compounds that are listed in the Tables were obtained through commercial sources and used without purification. However, it was discovered that a number of preparations contained easily separable components. The main spot in these instances was considered to be the compound named on the supplier's label. All the aldoximes and ketoximes appearing in this supplement were synthesized from the corresponding aldehydes or ketones. All the derivatives were obtained in water–ethanol mixtures by adding slightly alkaline hydroxylamine hydrochloride and heating the mixture. The oximes were then recrystallized from ethanol–water mixture. It can be seen from the Tables that in a few instances the *cis* and *trans* isomers separated in certain solvents. Aromatic amine N-oxides were synthesized according to methods already described[23]. This essentially involved oxidation with 33% hydrogen peroxide in glacial acetic acid at 60°. N-Nitroso derivatives were prepared from the corresponding secondary amines by nitrosation with sodium nitrite in glacial acetic acid[24]. Unsubstituted and substituted benzamides were prepared by the action of ammonia or the corresponding amine on freshly distilled acid chlorides[25]. Preparations were used without purification.

RESULTS

Tables I–X contain information on 180 organic compounds investigated by the above procedure. The R_F values were recorded in six different solvent systems arranged in a special order, designated by F, E, A, B, C and D. The detection as colour reactions is recorded for the twelve different spray reagents used for the identification of each compound; the colour produced under ultraviolet light is also indicated. In addition, all compounds were also tested with NH, DAB and Bi reagents (for abbreviations, see below). When positive reactions were observed, the results were labelled with the superscript a, b or c, as can be seen in the footnotes to the tables. The amount of substances used in these experiments was about 25 μg per spot. When the reagents were applied, the colours produced are referred to

in the tables by numbers; the explanation of the code used is given in the colour index.

Owing to lack of space in the tables, the R_F values have been multiplied by 100; *e.g.* values recorded as 12, 56 and 88 refer to true R_F values of 0.12, 0.56 and 0.88. In order to facilitate the 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 that was too weak to deserve colour estimation. In a few instances, the uncertainty in colour shades is expressed by a + sign. Reactions with the reagents Mn and Ind are, as a rule, indicated by the signs –, + or + +. The + + sign indicates that a positive reaction was immediately obtained. However, in this investigation, the number 33 often appears for the reagent Ind. This indicates that certain basic compounds become visible owing to the strong absorption of the reagent to the compound, when compared with the background shade. Reagent Bi is indicated as positive without differentiating between the shades. When colours are recorded by a number placed on top of another number, *e.g.* $\frac{24}{57}$, upon spraying a coloured spot (24 in the colour index) immediately appeared, which changed colour (57 in the colour index) within a few seconds. Usually, most colours are unstable and after some time take on a brownish tone; this is caused to some extent by the chemical influence of other reagents used in the vicinity. This change in colour is not recorded in the tables nor are those compounds indicated that, at this low concentration, are visible on the chromatograms because of their own colour. Exceptions are made for DAB and NH reagents, where the colour development is followed at room temperature, and the first observation is made in about 1 h and the final check within 24 h.

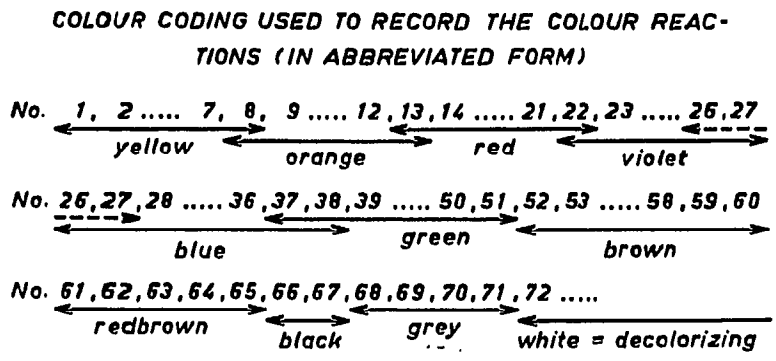

Fig. 1. Colour coding used to record colour reactions (in abbreviated form) as a complement to the colour index for the tables.

DISCUSSION

The earlier finding[5] that aromatic and heterocyclic aldoximes could conveniently be identified with *p*-dimethylaminobenzaldehyde in acetic anhydride (reagent DAB) is confirmed by the series of compounds in Table II. The general pattern of reactivity towards this reagent seems to be that aliphatic aldoximes produce faint yellow or no colour with this reagent. Aromatic aldoximes give preferentially orange colours with compounds containing free hydroxyl groups

in the benzene ring, and in some instances a pink colour is produced (oximes of vanillin, isovanillin and 3-cinnolinealdehyde type). However, many aldoximes lacking free hydroxyl groups in the benzene ring failed to react. Aromatic ketoximes (Table III) seem to be less reactive and only yellow to orange colours are produced. For aliphatic ketoximes no reactions with DAB reagent were recorded.

As before one of the features has been to record unusual colour reactions with compounds that in the literature are not usually described as Ehrlich positive compounds, *e.g.* compounds not containing nitrogen. In order to provide a basis for this cautious interpretation of Ehrlich positive responses (reagents EH and DAC) the following observations were made. Flavonoid compounds have been suspected to interfere with the Ehrlich colour reactions obtained with amino compounds or heterocyclic derivatives of indoles and pyrroles. Since the reports of purple colours with Ehrlich reagent with resorcinol derivatives[3,26] appeared, there have been no direct data indicating flavonoid compounds. As seen in Table I, L-epicatechin (3,3',4',5,7-pentahydroxyflavan, a *cis* diastereomer of D-catechin) gives a magenta colour with EH reagent and a green colour with DAC reagent. Catechin itself is also recorded as positive, although with less pronounced colours. Only the trihydroxy derivatives, hesperetin and naringenin, and the pentahydroxy derivative of flavanone, robinetin, gave positive EH and DAC reactions, in yellow and brown shades, respectively. The presence of a resorcinol configuration of the hydroxyl groups in the molecule was not immediately held responsible for this colour reaction, since daticetin (tetrahydroxyflavone) and quercetin (pentahydroxyflavone), both of which are also 5,7-dihydroxy derivatives, are negative and this might indicate a more complicated colour-forming mechanism than the requirement of a free 4 or 6 position for simple resorcinol derivatives giving purple colours.

Among other interfering colour reactions, the following compounds produced yellow colours with EH reagent: glyceraldoxime (Table II), *p*-benzoquinonedioxime (Table III), salicylamide, *trans*-cinnamide (Table IV), gramine-N-oxide and phenazine-N-5-oxide (Table VII). A brown colour was produced by 2,6-dihydroxybenzamide (Table IV) and 2-hydroxypyrimidine (Table VIII). Pink shades were obtained with 4,6-dihydroxypyrimidine and 2-mercapto-4,6-dihydroxypyrimidine. The pink colour obtained with the 2,4,6-trihydroxypyrimidine derivative is reminiscent of the activity of the 2,4,6-trihydroxyacetophenone[27] and seems to be positively correlated with this compound. Even 4,6-dihydroxypyrimidine (hydroxyl groups in the *meta* position), because of the pink shade developed, belongs to the same group of "erroneous" Ehrlich positive compounds as resorcinol derivatives[26].

