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'OURTH SUPPLEMENT FOR THE PAPER CHROMATOGRAPHIC EPARATION AND IDENTIFICATION OF PHENOL DERIVATIVES .ND RELATED COMPOUNDS OF BIOCHEMICAL INTEREST, 'SING A "REFERENCE SYSTEM"

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UMMARY

Paper chromatographic mobility data in six solvent systems are given for further 160 compounds. All the compounds were also checked against fifteen standard colour reagents and positive reactions are recorded in ten tables. The following types of compounds are covered: phenolic natural products; aliphatic and aromatic aldoximes and ketoximes; benzoic acid derivatives; aliphatic, aromatic and heterocyclic amino acid derivatives, pyrimidine and purine derivatives; and alkaloids and drugs used mainly in psychiatry. The paper chromatographic mobility patterns are discussed with reference to earlier results. Interesting similarities in paper chromatographic mobilities were observed for 1,3- and 1,4-monohydroxybenzaldoximes, which showed the typical patterns recorded earlier for 1,3- and 1,4-dihydric phenols. All the bases from the nucleic acids series showed very low mobilities in all solvents, as expected. It was shown that a small degree of substitution of the bases can alter considerably the characteristics of the mobility patterns and increase the general mobility in all solvents. In particular, N-substituted purines produce mobility patterns that are similar to those recorded earlier for alkaloids in general. DAB reagent (*p*-dimethylaminobenzaldehyde in acetic anhydride) was found to be useful for the detection of aromatic and heterocyclic aldoximes by the production of a pink colour.

NTRODUCTION

This supplement is a continuation of a previous series of investigations on phenolic natural products and compounds involved in the general metabolism of mammals and plants. The emphasis on the selection of the vast number of compounds involved has varied in the different supplements in the series, so as to cover as many types of compounds as possible and yet to present for these compounds a uniform collective data source for their paper chromatographic (PC) mobility characteristics in six selected PC solvent systems together with the information provided by the use of fteen standard spray reagents for detection purposes.

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In the original introductory work[1], results were presented for most of the commonly occurring phenolic compounds of both natural and synthetic origins (in order to find distinguishing features among these compounds, as far as the detection is concerned), together with a great number of metabolic products from moulds. This work was later extended in the first[2] and second[3] supplements, which also contained results for a great number of hydroxy, methoxy, etc., substituted indole and hippuric acid derivatives, in order to demonstrate that this systematic approach could be extended to the detection of metabolic products based on their individual PC mobility patterns in combination with certain spray reagents. Several new and very characteristic mobility patterns were disclosed. The most interesting patterns were those for monohydroxyindole derivatives and for alkaloids, discussed in a recent survey[4]. The third supplement[5] added results for more indoles to the collection, and more alkaloids were investigated in relation to the earlier finding that a minor group of synthetic organic bases (some of which were drugs) produced PC mobility patterns similar to those recorded previously for alkaloids.

The present supplement, the fourth in the series, gives data for oxime derivatives of aromatic and aliphatic aldehydes and ketones. Although no oximes of these parent compounds occur naturally, according to present knowledge, it may be possible to use these oxime derivatives for the analysis of their parent compounds. Among simple naturally occurring aldehydes and ketones are *o*- and *p*-hydroxybenzaldehyde[6,7] and *o*- and *p*-hydroxyacetophenone[8,9]. After converting these compounds, prior to the chromatographic separation, into their corresponding oxime derivatives, *p*-hydroxybenzaldoxime and *p*-hydroxyacetophenone oxime (and also their corresponding *meta* isomers) showed an increase in their R_F values in solvent B, according to the pattern A < B > C < D (see Tables for explanation of abbreviations), which was similar to and characteristic of the patterns recorded for 1,3- and 1,4-dihydric phenols. *o*-Hydroxybenzaldoxime and *o*-hydroxyacetophenone oxime showed no increase in their R_F values in solvent B, which was in accordance with the same observation for 1,2-dihydric phenols. By forming oximes of this type of compound, the entire R_F pattern was lowered, particularly in solvents A, B, C and D. This result gives a powerful tool for use in the detection of related isomers that normally are not separated or produce very similar R_F patterns.

Recently, there has been an increasing interest in the separation of carbonyl compounds as oximes by gas chromatography[10]. The use of the *trans*-aldoxime of 1-perillaldehyde[11] as a sweetening agent in foods in Japan is well known. However, the biochemistry of oximes has not been exploited. Reports on the conversion of creatinine to creatinine-5-oxime and to 1-methylhydantoin-5-oxime by nitrite (food preservative) *in vitro*[12], leading to compounds that have unknown biological effects and unknown pharmacology, indicate the necessity for further analytical and biochemical studies in this field. The treatment of creatine with nitrite gives N-nitrososarcosine[12], which has been shown to be a carcinogen[13]. In general, the nitrosamines are carcinogens[14] and results for a highly carcinogenic member of this group of compounds, nitrosoproline[15], are given in this paper.

Another group of compounds that was investigated more closely were pyrimidine and purine derivatives. It was noticed earlier that bases which are regular constituents of nucleic acids have very low mobilities in all solvents. In recent years, several new antimetabolites have been synthetized, including mono- and dimercapto

analogues of purines and pyrimidines, some of them having drug action (*e.g.*, 6-mercaptopurine[16]). These compounds showed a markedly increased mobility in all solvents, and separated in well defined spots. It is shown here that through N-methylation and S-mercaptomethylation of the bases, the mobility patterns for these purines and pyrimidines are close to the patterns produced by alkaloids, involving a characteristic elevation of the R_F value in solvent B. A good example of this type of compound is 6-furfurylaminopurine (kinetin)[17,18], which is known to initiate vigorous cell division in plant tissues. Results for a number of drugs that are used mainly as tranquillizers in psychiatry, with interesting mobility characteristics, completes this supplement.

MATERIALS AND METHODS

For one-dimensional descending chromatography, rectangular glass jars of dimensions 20 × 30 × 60 cm were used. Whatman No. 1 filter-papers (chromatography grade), 24 × 48 cm, were 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*.