The DAC reagent, which is more sensitive and with a broader colour range, produced the following unusual colour reactions: yellow with *p*-benzoquinonedioxime, 2,5-dihydroxyacetophenone oxime and menadione monoxime (Table III) and magenta with benzamide, *m*-nitrobenzamide, *o*-hydroxybenzamide, *o*- and *p*-methoxybenzamides, 2,6-dihydroxybenzamide, piperonylamide and *trans*-cinnamide, but not with mono- or di-N-alkyl-substituted benzamides (Table IV). The usefulness of DAC reagent for detecting unsubstituted aromatic acid amides is thereby clearly indicated. On average the colour response reached its maximum intensity in about two hours at room temperature, although the single benzamides showed great variations in speed of colour development. Several N-nitroso compounds (Table VI), as well as all N-oxides of tertiary amines (Table VII), also gave magenta

colours in contrast with the corresponding parent bases, which were DAC negative. Blue colours were obtained with 4,6-dihydroxypyrimidine, 6-aminouracil, 2-mercapto-6-aminouracil, 2-mercapto-4-amino-6-hydroxypyrimidine, 4-amino-2,6-dihydroxypyrimidine and 2,4-diamino-6-hydroxypyrimidine. Rare green colours were obtained with 2-mercapto-4,6-dihydroxypyrimidine and 2-amino-4,6-pyrimidinedione (Table VIII).

Dragendorff reagent (Bi) was reported in previous supplements to be a valuable adjunct in detecting alkaloid compounds, aromatic bases in general and certain N-containing drugs[3–5]. From Table I it can be seen that the flavonoid derivatives, datiscetin, malvin chloride, myricetin and pelargonidin chloride, are Bi positive. A few other compounds, benzoylacetone oxime (Table III), N-nitrososarcosine (Table VI), 2-methylmercaptopyrimidine, 2-mercapto-4-methylpyrimidine and 4,6-diaminopyrimidine (one of the few Bi positive aminopyrimidines, Table VIII) and 3-methylindole (Table IX) gave Bi positive reactions which can in certain circumstances be indicative of alkaloids.

Concerning the unexpected positive responses with the hippuric acid reagent (DAB) with oxime derivatives, as mentioned earlier, the following compounds were also recorded as DAB positive: epicatechin, magenta (Table I); *p*-chloroaniline and *o*-ethylaniline, pale yellow (Table V); nicotinic acid N-oxide, pale yellow (Table VII); 2-mercaptopyrimidine, yellow; 6-aminouracil, 2-mercapto-6-aminouracil and 2-amino-4,6-pyrimidinedione, pink (Table VIII); and 7-methylindole, pink (Table IX). Ninhydrin reagent (NH) was not expected to reveal the compounds studied in this supplement; however, the following reactions were noted: thiophene-2-aldoxime, pale brown (Table II); *o*-acetoacetylphenyloxime and menadione monoxime, red-violet (Table III); *m*- and *p*-chloroanilines and mesidine, pink (Table V); 2-mercaptopyrimidine and 2,5-diamino-4,6-dihydroxypyrimidine, red-violet (Table VIII); and 5-amino-2,3-dimethylindole, brown (Table IX). The red-violet colour produced with some of these compounds can easily be confused with that of an amino acid derivative.

With the other detection reagents used for screening these compounds the most interesting results were as follows. With diazonium reagents (D_3 and D_4) a blue colour was obtained with vanillin oxime, ethylvanillin oxime and monophthalaldoxime (Table II). Orange colours were produced by 4,6-diaminopyrimidine, 2-mercapto-6-aminouracil, 4,6-dihydroxypyrimidine and 2,4-diamino-6-hydroxypyrimidine (Table VIII). Most of the mercaptopyrimidine derivatives (Table VIII) gave brownish colours with 2,6-dibromoquinone-4-chloroimide reagent (DB). Similar brownish and red-brownish colours were observed earlier with compounds which contained a free mercapto group; S-methylation as a rule depresses the colour formation. A green colour with DB reagent was obtained with 2-hydroxy-3-methylbenzaldoxime (Table II) and with *o*-hydroxybenzamide (Table IV), which was regarded as a more unusual response. Ferric chloride reagent (Fe) gave red-brown colours with 2-methylmercaptopyrimidine, 2-mercapto-4,6-dihydroxypyrimidine (Table VIII), all N-nitroso compounds (Table VI) and most aldoximes (Table II) and ketoximes (Table III). A few N-oxides of tertiary amines gave brownish or red-brown colours with Fe reagent.

Coming to the results obtained by the analysis of the approximately 1000 R_F values listed in this supplement, a few introductory comments are appropriate. When

presenting the six R_F values for the different solvent systems in a certain order for each compound (preferentially in a diagram), a number of characteristic mobility patterns was obtained. The majority of the compounds followed a regular pattern, but for certain compounds still very individual patterns, dependent on the solubility properties of a particular compound in these solvents. From these simple characteristics another selection of compounds could be made on the basis of larger deviations from this regular mobility pattern due to the total number and positions of hydrophilic and hydrophobic substituents in the molecule. In the first place, the compounds which showed a sudden drop or elevation of R_F values for certain solvents, compared with the regular decrease from one solvent to another, in a given order, were of great interest.

Regular mobility was defined in an earlier review on this subject[6] as occurring when the R_F values for neutral compounds for these solvents, arranged in the order F>E>A>B>C>D, showed a continuous decrease. The presence of an acidic function in the molecule, not masked by a basic function, was indicated by a change in the partial sequence of the R_F values for these solvents, F>E<A. For a basic function, a partial sequence of R_F values, F<E>A, was found to be highly significant. Any other variations in these three basic criteria were defined as irregular, and in numerous cases could be correlated with the position, number and type of substituents present.

It was found earlier that simple 1,3- and 1,4-dihydric phenol derivatives showed a sudden R_F value increase in solvent C, according to the partial sequence B<C>D, in conjunction with neutral or acidic compounds. For more complicated structures such as flavone derivatives (Table I), which are polyhydroxy compounds and several of which contain 5,7-dihydroxy (compared with 1,3-dihydroxy) substituents, the mobility pattern turns out to be more like a regular one. Naringenin only shows B<C>D elevation due to the hydroxyl groups in positions 5 and 7, and produces an irregular pattern. Any greater number of hydroxyl groups seems to abolish this irregularity and the R_F elevating effect in solvent C is proved valid and is limited to a maximum of three hydroxyl groups per molecule. This is also seen from the mobility of the penta- and hexa-hydroxy derivatives, where the R_F values in solvents B, C and D are close to zero. Compounds of this type, with a sugar moiety attached to a hydroxyl group, show very low mobilities even in solvents F, E and A.

On the other hand, when the aromatic aldehydes are converted to aldoximes (ketones to ketoximes) an interesting increase in the mobility in solvent C is obtained for 1,3- and 1,4-hydroxyaldoximes (and the corresponding ketoximes), similar to the mobility pattern found earlier for 1,3- and 1,4-dihydric phenols. 5-Formaldoximosalicylic acid, isophthaldoxime (Table II), 2,5-dihydroxyacetophenone oxime, 3,4-dihydroxypropiophenone oxime and *m*-hydroxypropiophenone (Table III) show an irregular mobility in the sequence B<C>D, which does not occur with the corresponding parent compounds in these solvents. This conversion of aldehydes to oximes before chromatography provides a helpful and selective means for the identification of hydroxyaldehydes. Two indolealdoximes, 3-indolealdoxime and 3-indoleacetaldoxime (Table II), also followed this pattern. The seemingly analogous R_F elevating effect in solvent C, recorded earlier for hydroxyindoles (without a carboxyl function), seems to be in accordance with this finding; thus indolealdehydes

TABLE I

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME FLAVONOIDS

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		UV	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
84	67	58	0	0	0	D-Catechin (3,3′,4′,5,7-pentahydroxyflavan)[b]	–	10	65	65	11	24	–	51	9 42	++	–	17	44
95	42	93	84	76	15	Datiscetin (2′,3,5,7-tetrahydroxyflavone)[a]	60	62	54 56	53 55	60 63	42	–	50	3 51	++	–	–	–
82	3	35	0	0	0	2,3-Dihydrorobinetin	48	3 58	63 23	13 63	7 59	57	–	70	51 49	+	+	+	–
87	32	81	5	4	0	2,3-Dihydrofisetin	48	+	63	62	7	28	–	51	8 50	+	–	5	+
79	70	46	0	0	0	L-Epicatechin (*cis* diastereomer of catechin)[b]	–	9 62	65	23	10 62	25 68	–	60	68	++	–	22	41
92	9	80	16	2	0	Fisetin (3,3′,4′,7-tetrahydroxyflavanone)	48	9	13	13	9 62	51	–	51	56 43	+	–	–	60
93	12	92	80	76	19	Hesperetin (3′,5,7-trihydroxy-4′methoxyflavanone)	–	3	13	64	8	28	–	–	–	+	–	5	52 23
1	3	0	0	0	0	Hesperidin (hesperetin-7-rutinoside)	–	3	13	64	8	28	–	–	–	+	–	5	52 23
2	0	0	0	0	0	Malvin chloride (3,5-bisglucosyloxy-4′,7-dihydroxy-3′,5′-dimethoxyflavylium chloride)[a,c]	+	22	63	63	17	33	+	+	59	–	–	–	–

88	0	65	0	0	0	Myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone)[a]	3	51 62	55 63	56 63	58 62	42	–	52	3 68	++	–	–	–
93	74	91	79	81	12	Naringenin (4′,5,7-trihydroxyflavanone)	+	8	19	23 65	10	68	–	52 51	40	+	–	17 64	70 26
37	13	1	0	0	0	Neohesperidin (hesperetin-7-neohesperidoside)	–	7	11	63	8 65	33 35	–	57 56	71	+	–	–	–
94	4	22	0	0	0	Pelargonidin chloride (3,4′,5,7-tetrahydroxy-flavylium chloride)[a,c]	64	13	63	63	14	28	+	+	70	+	–	+	+
92	14	91	5	4	0	Quercetin (3,3′,4′,5,7-pentahydroxyflavone)	48	6	63	64	11	48	–	50	3 43	+	–	–	–
76	3	23	5	0	0	Quercitrin (quercetin-3-L-rhamnoside)	56	7	15 63	62	58	47	–	50	8 51	++	–	–	6
75	3	37	0	0	0	Robinetin (3,3′,4′,5′,7-pentahydroxyflavone)	48	47 59	63	64	8 59	56 62	+	52	51 50	+	–	58	8
32	0	0	0	0	0	Rutin (quercetin-3-rutinoside)	65	6	65	64	11	48	–	50	7 69	+	–	58 8	60 23
94	21	90	5	9	0	Morin (2′,3,4′,5,7-pentahydroxyflavone)	10	60	65	66	11	3	–	51	51	++	–	57	51 27

[a] Bi reagent, positive.
[b] DAB reagent, magenta (colour index 22).
[c] Change of colour to 22 with the acidic reagents Fe, DN, EH and DAC.

TABLE II

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME ALDOXIMES

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		UV	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
43	39	8	1	0	0	D-Glyceraldoxime[a]	–	–	–	72	2	–	–	60 56	68	++	+	2	60
92	94	93	77	79	19	Phenylglyoxime[b]	–	–	–	–	5 9	–	–	63	68	+	–	–	–
93	92	92	90	95	79	*o*-Nitrobenzaldoxime	27	–	–	–	–	–	–	–	38	–	–	–	–
92	91	89	90	89	77	*m*-Nitrobenzaldoxime[a]	27	+	60	64	5	–	–	–	45	+	–	–	17
92	92	91	89	87	75	*p*-Nitrobenzaldoxime[a]	23	7	8	60	5	57	–	–	39	+	–	–	17
92	27	87	25	38	1	5-Formaldoximosalicylic acid[c]	34	–	–	–	–	–	–	24	38	+	+	–	–
94	94	92	93	91	90	2-Hydroxy-3-methylbenzaldoxime	–	+	60	56	60	43	+	53	50 68	++	–	–	–
94	93	92	87	89	80	2,3-Dimethoxybenzaldoxime	–	–	–	–	–	57	–	–	38	+	–	–	–
95	94	91	93	91	86	2,5-Dimethoxybenzaldoxime	33	–	–	–	–	–	–	–	38	+	–	–	–
86	86	85	66	57	26	Vanillin oxime[c]	–	22	25	28	28 68	69	–	27 70	52 68	++	–	–	–
91	89	87	88	83	68	Ethylvanillin oxime	–	9	67 25	35 65	28 68	60 69	–	52	68	++	–	–	–
87	86	85	60	46	16	Isovanillin oxime[c]	–	10 53	14 20	60 25	63 65	30 70	–	70	68	+	–	–	–
92	94	92	92	88	84	5-Allylvanillin oxime[b]	–	+	37	64 35	68 60	59	–	69	68	++	–	–	–