Spray reagents

The following twelve standard spray reagents were used to detect the compounds: diazotized sulphanilic acid (obtained from Th. Schuchart Co., G.F.R.); diazotized 4-benzoylamino-2,5-dimethoxyaniline (Koch-Light Laboratories Ltd., Great Britain); diazotized *o*-dianisidine (Koch-Light); *p*-nitrobenzenediazonium fluoborate (Eastman Kodak, U.S.A.); 2,6-dibromoquinone-4-chloroimide (British Drug Houses Ltd., Great Britain); 2,4-dinitrophenylhydrazine, ferric chloride, phosphomolybdic acid, potassium permanganate, bromophenol blue, *p*-dimethylaminobenzaldehyde and *p*-dimethylaminocinnamaldehyde (Heidenheimer Chemisches Laboratorium, Heidenheim-Brenz, G.F.R.). All compounds were also tested with ninhydrin, *p*-dimethylaminobenzaldehyde in acetic anhydride and Dragendorff reagent $KI \cdot BiI_3$ (Merck, G.F.R.). Positive reactions were recorded, and these are indicated by an asterisk in the tables. For the compositions 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 were used without purification. However, it was discovered that a number of preparations contained several components that generally separated well. The main spot, in these instances, was considered to be representative of the compound named on the supplier's label. Most of the aldoximes and ketoximes were synthesized from the corresponding aldehyde or ketone. All the derivatives were obtained in water–ethanol mixture by adding slightly alkaline hydroxylamino hydrochloride and heating the mixture. The oximes were then recrystallized. It can be seen from the tables that in a few instances the *cis* and *trans* isomers separated in certain solvents.

RESULTS

Guide to Tables I–X

Tables I–X contain condensed information for 160 organic compounds investigated by the above procedure. The R_F values were recorded in six different solvent

systems, but are arranged in a special order and designated by F, E, A, B, C and D. (For the composition of the solvents, see the list of abbreviations given below.) Under *Detection* the colour reactions are recorded for twelve different spray reagents used for the identification of each compound; the colour produced under UV light is indicated in the first column under this heading. In addition, all compounds were also tested with NH, DAB and Bi reagents. When positive reactions were observed, the results are labelled with the superscript a, b or c, respectively, as can be seen in the footnotes to the tables. The amount of substance 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 only as positive, without differentiating between the shades. In a few instances in which the colours are recorded by a number placed on top of another number, *e.g.*, $\left\{\begin{smallmatrix}24\\57\end{smallmatrix}\right\}$, upon spraying, a coloured spot (24 in the colour index) immediately appears, which changes 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 neither recorded in the tables nor is there any column for those compounds that, at this low concentration, are visible on the chromatograms because of their own colour. 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.

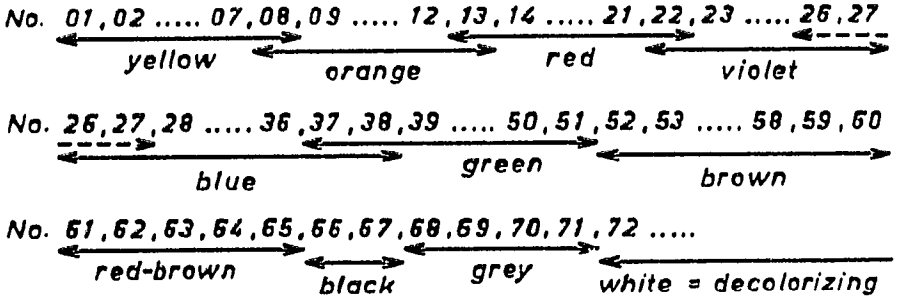

Fig. 1. Colour coding used to record the colour reactions (in abbreviated form) as a complement to the colour index for the tables.

Abbreviations used in Tables I–X

The chromatographic solvent systems used are identified in the tables as follows.

A = Methyl isobutyl ketone–formic acid–water (10 parts of ketone saturated with 1 part of 4% formic acid).
B = Chloroform–methanol–formic acid–water (10 parts of chloroform saturated with a mixture of 1 part of methanol and 1 part of 4% formic acid).
C = Benzene–methyl ethyl ketone–formic acid–water (a mixture of 9 parts of benzene and 1 part of ketone saturated with 1 part of 2% formic acid).
D = Benzene–formic acid–water (10 parts of benzene saturated with 1 part of 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 1:2 dioxan–water).
D_2 = Diazotized 4-benzoylamino-2,5-dimethoxyaniline (0.6% solution in 1:2 dioxan–water).
D_3 = Diazotized *o*-dianisidine (0.6% solution in 1:2 dioxan–water).
D_4 = *p*-Nitrobenzenediazonium fluoborate (0.4% solution in 1:2 dioxan–water).
DB = 2,6-Dibromoquinone-4-chloroimide (0.5% solution in 4:1 dioxan–acetone).
DN = 2,4-Dinitrophenylhydrazine (*ca.* 0.1% solution in 1 *N* HCl).
Fe = Ferric chloride (2% aqueous solution).
Mo = Phosphomolybdic acid (2% aqueous solution).
Mn = Potassium permanganate (1% aqueous solution).
Ind = Bromophenol blue (*ca.* 0.05% solution in ethanol).
EH = Ehrlich reagent (1% *p*-dimethylaminobenzaldehyde in 1 *N* HCl).
DAC = *p*-Dimethylaminocinnamaldehyde (0.1% solution in 1 *N* HCl).
DAB = *p*-Dimethylaminobenzaldehyde (2% solution in acetic anhydride).
NH = Ninhydrin reagent (2% solution in butanol saturated with water).
Bi = Dragendorff reagent (2% solution of potassium bismuth tetraiodide in 0.01 *N* HCl).

Colour index for Tables I–X

The colours produced by the action of different reagents on the investigated compounds are recorded in Tables I–X as numbers according to the following code.