87	92	88	92	89	85	5-Propenylvanillin oxime[b]	–	23	43 35	28	26 39	60	–	70	68	++	–	7	–
92	29	89	90	85	56	5-Nitrovanillin oxime[a]	57	–	64	–	70	–	–	–	50 68	+	–	–	–
74	76	58	17	8	0	Phthalaldoxime	–	8	7 43	28	28 23	–	–	–	52	+	–	–	–
88	93	91	63	65	10	Isophthalaldoxime	–	–	–	–	57	–	–	–	–	–	–	–	–
90	95	92	94	93	91	2-Hydroxy-1-naphthaldoxime[a]	–	–	–	64	13	–	–	51	68	+	–	–	–
89	93	91	89	86	78	Thiophene-2-aldoxime[d]	–	–	–	–	–	–	–	62 57	34	+	–	–	–
91	91	88	64	68	36	3-Indolealdoxime[a]	–	12	14	24	23 65	+	–	26 70	68	++	–	–	–
91	91	88	64	56	25	3-Indolealdoxime[a] (double spot, *cis* or *trans* analogue)	–	12	14	24	23	+	–	26	68	++	–	–	–
89	90	87	74	72	10	5-Methoxy-3-indolealdoxime[a]	–	–	+	–	–	+	–	–	38	+	–	–	–
89	90	87	65	50	10	5-Methoxy-3-indolealdoxime[a] (double spot, *cis* or *trans* analogue)	–	–	+	–	–	+	–	–	38	+	–	–	–
85	89	84	89	90	60	3-Indoleacetaldoxime	–	–	–	–	–	–	–	–	39	+	–	23	27 69
86	87	85	82	57	17	3-Cinnolinealdoxime[c]	–	6	7	61	–	–	–	59 62	34	–	–	–	–

[a] DAB reagent, pale yellow (colour index 3–6).
[b] DAB reagent, orange (colour index 9–13).
[c] DAB reagent, pink (colour index 17).
[d] NH reagent, pale brown.

TABLE III

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME KETOXIMES

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		UV	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
18	34	1	2	0	0	Formamide oxime	–	–	–	60 72	6 72	+	–	63	40 68	++	33	–	–
73	8	43	22	5	0	1-Methylhydantoin-5-oxime	–	–	–	–	–	–	–	–	34	+	–	–	–
87	88	84	87	87	84	Cyclopentanone oxime[c]	–	–	–	–	–	–	–	58	–	+	–	–	–
91	93	92	94	91	70	1,3-Cyclohexanedione dioxime[b]	–	–	–	–	–	–	–	–	46 68	+	–	–	–
87	87	86	43	33	5	*p*-Benzoquinone dioxime	56	+	64 65	64 65	7 28	–	–	–	68	++	–	6 9	3 25
86	16	85	31	38	0	Benzoylformic acid oxime[b]	–	–	–	17 72	+	–	–	62	68	++	+	+	+
86	5	85	74	52	3	Benzoylformic acid oxime[b] (double spot, *cis* or *trans* analogue)	–	–	–	17 72	+	–	–	3	68	++	+	–	–
93	92	93	93	87	85	Acetophenone oxime[c]	–	–	+	60	4	+	–	+	68	+	–	–	–
93	94	90	92	88	86	*o*-Nitroacetophenone oxime[b]	+	–	51	51	53	–	–	–	38	+	–	–	–
93	92	91	94	90	84	*m*-Nitroacetophenone oxime[b]	27	–	–	–	–	–	–	–	–	+	–	58	+
94	92	91	94	88	82	*p*-Nitroacetophenone oxime[b]	54	–	–	–	–	–	–	–	–	–	–	–	–
94	92	89	94	91	85	*p*-Methoxyacetophenone oxime[c]	–	–	–	–	–	–	–	+	38	–	–	–	–
65	88	48	76	16	7	*m*-Aminoacetophenone oxime[c]	34	–	+	64 5	12 64	–	–	60	68	++	–	8	19 64
77	85	61	66	25	6	*p*-Aminoacetophenone oxime[c]	–	+	+	62	8 62	+	–	+	68	+	–	9	22 24
92	90	88	58	69	10	2,5-Dihydroxyacetophenone oxime[b]	+	+	+	17 58	59	57 52	–	23 63	49 68	++	–	–	3

93	92	88	86	83	27	3-Methyl-4-hydroxyacetophenone oxime[b]	–	–	+	64 72	8	51	–	62	38 49	++	–	–	60
89	86	82	88	78	37	*m*-Acetovanillone oxime	–	8	64 63	5 25	7 23	37	–	60	68	+	–	–	–
92	93	92	88	91	32	*m*-Hydroxypropiophenone oxime[b]	–	–	–	64	10	+	–	+	49 68	++	–	–	–
87	88	83	33	40	3	3,4-Dihydroxypropiophenone oxime[b]	–	–	62	64 72	62 65	+	–	68	56 68	++	–	–	–
89	91	87	93	77	73	Benzoylacetone oxime[a]	–	–	–	63 5	5 52	–	–	40	40	++	–	–	–
89	91	87	83	77	73	Benzoylacetone oxime[a] (double spot, *cis* or *trans* analogue)	–	–	–	63 5	5 52	–	–	40	40	++	–	–	–
93	92	87	84	82	55	*o*-Acetoacetylphenol oxime[b,d]	–	–	+	9	5	34 71	–	69 23	68	++	–	–	–
93	94	91	89	90	85	2-Hydroxy-4-methoxybenzophenone oxime	–	–	+	63	7	39	–	59	34	–	–	–	–
95	95	92	96	94	91	2-Aminobenzophenone oxime[a,c]	–	–	–	–	7	+	–	–	38	+	–	9	22 24
93	94	92	88	82	80	1-Acetylnaphthalene oxime[a]	–	–	–	–	–	–	–	–	–	–	–	–	–
94	94	93	95	95	92	2-Acetylnaphthalene oxime[c]	–	–	–	–	–	–	–	–	39	+	–	–	–
93	92	92	90	89	83	Menadione monoxime[b,d]	70	+	+	62 5	6 8	52 59	–	63 27	68	++	–	7	60
69	27	39	4	0	0	Saccharin oxime[b] (2-sulphobenzoic acid imidoxime)	34	–	–	–	8	–	–	63	68	+	–	–	–
87	79	76	30	10	0	Ninhydrin oxime[b]	–	–	–	–	–	–	–	57 62	34	+	–	–	–
91	96	90	87	92	88	2-Pyrithione oxime[a]	–	–	–	5	–	38	–	71 28	38	+	–	–	–

[a] Bi reagent, positive.
[b] DAB reagent, pale yellow (colour index 3–6).
[c] DAB reagent, orange (colour index 9–13).
[d] NH reagent, red-violet.

TABLE IV

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME BENZAMIDE DERIVATIVES

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		UV	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
84	82	77	86	55	42	Benzamide	–	–	–	–	–	–	–	–	–	–	–	+	22
85	84	85	92	92	70	N-Methylbenzamide[a]	–	–	–	–	–	–	–	–	–	–	–	–	17
86	86	83	96	94	89	N-Dimethylbenzamide[a]	–	–	–	–	–	–	–	–	–	–	–	–	–
92	91	87	95	94	90	N-Diethylbenzamide[a]	–	–	–	–	–	–	–	–	–	–	–	–	–
87	88	84	85	79	33	*m*-Nitrobenzamide[b]	+	–	–	–	–	–	–	–	–	–	–	–	22
92	90	86	94	94	88	*m*-Nitro-N-dimethylbenzamide[a,b]	+	–	–	–	–	–	–	–	–	+	–	–	–
92	79	86	76	69	44	*o*-Hydroxybenzamide (salicylamide)	33	7	8	65	10	41	–	23	–	++	–	6	22
83	82	82	87	79	64	*o*-Methoxybenzamide	–	–	–	–	–	–	–	–	–	+	–	+	22
87	85	79	96	91	85	*o*-Methoxy-N-dimethylbenzamide[a]	–	–	–	–	–	–	–	–	–	+	–	–	–
89	92	83	95	89	86	*o*-Methoxy-N-diethylbenzamide[a]	–	–	–	–	–	–	–	–	–	–	–	–	–
82	79	77	85	52	41	*p*-Methoxybenzamide[a]	–	–	–	–	–	–	–	–	–	+	–	–	22

88	89	87	96	90	88	*p*-Methoxy-N-dimethylbenzamide[a]	–	–	–	–	–	–	–	–	–	–	–	–	–
90	92	90	96	90	92	*p*-Methoxy-N-diethylbenzamide[a]	–	–	–	–	–	–	–	–	–	–	–	–	–
90	54	90	75	45	5	2,6-Dihydroxybenzamide	34	8 63	19	19 23	10 63	28	–	24	68	++	–	26 62	22
87	93	76	85	48	35	Piperonylamide	–	–	–	–	–	–	–	–	–	–	–	–	17 22
88	89	88	96	90	86	Phenyl-N-dimethylacetamide[a,b]	–	–	–	–	–	–	–	–	–	–	–	–	–
94	93	90	95	90	88	Phenyl-N-diethylacetamide[a]	+	–	–	–	–	–	–	–	–	–	–	–	–
87	87	83	85	58	29	*trans*-Cinnamamide[a]	–	–	–	–	–	–	–	–	–	++	–	9	22
87	88	87	96	90	90	N-Dimethylcinnamamide[a,b]	+	–	–	9	5	–	–	7	+	++	–	–	–
94	92	90	95	91	88	N-Diethylcinnamamide[a]	34	–	–	9	6	–	–	–	–	+	–	–	–
85	87	81	86	77	54	Tetrahydrophthalimide	+	–	–	–	–	–	–	–	–	++	–	–	–
73	5	42	8	4	2	3,6-Dihydroxyphthalimide	40	7	11	11	60	56	–	51	69 3	++	–	–	–

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

TABLE V

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME AMINOBENZENE DERIVATIVES AND HETEROCYCLIC BASES

R_F values × 100						*Compounds*	*Detection*												
F	*E*	*A*	*B*	*C*	*D*		*UV*	*D_1*	*D_2*	*D_3*	*D_4*	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
94	95	90	96	88	87	*o*-Chloroaniline	–	–	+	60	6	–	–	–	–	+	–	8	22 19
94	94	90	94	91	90	*m*-Chloroaniline[a]	–	7	+	64	8	–	+	–	–	++	–	9	15 19
92	93	90	95	84	83	*p*-Chloroaniline[a,c]	–	–	–	8 64	6	+	–	–	34	++	–	9	15 19
93	95	90	96	91	89	*o*-Bromoaniline[a]	–	–	+	60	6	–	–	–	34	+	–	8 17	17 22
92	92	88	85	91	83	*m*-Bromoaniline[c,e]	–	–	+	60	8	–	–	–	–	++	–	8	13 65
93	95	92	93	93	90	4-Amino-2-nitrotoluene	56	–	–	11	+	+	–	–	–	+	–	11	19 23
95	95	94	91	89	84	2,4-Dinitroaniline	3	–	–	–	–	–	–	–	–	–	–	–	–
85	95	84	94	84	79	*o*-Ethylaniline[a,c]	–	17	+	63 64	13 8	53 38	–	–	56 70	+	–	2	62 19
79	96	84	92	87	85	2,4,6-Trimethylaniline (mesidine)[a,c]	–	–	+	64	8	–	–	+	59 68	++	–	7	12
40	81	16	43	1	0	2,6-Diaminotoluene	27	63	65 64	65 19	63	28	+	–	68	++	–	9	65
95	97	94	93	93	90	4-Aminoazobenzene[e]	52	–	–	–	4	–	12	11	+	+	–	12	65 25
45	93	29	88	23	18	4,4'-Diaminodiphenylmethane[b,c,e]	–	–	+	12	10	56	–	57	56 68	++	–	11	64 65

90	94	92	77	58	19	4,4′-Diaminodiphenylsulphone[b,c]	–	–	–	60	7	+	17	–	–	+	–	11	65
58	60	3	0	0	0	2-Aminobenzimidazole	+	–	–	–	+	+	–	–	71	+	33	–	–
76	84	67	90	67	60	2-Acetylaminopyridine[e]	34	–	–	–	–	+	17	+	–	–	–	–	–
34	88	17	89	24	18	6-Methyl-2-pyridinealdehyde[e]	–	–	–	–	–	–	+	–	–	+	–	–	–
70	89	58	78	37	31	2-Amino-5-chloropyridine[b,c,e]	34	–	–	–	–	+	–	7 59	60 34	–	33	9	22 23
74	96	68	93	80	48	Quinoline[e]	–	–	–	63	8	27	+	–	+	+	–	59	57
57	91	44	92	58	54	Isoquinoline[e]	+	–	–	–	–	–	–	–	–	–	–	–	–
80	94	79	94	83	82	1-Hydroxyacridine[e]	71	15 65	56	33 68	13	62 33	8	58 60	62 52	+	–	8	8
96	97	90	96	95	90	Phenazine[e]		+	60 62	71	70	+ 50	17 26	28 50	23 63	+	–	17 44	59 25
18	90	2	20	0	0	Pyrrolidine[b,e]	–	–	–	10	7	24	–	60	–	+	33	–	–
63	95	5	78	3	2	Pyrrole[b,e]	57	60 5	64	64	10 64	69	59 27	57 52	52 68	+	33	22 23	69 28
71	78	55	78	34	24	4-Methyl-5-β-hydroxyethylthiazole[e]	–	–	–	–	–	–	–	7 58	8 40	+	–	–	+
54	84	4	7	0	0	1-Adamantanamine[e]	–	–	–	–	–	–	–	–	–	–	33	–	–
87	93	87	93	92	86	1-Azapyrene (1-aza-benzo(d,e,f)phenanthrene)[e]	7	–	–	–	–	–	+	3	–	–	–	5	59
52	45	24	90	18	12	1,10-Phenanthroline[d,e]	34	–	–	–	–	–	–	57	–	+	33	–	–

[a] NH reagent, pink.
[b] NH reagent, pale brown.
[c] DAB reagent, pale yellow (colour index 3–7).
[d] DAB reagent, pale violet.
[e] Bi reagent, positive.