01 Zinc yellow
02 Lemon cadmium
03 Gold
04 Primrose yellow
05 Straw yellow
06 Deep cadmium
07 Naples yellow
08 Middle chrome
09 Deep chrome
10 Orange chrome
11 Spectrum orange
12 Scarlet lake
13 Pale vermilion
14 Deep vermilion
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 Terra cotta
65 Burnt carmine
66 Chocolate
67 Ivory black
68 Blue grey
69 Gunmetal
70 French grey
71 Silver grey
72 White = colourless

TABLE I

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME PHENOLIC NATURAL PRODUCTS OF VEGETABLE ORIGIN

$R_F \times 100$						*Compound*
F	*E*	*A*	*B*	*C*	*D*	
95	95	93	82	88	93	Alkannin [(1-hydroxy-3-isohexenyl)-naphthazarin]
94	95	93	95	93	84	Atranoric acid
73	02	01	00	00	00	Cynarin (1,5-dicaffeoylquinic acid)[a]
84	34	77	58	24	04	7,8-Dihydroxycoumarin (daphnetin)
34	02	03	00	00	00	Esculin (6,7-dihydroxycoumarin-6-glucoside)
95	28	94	88	87	75	Evernic acid
95	94	95	94	96	95	Imperatorin (8-isoamyleneoxypsoralen)
87	04	84	83	39	08	Isoferulic acid (hesperetinic acid)
94	97	94	97	96	92	*dl*-Kawain
95	97	93	96	96	93	*dl*-Dihydrokawain (marindinin)
89	88	83	91	87	83	Khellin (5,8-dimethoxy-6,7-furo-2-methyl-1,4-chromone)
96	95	93	94	94	93	*dl*-Methysticin
96	95	93	96	93	93	*dl*-Dihydromethysticin
17	08	00	00	00	00	Ouabain (G-strophanthin)
41	35	06	00	00	00	Rhapontin (rhaponticin)
95	93	95	94	92	90	Rotenone[a]
95	96	95	96	96	91	Rottlerin (mallotoxin)
93	94	93	88	94	91	α-Santonin
88	42	85	88	51	39	Scopoletin (7-hydroxy-6-methoxycoumarin)
92	93	93	93	95	95	Xanthotoxin (8-methoxypsoralen)

[a] DAB reagent, pale yellow.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
17	+	51	+	+	−	−	+	30	+	−	60	−
−	−	−	−	07	−	+	70	−	+	−	−	−
71	59	55	51 03	52	43 50	−	70	68 60	++	+	−	−
48	62	63	03	62 55	35 38	−	40 70	68	++	−	−	−
33	57 63	57 63	17 62	62	23	−	+	38	++	−	−	−
−	62	63 23	23	08	35 33	−	−	34	+	+	−	−
48	−	−	−	−	58	+	−	−	++	−	−	−
32	63	19 64	25	08 25	35	−	−	69	++	+	−	−
−	−	−	−	−	−	−	−	−	+	−	−	−
+	−	−	−	−	−	−	−	−	+	−	+	−
33	−	−	−	−	58	+	+	03	++	−	06	60
34	−	−	−	−	−	+	−	−	+	−	05	57
34	−	−	−	−	−	−	−	−	+	−	+	+
−	−	−	−	−	−	−	−	−	+	−	−	−
33	13 08	63	25 65	08 63	38 70	−	+	70	+	−	−	−
−	−	−	−	−	−	−	−	−	+	−	−	−
59	−	−	−	−	58	−	−	40	+	−	−	−
−	−	−	−	−	−	−	−	−	+	−	−	−
33	48	52 65	56 66	65 56	−	−	+	49	+	−	+	−
48	−	−	−	−	+	+	−	−	++	−	−	−

TABLE II

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME ALDOXIMES

$R_F \times 100$						*Compound*
F	*E*	*A*	*B*	*C*	*D*	
36	00	00	00	00	00	Formaldoxime[a,c]
82	73	47	32	02	01	Acetaldoxime[a,c]
62	65	29	06	00	00	Glycolaldoxime[a,c]
81	85	73	35	24	04	5-Hydroxymethylfuraldoxime[a,c]
95	95	93	91	93	83	Perillartine (1-perillaldehyde-α-antioxime)
93	91	92	94	88	85	Benzaldoxime[a,c]
92	91	93	93	89	86	*p*-Tolualdoxime[b]
95	91	93	89	88	68	*o*-Hydroxybenzaldoxime[a]
92	91	93	94	94	91	*o*-Methoxybenzaldoxime[b]
88	90	88	44	48	05	*m*-Hydroxybenzaldoxime[b]
88	87	83	28	33	02	*p*-Hydroxybenzaldoxime[b]
92	92	93	93	87	84	*p*-Methoxybenzaldoxime[a]
93	91	88	28	47	03	2,4-Dihydroxybenzaldoxime[b]
91	92	83	25	48	04	2,5-Dihydroxybenzaldoxime
84	74	72	06	15	01	3,4-Dihydroxybenzaldoxime[b]
92	93	92	90	87	80	Piperonaldoxime[b]
74	87	55	86	27	10	6-Methylpyridine-2-aldoxime[b]
89	88	85	88	84	65	N-Methylpyrrole-2-aldoxime[b]
89	88	85	88	73	52	N-Methylpyrrole-2-aldoxime[b] double spot, *cis* or *trans* analogue

[a] DAB reagent, pale yellow (colour index 03 to 06).
[b] DAB reagent, orange to pink (colour index 09 to 13 and 17).
[c] NH reagent, pale brown.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
–	–	–	62	60	–	–	63	68	+	–	05	62
–	–	–	–	60	–	–	05, 59	68	++	–	–	–
–	–	–	17, 72	07	–	–	03, 23	68	++	–	05	60
–	–	–	14, 64	06	–	–	63	68	+	–	–	60
–	–	–	–	07	–	–	–	–	+	–	+	–
–	+	60	17	08	–	–	27	68	++	–	–	–
–	–	17	17	08	–	–	27	68	++	–	–	–
–	–	–	63	–	03	–	54	68	++	–	–	–
–	–	–	62	08	–	–	62	03, 44	+	–	–	–
+	+	62	62	64	43	–	24, 70	68	++	–	–	–
–	15	63	23	25, 65	–	–	27	68	++	–	–	–
–	07	11, 59	64	08	+	–	58	38	+	–	–	–
+	53, 66	23, 54	23	23, 65	69	–	69	45	+	–	–	–
27	–	–	13, 64	07	–	–	45	49, 68	++	–	+	03
–	62	56	62	55	43	–	51, 52	68	++	–	–	–
–	–	09	+	62	–	–	–	–	+	–	–	–
–	–	–	–	–	–	–	60	+	+	–	–	–
–	11, 14	15	19	12, 64	62	–	66	59, 50	++	–	27, 25	70
–	11, 14	15	19	12, 64	62	–	66	59, 50	++	–	27, 25	70

TABLE III

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF KETOXIMES

$R_F \times 100$						*Compound*
F	*E*	*A*	*B*	*C*	*D*	
89	92	90	64	65	18	Diacetyl monoxime
88	88	88	48	67	10	Diacetyl dioxime (dimethylglyoxime)
88	90	88	44	55	16	Acetoin oxime[a, c]
74	74	52	27	07	03	Acetoin oxime double spot, *cis* or *trans* analogue
86	88	80	25	29	04	Dihydroxyacetone oxime[a]
89	93	92	71	92	48	2,3-Pentanedione oxime[b]
82	84	69	44	24	05	2,4-Pentanedione oxime[a] (acetylacetone dioxime)
79	78	91	49	08	04	2,5-Hexanedione oxime[a] (acetonylacetone dioxime)
93	93	93	92	90	90	1-Methylcyclohexanone 3-oxime[a]
81	84	72	87	32	07	1,2-Cyclohexanedione dioxime (nioxime)
84	87	83	65	34	09	5,5-Dimethyldihydroresorcinol dioxime[a]
71	45	49	82	29	15	*o*-Aminoacetophenone oxime
91	91	90	68	67	12	*o*-Hydroxyacetophenone oxime[b]
71	57	70	50	57	07	*p*-Hydroxyacetophenone oxime[b]
93	92	89	49	61	07	2,4-Dihydroxyacetophenone oxime[a]
92	93	93	94	87	78	α-Benzoin oxime
92	92	92	93	92	94	Benzil dioxime
93	94	93	90	83	32	α-Furil dioxime
93	79	90	91	91	92	1,2-Naphthoquinone 1-oxime[d]
94	68	91	87	88	87	1,2-Naphthoquinone 2-oxime[d]

[a] DAB reagent, pale yellow (colour index 03 to 06).
[b] DAB reagent, orange to pink (colour index 09 to 13 and 17).
[c] NH reagent, pale yellow.
[d] Bi reagent, positive.

TABLE IV

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME BENZOIC AND PHTHALIC ACID DERIVATIVES

$R_F \times 100$						*Compound*
F	*E*	*A*	*B*	*C*	*D*	
86	08	79	61	29	04	Tetrahydrophthalic acid
89	87	89	89	75	38	*p*-Acetamidobenzaldehyde
94	24	92	91	91	65	2-Nitro-4-methylbenzoic acid[a]
94	28	93	94	92	80	3-Nitro-4-methylbenzoic acid[a]
96	33	90	84	83	27	2-Amino-4-nitrobenzoic acid
94	47	92	84	89	48	3,4-Dinitrobenzoic acid[a]
93	14	94	92	91	90	*p*-Methylcinnamic acid
74	02	46	15	04	00	3-Aminophthalhydrazide[a]
85	00	39	00	00	00	3,6-Dinitrophthalic acid

[a] DAB reagent, pale yellow (colour index 03 to 06).

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	—	—	—	—	—	+	+	—	—	—
—	—	—	60	—	—	—	—	+	+	—		—
—	—	+	64	07	—	—	63 60	68	++	—	+	60
—	—	—	64	07	—	—	03	68	++	—	+	60
—	—	59	60	62	—	—	60	68	++	—	—	—
—	—	70	62	06	—	—	58	38	++	—	—	—
—	—	—	10 72	07 62	—	—	05 63	68	++	—	—	05
—	—	—	05	05	—	—	23	58	++	—	17	+
—	—	—	17	—	—	—	—	—	+	—	—	—
—	—	—	—	—	—	—	62 55	40	++	—	—	—
—	45 09	59	53	47 60	52	—	58	43 68	+	—	03	—
48	+	+	62	08	70	—	—	38	+	—	07	—
—	07	52 56	23	09 64	68	—	—	38	+	—	—	—
—	62	56	28 53	25 10	—	—	57	38 68	+	—	—	—
—	12	19	23 25	08 63	25	—	66	03 68	++	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
—	60	07	63	08	—	—	—	40	+	—	—	—
56	—	—	—	—	—	—	52 55	60 56	++	—	+	+
52	—	—	—	—	62	—	56	70	+	—	—	—

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	—	—	—	—	—	—	++	++	—	—
04	—	—	—	—	—	07	—	—	—	—	—	—
56	—	—	—	—	—	—	—	—	+	++	—	—
56	—	—	—	—	—	—	—	—	—	+	—	—
60	—	+	—	—	—	—	—	—	+	+	07 11	64 65
60	—	—	—	—	—	—	—	—	—	++	—	—
—	—	—	—	—	—	—	—	—	++	+	—	—
33	—	+	+	—	—	—	—	37	+	33	10	23 63
53	—	—	—	—	—	—	—	—	—	—	—	—

TABLE V

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME ALIPHATIC AMINO ACID DERIVATIVES