TABLE VI

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME N-NITROSO COMPOUNDS

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		UV	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
4	0	0	0	0	0	N-Nitrosodiethylamine[a]	–	–	–	9	6	70	–	62	38	–	–	5	+
19	0	0	2	0	0	N-Nitrosopyrrolidine[b,c]	–	–	–	9	7	23	–	62	38	+	–	+	62 22
24	0	0	22	0	0	N-Nitrosopiperidine[a,c]	–	–	–	10	6	23	–	62	48	–	–	–	62 22
14	0	0	7	0	0	N-Nitrosomorpholine[a,c]	–	–	–	10	7	23	–	62	38	+	–	–	62 22
4	0	0	0	0	0	N-Nitroso-4-hydroxyproline[b,c]	–	–	–	9	6	+	–	62	38	–	–	17	–
85	4	64	45	19	1	N-Nitrososarcosine[c]	–	–	–	–	–	–	–	–	–	–	++	–	22

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

TABLE VII

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME N-OXIDES

R_F values × 100						*Compounds*	*Detection*												
F	*E*	*A*	*B*	*C*	*D*		*UV*	*D_1*	*D_2*	*D_3*	*D_4*	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
45	1	26	39	0	0	3-Hydroxypyridine-N-oxide	34	62	51 27	38 23	9 62	38	–	62	39	++	–	–	–
57	36	23	79	22	16	2-Picoline-N-oxide[a,b]	–	–	–	–	–	60	–	3	48	–	–	–	22
64	48	37	87	55	35	2,4-Lutidine-N-oxide[b]	–	–	–	–	–	–	–	–	–	–	–	3	22
41	0	7	41	0	0	Nicotinic acid N-oxide[a]	–	–	–	–	–	–	–	–	–	23	++	–	22
59	8	7	7	0	0	Gramine-N-oxide	–	–	–	64	7	+	17	58	59 71	+	33	58	22 24
71	64	62	83	67	64	Quinoline-N-oxide[b]	–	60	62	65	8 65	+	–	52	38	+	–	+	22
88	86	90	95	91	87	Phenazine-N-5-oxide[b]	58	–	–	–	–	–	8	–	8	–	–	8	9 60
42	3	3	78	3	2	Strychnine-N-oxide[b]	–	–	–	–	–	–	–	–	–	–	–	–	17 22

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

TABLE VIII

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME PYRIMIDINE DERIVATIVES

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		UV	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
31	15	3	4	0	0	2,4-Dichloropyrimidine	34	−	−	−	−	−	−	−	34	+	−	−	−
43	0	29	0	0	0	4,6-Dichloropyrimidine	27	−	−	−	−	−	−	−	43	−	−	−	−
21	5	0	7	0	0	2-Hydroxypyrimidine	+	—	−	−	−	+	−	−	−	++	−	60	−
47	2	1	23	7	2	2-Mercaptopyrimidine[a,b]	27	−	+	+	5	+	+	−	38	−	−	−	−
27	37	0	15	1	0	2-Methylmercaptopyrimidine[b,c]	+	8	+	+	60	+	+	63	45	+	−	−	−
28	39	0	14	1	0	2-Methylmercapto-4-mercaptopyrimidine[b,c]	+	−	−	−	6	59	−	−	38	+	−	−	−
56	39	30	42	20	13	2-Mercapto-4-methylmercaptopyrimidine[b,c]	+	−	−	−	6	58	−	−	38	+	−	−	−
91	95	90	92	90	87	2,4-Dimethylmercaptopyrimidine[c]	+	−	−	−	−	+	−	−	+	+	−	−	−
22	0	0	1	0	0	4,6-Dihydroxypyrimidine	34	6	62	12	6	−	−	+	−	−	−	14 22	25 35
44	0	9	3	0	0	6-Aminouracil (6-amino-2,4-dihydroxy-pyrimidine)[b,d]	−	−	+	9 65	6	52	−	−	−	+	−	14 22	32 35
48	5	12	5	0	0	2-Mercapto-6-aminouracil[d]	−	+	60 64	14 19	8	57	−	−	38	+	−	22	32 35
50	5	12	5	0	0	2-Mercapto-4-amino-6-hydroxypyrimidine[d]	−	−	62 65	64 65	7	+	−	−	−	+	−	22	32
51	2	14	1	0	0	2-Mercapto-4,6-dihydroxypyrimidine[d,e]	−	7	64 19	62 20	6	71	13	60	34	+	−	15	44 35
69	0	37	11	2	0	2-Mercapto-5-carboxyuracil	−	−	−	−	−	+	−	4	+	+	+	−	−

66	15	34	17	3	0	2-Mercapto-5-carbethoxyuracil[b]	+	–	–	–	–	71	–	–	–	+	–	–	–
72	28	52	39	8	2	Thiothymine (2-mercapto-4-hydroxy-5-methyl-pyrimidine)	–	–	–	–	–	+	–	–	34	+	–	–	–
13	13	0	1	0	0	5-Methyl-2-thiocytosine (2-mercapto-4-amino-5-methylpyrimidine)[b]	–	–	–	–	–	+	–	–	–	+	–	–	–
80	29	54	51	12	0	2,4-Dimercapto-5-methylpyrimidine[b,e]	8	–	–	8	+	+	–	34	42	++	–	–	–
2	11	0	0	0	0	2-Methyl-4-amino-5-aminomethylpyrimidine[e]	–	–	–	–	–	–	–	–	–	–	–	+	22
5	0	0	0	0	0	2-Amino-4,6-pyrimidinedione[d]	–	–	+	13	6	–	–	–	–	–	–	17 13	40 25
16	0	0	0	0	0	4-Amino-2,6-dihydroxypyrimidine[d]	34	+	63	13 65	5	–	–	+	–	–	–	17 14	33 29
23	48	0	22	0	0	2,4-Dimethyl-6-aminopyrimidine[c,e]	–	–	–	–	–	–	–	–	–	–	33	+	21
13	1	0	0	0	0	2,4-Diamino-6-hydroxypyrimidine[b]	–	–	+	13 65	6	–	–	–	–	–	–	–	30
0	0	0	0	0	0	2,5-Diamino-4,6-dihydroxypyrimidine[a]	–	–	62	–	–	+	–	–	45 38	–	–	–	22
0	0	0	0	0	0	4,5-Diamino-2,6-dihydroxypyrimidine	34	–	62	–	–	–	–	–	42	–	–	–	–
16	15	0	0	0	0	4,6-Diaminopyrimidine[b,c,e]	–	–	+	13	8	–	–	–	58	–	33	8	22
15	8	0	0	0	0	4,6-Diamino-2-mercaptopyrimidine	57	8	8 60	64 8	7	60	–	–	58 40	+	–	8	27

[a] NH reagent, red-violet.
[b] DAB reagent, pale yellow.
[c] Bi reagent, positive.
[d] DAB reagent, pink.
[e] NH reagent, yellow-brown.

TABLE IX

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME INDOLE DERIVATIVES

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		UV	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
87	83	81	69	42	18	2-Carbamylindole[a]	–	7	64	65	62	+	–	–	–	–	–	17 27	23
96	94	94	96	94	93	3-Methylindole (skatole)[a,c]	–	7	+	62	60	–	+	+	46	+	–	23 38	25
93	90	92	93	82	82	2-Hydroxyskatole	–	–	–	–	–	–	–	–	33	+	–	58	23
94	93	90	95	92	92	7-Methylindole[b,d]	34	59	+	64	9	24 25	17	71 51	43 68	+	–	22 23	43 35
74	69	37	17	3	1	5-Hydroxyoxindole	–	9 62	61 23	24	17 63	–	–	58	38	+	–	8	+
91	92	89	92	82	69	3-Acetylindole	34	–	–	–	57	+	+	–	34	+	–	+	–
93	94	88	78	79	53	2,3-Dimethyl-6-hydroxyindole[a]	–	8 13	63 19	23 64	11	17	–	70 52	45	+	–	37 40	23 61
92	93	89	77	78	52	2,3-Dimethyl-5-hydroxyindole[a]	34	6 13	23	70 24	7 62	38	–	+	45	+	–	71 53	27 70
95	96	92	94	93	91	2,3-Dimethyl-6-methylmercaptoindole[c]	–	8	7 59	23 61	13	69 70	58 34	48	38	++	–	71	27 25
76	88	17	20	2	0	5-Amino-2,3-dimethylindole[d,e]	50	23 63	64	65 63	53 66	55 69	+	68 55	38 68	++	–	10	62 65
94	95	92	92	93	91	1,2,3,4-Tetrahydro-7-methylmercaptocarbazole[c]	34	6	7	60	13	70	+	–	38	+	–	42	27 23

92	26	91	89	95	88	1-Methylindole-2-carboxylic acid	34	6 60	+	+	8	+	−	58	34	+	−	17 27	70 24
85	3	74	8	4	0	5-Hydroxyindole-2-carboxylic acid	33	12 56	52 24	25	17 64	−	−	+	39	+	−	17 71	40 26
92	20	94	87	81	47	5-Methylindole-2-carboxylic acid	34	8	58 24	63 65	64 10	−	−	+	−	+	−	17 27	71 23
94	7	88	84	61	17	6-Methoxyindole-2-carboxylic acid	−	8	52 68	28	64 65	+	70	26	−	+	+	26 70	70
88	16	89	88	75	34	7-Methoxyindole-2-carboxylic acid	33	8	27	63 23	62	−	−	58	34	+	+	27 71	40 24
86	9	92	68	64	45	Indole-3-carboxylic acid	−	−	−	−	−	+	−	58	−	+	+	17	24
85	89	84	89	90	60	3-Indoleacetaldoxime	−	−	−	−	−	−	−	−	39	+	−	23	27 69
90	93	88	85	77	56	5-Methoxy-3-indolealdehyde[a]	34	−	−	−	−	+	5	60	−	+	−	6 70	24 17
90	91	87	89	85	58	5-Methoxytryptophol	−	−	+	63	9	25	−	3	5 42	++	−	24 28	25 28
52	1	2	0	0	0	D,L-α-Methyltryptophan[d]	−	−	−	−	−	−	−	−	34	+	−	17 23	24 25
30	15	6	16	0	0	Adrenochrome semicarbazone	64	−	−	−	45	52	2	59	−	+	−	51	56

[a] DAB reagent, pale yellow.
[b] DAB reagent, pink.
[c] Bi reagent, positive.
[d] NH reagent, pale yellow-brown.
[e] DAB reagent, grey.

TABLE X

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME SULPHONAMIDES

R_F values × 100						Compounds	Detection												
F	E	A	B	C	D		UV	D_1	D_2	D_3	D_4	DB	DN	Fe	Mo	Mn	Ind	EH	DAC
84	82	67	8	4	0	Sulphanilamide[a]	–	–	–	64	6	+	–	–	–	+	–	9	19
95	92	90	96	91	81	N′,N′-Diethylsulphanilamide[a]	–	–	–	–	–	60	–	–	–	–	–	8	65
64	62	22	2	0	0	Sulphaguanidine (N′-amidinosulphanilamide)[a]	–	–	–	62	7	–	+	–	46	+	33	9	63 65
87	24	82	76	45	0	Sulphadiazine (2-sulphanilamidopyrimidine)[a]	+	–	–	–	–	–	–	–	–	–	33	7	62 65
90	48	89	92	63	0	Sulphameter (5-methoxysulphadiazine)[a]	–	–	–	–	17	–	+	–	–	+	33	8	62 65
91	46	88	83	54	2	Sulphamethazine (4,6-dimethyl-2-sulphanilamidopyrimidine)[a]	+	–	–	–	–	–	–	–	–	+	33	8 51	63 65
84	38	70	40	9	2	Sulphathiazole (2-sulphanilamidothiazole)[a]	–	8	10 64	23 65	8	–	–	–	34	+	–	8	19
94	23	88	79	40	6	Sulphamethizole (2-sulphanilamido-5-methyl-1,3,4-thiadiazole)[a]	–	–	–	–	–	43	–	–	–	+	–	8	65

90	37	82	83	44	15	Sulphamerazine (2-sulphanilamido-4-methylpyrimidine)[a,b]	–	–	–	–	–	–	–	–	34	+	–	9	19
78	47	82	63	16	2	N′-Acetylsulphapyridine (2-sulphanilamido-pyridine)[a,c]	33	–	–	–	–	–	–	–	5 34	–	–	6	22
95	32	88	81	92	2	Salazopyrin (salicylazosulphapyridine)	56	–	–	–	–	–	–	59	48	+	–	6	+
94	22	90	68	24	0	Salazothiazole (salicylazosulphathiazole)	56	–	+	62	10	8 56	–	61 53	8	+	–	–	–
95	28	94	92	92	89	Acetyl sulphisoxazole (N′-acetyl-N′-(3,4-dimethyl-5-isoxazolyl)sulphanilamide)[a]	–	–	–	–	–	–	–	+	+	+	–	8	62 22

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

Abbreviations used in Tables I–X.

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 = Diazotized sulphanilic acid (0.3% solution in dioxan–water, 1:2).
D_2 = Diazotized 4-benzoylamino-2,5-dimethoxyaniline (0.6% solution in dioxan–water, 1:2).
D_3 = Diazotized *o*-dianisidine (0.6% solution in dioxan–water, 1:2).
D_4 = *p*-Nitrobenzenediazonium fluoborate (0.4% solution in dioxan–water, 1:2).
DB = 2,6-Dibromoquinone-4-chloroimide (0.5% solution in dioxan–acetone, 4:1).
DN = 2,4-Dinitrophenylhydrazine (approx. 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 (approx. 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

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

converted to N-hydroxy derivatives simulate the hydroxyindole pattern in their partition properties in these solvents.

Table IV contains results for a number of benzamides, which are approximately neutral compounds, produce very regular mobility patterns and can only be distinguished from other types of neutral compounds by a positive DAC reaction. Mono- or dihydroxy substitution of the benzene ring, however, introduces acidic properties, as seen by a considerable decrease in the R_F value in solvent E, producing a more acid-like partial mobility pattern, F>E<A.