$R_F \times 100$						*Compound*
F	*E*	*A*	*B*	*C*	*D*	
27	00	01	01	00	00	N-Acetyl-*dl*-asparagine
05	00	00	00	00	00	Albizziin (1-2-amino-3-ureido-propionic acid)[b]
09	00	00	00	00	00	α-Aminoadipic acid
12	00	00	00	00	00	Δ-Aminolaevulinic acid
00	00	00	00	00	00	*l*-Arginine
01	00	00	00	00	00	*l*-Asparagine
02	00	00	00	00	00	*dl*-Aspartic acid
00	00	00	00	00	00	*l*-Canavanine [2-amino-4-(guanidinoxy)butyric acid][b]
02	00	05	00	00	00	S-Carbamyl-*l*-cysteine[b]
08	00	00	00	00	00	S-Carboxymethyl-*l*-cysteine
05	00	00	00	00	00	*dl*-Citrulline (Δ-ureidonorvaline)
06	00	00	00	00	00	Formiminoglycine
82	05	66	40	10	01	N-Formyl-*dl*-methionine[b]
07	00	00	00	00	00	*l*-Glutamic acid
03	00	00	00	00	00	*l*-Glutamine[b]
17	04	00	00	00	00	Guanidinosuccinic acid[b]
00	00	00	00	00	00	*l*-Homocarnosine[b]
34	00	01	00	00	00	Kainic acid (2-carboxy-4-isopropenyl-3-pyrrolidineacetic acid)
22	02	00	00	00	00	*l*-Leucinamide

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

TABLE VI

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

$R_F \times 100$						*Compound*
F	*E*	*A*	*B*	*C*	*D*	
44	00	01	00	00	00	3-Chloro-*l*-tyrosine[b]
23	03	01	00	00	00	O-Methyl-*l*-tyrosine[b]
30	02	01	00	00	00	β-2-Thienylalanine[b]
20	00	00	00	00	00	2-Methyl-3-(3,4-dihydroxyphenyl)-alanine (α-methyldopa)
58	93	08	81	02	01	*l*-Phenylalanine ethyl ester[b]
57	92	04	11	00	00	*l*-Tyrosine ethyl ester
02	15	00	01	00	00	*l*-Histidine methyl ester[b]
51	49	19	33	03	02	Nicotinamide[c]
62	59	27	74	11	05	N'-Methylnicotinamide[c]
84	00	70	68	24	04	Nitrosoproline[c]

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

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*NH*[a]	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	—	—	—	—	—	—	—	+	06	17
—	—	—	—	—	—	23	—	—	—	—	06	17 19
—	—	—	—	—	—	23	—	—	—	—	—	—
—	—	—	—	—	—	60	—	—	—	—	—	—
—	—	—	—	—	—	24	—	—	+	33	—	—
—	—	—	—	—	—	59 23	—	—	—	33	04	—
—	—	—	—	—	—	23	—	—	—	—	04	—
—	—	—	—	—	—	23	—	—	—	—	—	—
—	—	—	—	—	—	23	—	34	+	33	05	17 15
—	—	—	—	—	—	23	—	—	+	—	—	—
—	—	—	—	—	—	23	—	—	—	33	06	17
—	—	—	—	—	—	—	—	—	—	33	—	—
—	—	—	—	—	52	—	—	—	++	+	—	—
—	—	—	—	—	—	24	—	—	—	+	04	—
—	—	—	—	—	—	23	—	—	—	—	04	—
—	—	—	—	—	+	—	—	—	—	—	—	—
—	14	+	63	63	+	23	—	—	—	—	—	—
—	—	—	—	—	—	03 62	—	—	+	+	—	—
—	—	—	—	—	—	23	—	—	—	33	—	—

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*NH*[a]	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	09	+	+	62	—	23	—	38	++	33	06	13
—	—	—	—	—	—	23	—	—	—	—	06	+
—	—	—	—	—	—	23 39	—	—	+	—	06	+
—	11	19 62	60 63	23	62	23	45 51	68	++	—	—	—
—	—	—	—	—	—	23	—	—	+	—	—	—
—	+	60	63	64	+	23	+	38	+	—	—	—
—	+	60	64	07	—	23	—	—	+	—	06	—
—	—	—	—	—	—	—	58	—	—	—	—	—
34	—	—	—	—	—	57	60	+	—	33	—	—
—	—	—	—	—	—	17	—	—	—	++	—	—

TABLE VII

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME PYRIMIDINE DERIVATIVES

$R_F \times 100$						*Compound*
F	*E*	*A*	*B*	*C*	*D*	
25	12	04	01	00	00	Uracil (2,4-dihydroxypyrimidine)
62	34	34	08	04	00	2-Thiouracil (2-mercapto-4-hydroxy-pyrimidine)
72	41	44	32	07	01	6-Methyl-2-thiouracil
07	01	02	00	00	00	Orotic acid (uracil-6-carboxylic acid)
54	01	00	00	00	00	Dihydro-*dl*-orotic acid
05	05	00	00	00	00	5-Aminouracil[a, b]
56	09	18	02	00	00	5-Nitrouracil
43	31	14	16	00	00	Thymine (5-methyluracil)
44	24	07	04	00	00	Thymine-2-desoxyriboside (2'-thymidine)
72	76	57	86	62	57	1,3-Dimethyluracil[c]
12	03	02	00	00	00	Cytosine (2-hydroxy-4-aminopyrimidine)
17	12	02	06	00	00	Isocytosine (2-amino-4-hydroxypyrimidine)
34	15	03	01	00	00	2-Thiocytosine[b]
13	04	00	02	00	00	5-Methylcytosine
84	40	80	62	31	07	2,4-Dimercaptopyrimidine[a, b]

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

TABLE VIII

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME PURINE DERIVATIVES

$R_F \times 100$						*Compound*
F	*E*	*A*	*B*	*C*	*D*	
34	09	08	20	02	00	Purine[a, c]
38	03	08	03	00	00	6-Mercaptopurine
67	32	48	75	22	06	6-Methylmercaptopurine[c]
78	47	68	86	49	22	6-Ethylmercaptopurine[c]
31	01	08	06	00	00	6-Hydroxy-2-mercaptopurine (2-thioxanthine)
57	06	27	10	02	00	2,6-Dimercaptopurine[a] (dithioxanthine)
19	08	01	03	00	00	Adenine (6-aminopurine)[a]
13	02	00	00	00	00	1-Methyladenine[a]
24	18	04	25	00	00	6-Methylaminopurine[c]
72	74	47	88	19	04	6-Furfurylaminopurine[a, c] (kinetin)
00	02	00	00	00	00	2-Mercapto-6-aminopurine[a, c]
03	02	00	00	00	00	Guanine (2-amino-6-hydroxypurine)[a]
05	02	00	00	00	00	Guanosine[a]
08	00	00	00	00	00	Uric acid (2,6,8-trihydroxypurine)

[a] DAB reagent, pale yellow.
[b] NH reagent, no positive reactions recorded.
[c] Bi reagent, positive.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	—	—	—	—	—	—	+	—	—	17
—	—	—	—	—	62 52	—	—	40	++	33	—	—
—	—	—	—	—	62 59	—	—	71	++	33	—	—
—	—	—	—	—	—	—	+	—	—	+	—	—
—	—	—	—	—	—	—	—	—	+	+	—	—
34	14	43 28	35 28	14 23	63	—	—	68	++	—	08	60 65
60	—	—	—	—	—	—	—	—	+	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
—	—	—	—	—	—	—	—	—	++	—	—	—
—	—	—	—	—	—	—	—	—	+	33	—	—
—	—	—	—	—	—	—	—	—	+	33	—	—
—	—	—	—	—	—	—	—	—	+	33	—	—
+	—	—	—	58	59	—	—	71	++	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
57	—	—	—	06	60 06	—	—	38	+	—	—	—

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	—	—	—	—	—	—	—	—	—	—
+	—	—	—	—	03 60	—	—	71	+	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	60	—	64	—	—	71	+	—	—	—
03	—	—	+	+	63 59	—	—	38	+	—	—	—
+	—	—	—	—	—	—	—	—	—	33	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
+	—	—	—	—	—	—	—	—	—	—	17	26
+	—	—	06	05	62	—	—	38	+	—	—	—
+	—	—	—	—	—	—	+	—	—	—	04	+
+	—	—	—	—	—	—	—	—	+	—	—	—
—	—	—	—	08	12	—	—	28	—	—	—	—

TABLE IX

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SOME ALKALOIDS

$R_F \times 100$						*Compound*
F	*E*	*A*	*B*	*C*	*D*	
39	96	02	14	00	00	Berbamine[a]
56	93	01	82	00	00	Bulbocapnine[a]
92	31	86	93	64	42	Colchiceine[a]
84	87	72	95	43	30	Colchicine[a]
88	96	47	95	09	03	Dihydroergocornin[a]
88	96	57	92	08	01	Ergocristine[a]
94	96	52	93	06	01	Dihydroergocristine[a]
91	95	56	96	10	03	Dihydroergocryptine[a]
86	93	29	95	02	00	Dihydroergotamine[a]
80	91	16	47	00	00	Ergotamine[a]
56	85	04	08	00	00	Methylergometrine[a]
47	93	03	50	00	00	N,N-Dimethylmescaline[a]
78	79	03	16	00	00	N-Methylmescaline[a]
37	86	03	28	00	00	Ethylmorphine[a]
39	82	01	22	00	00	Codeine[a]
38	76	02	30	00	00	Pilocarpine[a]
39	73	02	38	00	00	Isopilocarpine[a]
74	95	43	87	52	50	*l*-β-Narcotine[a]
95	96	95	93	91	90	Piperine[a]

[a] Bi reagent, positive.

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
—	—	—	+	—	60	—	—	—	+	33	—	—
34	+	64 63	62 25	13 63	33 38	—	52	68	+	33	—	—
07	—	—	—	—	—	04	51	—	+	—	06	03 60
07	—	—	—	—	—	05	59	—	+	—	06	03 60
—	—	59	53	10	+	—	—	—	+	—	24 27	26 25
27	—	—	—	62	70	—	—	03	+	—	24 71	27 25
—	—	—	—	09	+	—	—	—	—	—	26 64	71
—	—	—	—	09	+	—	—	—	+	—	24 70	71
—	—	—	—	+	—	—	—	—	—	—	22 62	71
34	—	—	—	—	+	—	—	—	+	—	26 70	+
27	—	—	+	62	+	—	—	—	+	—	24 27	25
—	—	—	—	—	—	—	—	—	—	33	—	—
—	—	—	—	—	—	—	—	—	—	33	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	33	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
38	—	—	—	—	+	04	—	03	+	—	06	07 60

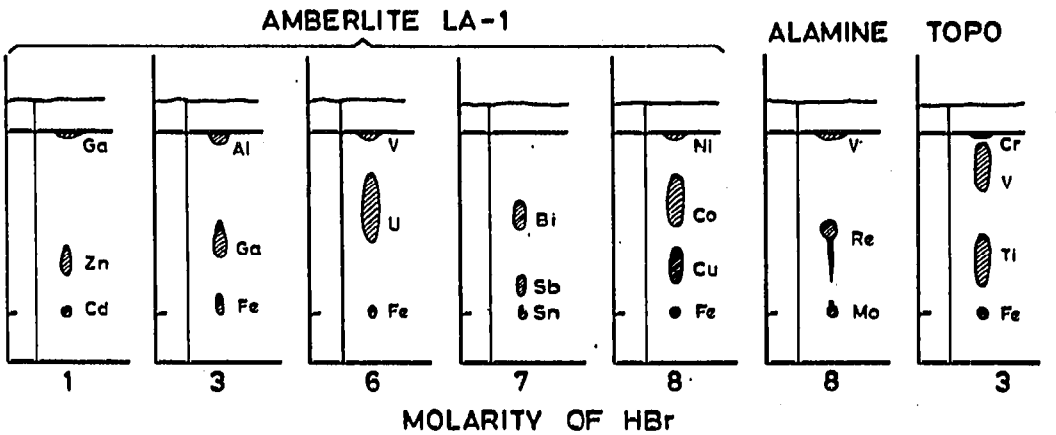

Fig. 4. Examples of separations carried out in various extractant–HBr systems. V = V(V); Cr = Cr(III).

moderately sorbing Amberlite LA-1 offers the best opportunities in analysis. One may add that for special purposes, *e.g.* the separation of Sc(III), Zr(IV) or Th(IV) (R_F = 0.0) from a rather large number of elements (R_F = 1.0), TOPO will come in useful.