All the bases recorded in Table V showed in addition to an increase in their R_F values in solvent E, elevation in solvent B according to a total mobility pattern F<E>A<B>C>D. This type of mobility was earlier found to be valid for alkaloids in general. By closer studies of these mobilities (see ref. 6), it was possible to subdivide these apparently similar patterns into four characteristic R_F patterns, depending on how the R_F value behaved in solvent F, A or C in relation to the peak heights in solvents E and B. If all these patterns are presented in diagrams, it becomes evident that three of the four basic patterns for both alkaloids and aromatic bases of non-biological origin were different. Only one pattern overlapped in this respect, but several of the amines which gave irregular patterns were also Dragendorff (Bi) reagent positive, which can make judgements for the presence of alkaloids unspecific when basic drugs or their decomposition products are present in a biological sample.

N-Nitrosoamines (Table VI), not previously studied in detail in these solvent systems, showed very low mobility in all solvents except F. In a few cases an R_F elevation effect (*e.g.*, N-nitrosopiperidine) in solvent B was noticed. More compounds in this series need to be investigated in order to establish the limits of possible separation possibilities for these solvents. A good identification seemed to be a red-brown colour with Fe reagent. N-Nitrososarcosine, however, gave a relatively normal mobility pattern in all solvents.

N-Oxides of heterocyclic bases (Table VII) lost their double peak character (in comparison with their parent compounds) in solvent E, consequently exhibiting an F>E>A partial mobility pattern, indicating much lower basicity than the parent compounds, but still exhibiting an A<B>C elevation, indicative of basic heterocyclic structures.

Coming to the R_F value distribution of pyrimidine derivatives, the mono- and diamino and mono- and dihydroxy derivatives showed very low, but regular, mobilities in the solvents, particularly in A, B, C and D. The separation of these compounds occurred in solvents F and E in the range of R_F values 0.0–0.50. Only a few of the listed pyrimidine derivatives, *e.g.* 2,4-dimethyl-6-aminopyrimidine, showed an alkaloid type mobility pattern, being also Bi reagent positive. 2-Mercaptopyrimidine and 2-methylmercaptopyrimidine also belonged to this mobility group.

Indole derivatives presented in Table IX were collected in an attempt to study more closely the remarkable R_F value shift in solvents B<C>D, which was earlier observed[3] for hydroxyindoles and hydroxyskatoles. It was shown that by the monomethylation of a hydroxyindole to a skatole derivative, this R_F value elevation effect remained unchanged[4]. By dimethylation, examples being 2,3-dimethyl-6-hydroxyindole and 2,3-dimethyl-5-hydroxyindole, it becomes evident

that this characteristic feature still exists, but is reduced in that the R_F value in solvent B almost equals that in solvent C. In this connection, it is of interest to note that other indole derivatives in general show regular mobility patterns. Only a few indole derivatives, so far, were found to give an alkaloid pattern. From Table IX the following indoles showed this double peak mobility: 5-amino-2,3-dimethyl indole and 3-indoleacetaldoxime, the latter being in this respect slightly masked by another effect, described earlier for hydroxyindoles.

It has been customary to present in these supplements some mobility data for certain types of drugs which are commonly used and in wide circulation. Table X gives the mobility data for sulphonamide derivatives; in previous supplements many tranquillizers of the phenothiazine type were analyzed. The main reason for this change has been to record some unusual colour reactions and possible irregular mobilities, which taken together demonstrate the need for caution when examining and interpreting results from biological extracts where compounds of this type, and others, could interfere in the search for indoles and normal metabolites.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. T. Gustafson, Department of Developmental Biology, Wenner-Gren Institute, Stockholm, Sweden, for the gift of a number of pyrimidine derivatives. Thanks are also due to Dr. N. Bauman, Department of Metabolic Chemotherapy, Lederle Laboratories, Pearl River, N.Y., U.S.A., for samples of indole-2-carboxylic acid derivatives, to Dr. N. J. Roth, Pharmaceutical Institute, University of Bonn, Bonn, G.F.R., for 2,3-dimethylindole derivatives, to Dr. M. C. Archer, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A., for 1-methylhydantoin-5-oxime and N-nitrososarcosine, to Dr. H. B. Bosmann, Department of Pharmacology, University of Rochester School of Medicine, Rochester, N.Y., U.S.A., for formamidoxime and to Lonza AG, Basel, Switzerland, for samples of α-picoline-N-oxide and nicotinic acid N-oxide.

REFERENCES

1 L. Reio, *J. Chromatogr.*, 1(1958) 338; *Chromatogr. Rev.*, 1 (1959) 39.
2 L. Reio, *J. Chromatogr.*, 4 (1960) 548; *Chromatogr. Rev.*, 3 (1961) 92.
3 L. Reio, *J. Chromatogr.*, 13 (1964) 475.
4 L. Reio, *J. Chromatogr.*, 47 (1970) 60.
5 L. Reio, *J. Chromatogr.*, 68 (1972) 183.
6 L. Reio, *J. Chromatogr.*, 48 (1970) 11.
7 J. B. Harborne, *Comparative Biochemistry of Flavonoids*, Academic Press, New York, 1967, p. 126.
8 K. Herrmann, *Z. Lebensm.-Unters.-Forsch.*, 148 (1972) 290.
9 B. Heinemann, *Appl. Microbiol.*, 23 (1972) 91.
10 Anonymous, Report of a colloquium, *Arzneim.-Forsch.*, 21 (1971) 752.
11 M. C. Archer, S. D. Clark, J. E. Thilly and S. R. Tannebaum, *Science*, 174 (1971) 1341.
12 S. S. Mirvish, L. Wallcave, M. Eagen and P. Shubik, *Science*, 177 (1972) 65.
13 H. Herrmann, *Naturwissenschaften*, 47 (1966) 162.
14 M. Polonovski, *Bull. Soc. Chim. Fr.*, 21 (1917) 191.
15 J. G. Davies, *Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg.*, Abt. 2, 100 (1939) 273.
16 E. C. White and H. J. Hill, *J. Bacteriol.*, 45 (1943) 433.

17 A. J. Kluyver, J. P. van der Welt and A. J. van Triet, *Proc. Nat. Acad. Sci. U.S.*, 39 (1953) 583.
18 R. Nery, *Biochem. J.*, 122 (1971) 503.
19 S. S. Epstein and I. B. Saporoschetz, *Experientia*, 24 (1968) 1245.
20 H. B. Bossmann, *Chem.-Biol. Interactions*, 3 (1971) 429.
21 K. W. Bentley, *Natural Pigments*, Vol. IV, Interscience, New York, 1960, p. 178.
22 E. Stephen-Sherwood, J. Oro and A. P. Kimbell, *Science*, 173 (1971) 446.
23 E. Ochiai, *Aromatic Amine Oxides*, Elsevier, Amsterdam, 1967, p. 26.
24 E. H. Rodd, *Chemistry of Carbon Compounds*, Vol. III A, Elsevier, Amsterdam, 1954, p. 247.
25 E. H. Rodd, *Chemistry of Carbon Compounds*, Vol. III A, Elsevier, Amsterdam, 1954, p. 551.
26 R. M. Acheson and I. Turner, *J. Chromatogr.*, 7 (1962) 520.
27 C. Steelink, *Nature*, 184 (1959) 720.