Liquid–liquid extraction

Data on liquid–liquid extraction of metal ions from aqueous bromide solutions are comparatively sparse. Reference may be made here to studies by SUZUKI AND SOTOBAYASHI[22], ALIAN[23] and BRINKMAN[14] on extraction by amines. As an illustration, some data from refs. 14 and 22 are summarized in Table II. Bearing in mind that the results of reversed-phase chromatography may be compared with data obtained in liquid–liquid extraction on the basis of the well-known relationship

$$D = k(1/R_F - 1) \qquad (3)$$

where D is distribution coefficient and k is constant, we may conclude that a good qualitative agreement exists. The same conclusion is reached when considering the results reported by ALIAN.

A study has been made of the systems extractant–Co(II)–Br^-. Percentage extraction *versus* M Br^- curves are shown in Fig. 5. The organic phases obtained in the three amine–Co(II)–Br^- and the AlamO–Co(II)–Br^- systems have identical

TABLE II

COMPARISON OF PERCENTAGE EXTRACTION AND R_F VALUES FOR NINE ELEMENTS IN THE AMBERLITE LA-1–HBr SYSTEM

Percentage extraction and R_F values correspond to refs. 22 and 14, respectively.

Ion	*Percentage extraction*			R_F		
N HBr	*0.3*	*0.5*	*7.0*	*0.3*	*0.5*	*7.0*
Mn(II)	0.0	0.0		1.0	1.0	
Ni(II)	0.0	0.0	0.8	1.0	1.0	1.0
Co(II)	0.0	0.0	1.4	1.0	1.0	0.7
Fe(III)	0.4	0.5	99.7	0.9	0.8	0.0
Cu(II)	0.5	0.5	39.4	0.9	0.9	0.3
Zn(II)	1.9	3.4		0.6	0.2	
Pb(II)	87.8	89.4		0.02	0.01	
Bi(III)	100.0	100.0		0.0	0.0	
Cd(II)	100.0	100.0		0.0	0.0	

Detection												
UV	D_1	D_2	D_3	D_4	*DB*	*DN*	*Fe*	*Mo*	*Mn*	*Ind*	*EH*	*DAC*
+	—	—	—	—	—	—	—	—	—	—	02	+
+	+	60	23 63	08 63	54	26 58	23	42 68	++	—	07	08 62
—	+	62	63	07	23	27	62	—	+	—	17	48 46
—	—	—	—	—	—	—	—	—	—	—	08	23 21
—	—	—	62	+	69	—	—	—	—	—	—	—
34	—	—	—	—	+	—	—	—	+	—	—	—
+	—	—	—	—	—	—	—	—	+	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
27	—	—	—	—	—	—	—	—	—	—	—	—
33	—	—	—	06	+	—	—	71	—	—	—	—
—	—	—	—	—	—	—	—	—	+	—	—	—
—	—	—	—	—	—	—	—	—	—	—	02	17
—	—	—	—	—	—	—	—	—	+	—	—	—
38	—	+	53	60	33 27	32 34	40 37	48	+	33	58 40	70 23
27	—	—	—	—	—	+	—	+	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	+	62	07 59	24 71	+	+	60	+	—	09	19 65
13	—	—	—	—	—	—	—	—	—	—	60	—
—	—	+	24 63	19 63	70 27	17 30	+	52 71	+	—	37	70 30
—	—	—	—	—	—	—	+	60	+	—		

which was used earlier for the detection of hippuric acid derivatives[2,3]. This reagent produces yellow colours with aliphatic aldoximes, but aromatic and heterocyclic aldoximes (derivatives of benzene, pyridine and pyrrole) were sharply differentiated by their deep orange to pink colour. The formation of colour was slow (1–24 h at room temperature) and was comparable with the colour development with hippuric acid derivatives with the same reagent. Whether or not the ring formation for these strongly coloured compounds is similar to or identical with azlactone formation[10] [formation of 2-substituted-4-(*p*-dimethylamino)benzylidene-5-oxazolone], which occurs when hippuric acids react, remains to be investigated. Contrary to the reactions of aldoximes, which were all DAB-positive, only about half of the ketoximes gave positive reactions with DAB-reagent. A pink colour was produced by 2,3-pentadione oxime and *p*-hydroxyacetophenone oxime (Table III), which indicates that there is no clear differentiation between the pink and yellow colour as far as aromatic and aliphatic ketoximes are concerned.

Special attention was paid to compounds that reacted with Ehrlich reagents (EH and DAC), and which ordinarily were not recorded as Ehrlich-positive compounds, as a free amino group was usually not present. With DAC reagent (which is more sensitive than EH reagent), the following compounds produced yellow to brown colours: alkannin and khellin (Table I), 5-hydroxymethyl-2-furaldoxime, glycolaldoxime and 2,5-dihydroxybenzaldoxime (Table II), acetoin oxime (Table III), and colchiceine, colchicine and piperine (Table IX). Compounds that gave a pink shade with DAC reagent were albizzin, citrullin and S-carbamylcysteine — which are all carbamyl derivatives, and similar colours have been observed earlier with other ureido derivatives (Table V) — 3-chlorotyrosine (Table VI), uracil (Table VII), and hydroxyphenamate and procaine (Table X). A violet colour was produced by oxypertine and a clear blue colour by solypertine (which also gave a blue EH reaction, Table X). All the derivatives of ergot alkaloids, *viz.* dihydroergotamine, dihydroergocryptine, dihydroergocristine, ergocristine and dihydroergocornin, gave a grey to weak blue–violet reaction with DAC reagent. A very characteristic and unusual DAC colour, grass green, was given by amisometradine [6-amino-3-methyl-1-(2-methylallyl)uracil], and this reaction seems to be suitable for the colorimetric determination of this compound.

The colour reactions with hippuric acid (DAB), ninhydrin (NH) (with the exception of amino acid derivatives) and Dragendorff (Bi) reagents are recorded as footnotes to the tables, as these positive responses occurred very irregularly and only in connection with specific types of compounds. The most important colour reactions with DAB reagent have already been discussed in connection with the oxime derivatives. Of the other types of compounds, a yellow colour was produced with DAB reagent by cynarin (1,5-dicaffeoylquinic acid) (Table I), 3-aminophthalhydrazide (Table IV), N-formylmethionine and guanidinosuccinic acid (Table V), and β-2-thienylalanine and histidine methyl ester (Table VI), and a pink colour by amisometradine (Table X). In addition to the compounds that generally reacted with ninhydrin and which are listed in Tables V and VI, the following compounds were also recorded as ninhydrin-positive: a brown colour was obtained with 2-thiocytosine and 2,4-dimercaptopyrimidine (Table VII), and instant(!) magenta colour with 4-aminophenazone (Table X).

Dragendorff reagent (Bi) was used as before to trace the alkaloids in Table IX and was also found to react with a number of other compounds, *viz.* drugs of the type of thioxanthen derivatives, oxypertine, oxotremorine, dimethindene and eupaverin (Table X), nicotinamide, N′-methylnicotinamide and nitrosoproline (Table VI), 1,3-dimethyluracil (Table VII), and purine, 6-methylmercaptopurine, 1-methyladenine, 6-methylaminopurine and 6-furfurylaminopurine (Table VIII). This latter reaction with Bi reagent, together with the mobility characteristics for N-methylated and S-mercaptoalkylated purines and pyrimidines, proved to be useful for the identification of these substances in complicated biological extracts.

Of the remaining reagents, some of the most characteristic colour reactions will be mentioned. N-Methylpyrrolealdoxime (Table II) gave a strong red colour with all four diazonium reagents (D_1, D_2, D_3 and D_4), which is very unusual. Mercaptopyrimidine (Table VII) and mercaptopurine (Table VIII) derivatives gave red–brown colours with DB reagent (2,6-dibromoquinone-4-chloroimide), similar to those which were noticed earlier for other mercapto derivatives of the benzene series and with

some of the aliphatic thiolic compounds investigated. The colour shade seems to be indicative of the presence of at least one free mercapto group. So far, there have been only a few compounds recorded that were not mercapto derivatives and that produced interfering red–brown colours with this particular reagent. Of the compounds studied in this work the exceptions are N-methylpyrrole-2-aldoxime (Table II) and 5-aminouracil (Table VII), and from previous reports gallic acid methyl ester[5] (Table III in ref. 5) and indolon-2[5] (Table X in ref. 5).

For the identification of the compounds, a valuable mobility characteristic is best obtained by studying the irregular mobilities and grouping them with several well defined mobility patterns. In the previous papers of this investigation, the mobility was defined as being regular when the R_F values obtained for six different solvent systems for the almost neutral compounds were plotted as a diagram in a certain order of the solvents, whereby these R_F values decreased gradually from solvent F to solvent D according to the sequence $F > E > A > B > C > D$. The irregularities in the mobilities of the compounds and the production of other types of mobility patterns (by a sudden increase or decrease at a particular R_F value in the series of decreasing R_F values) were caused primarily by the presence of basic and acidic groups in the molecule. Solvent E has been indicative of both bases (always giving medium or high R_F values and illustrated by the partial sequence of R_F values $F < E > A$) and acids (giving medium or low R_F values and illustrated by the R_F sequence $F > E < A$, *e.g.*, the R_F values in solvents F and A are always higher than in solvent E).

For alkaloids, which in general are bases by definition, and for some basic drugs, another interesting irregularity in the mobility was enhanced in solvent B, where the mobility suddenly increased according to the partial sequence of R_F values $A < B > C$. This second peak value, together with an increase in the R_F values in solvent E, has given fairly definite characteristics for alkaloids and a number of basic drugs, derivatives of thioxanthene and phenothiazine, etc., as far as Dragendorff-positive compounds are concerned. The interference with this interpretation of R_F data from synthetic bases has been discussed in greater detail earlier[4]. The similar double R_F value peaks differentiate these two types of compounds, however, when the R_F values are presented in diagrammatic form and subdivided to give four sub-patterns on the basis of the changes in R_F values in solvents F, A and C. All the alkaloids investigated in this work follow strictly this double-peak pattern, whereby great variations of their relative R_F peak heights in solvents E and B were observed. The drugs listed in Table X mainly follow a similar pattern, and for half of them the R_F value peak in solvent E is replaced by a regular decrease in the R_F values in the solvents in the order $F > E > A$. Complications in the identification of these compounds as a result of the mobility arise only when biological extracts from human material are being considered, where the subjects are taking or are suspected of taking alkaloids (some of them are narcotics) along with a number of certain drugs of a basic character, *e.g.*, tranquillizers. When screening plant extracts for the presence of alkaloids no problems should arise from interfering substances of non-biological origin.

Tables VII and VIII give mobility data for a number of pyrimidine and purine derivatives. It can be seen that generally these monohydroxy, dihydroxy, amino, nitro and mercapto derivatives have very low mobilities in all the solvents except

solvent F, and their R_F values mainly follow the regular pattern for neutral, weakly basic or weakly acidic compounds, F > E > A > B > C > D. By N-methylation, and in some instances C-methylation and S-mercaptoalkylation, the mobilities of these compounds are considerably increased and compounds such as thymine, 5-methylcytosine (Table VII), and 6-methylmercaptopurine and 6-methylaminopurine (Table VIII), show a change from the regular R_F pattern of their non-methylated parent compounds to a pattern with an R_F value peak in solvent B, according to the sequence A < B > C, similar to the increase that occurred with alkaloids. However, the increase in the R_F values in solvent E is not pronounced enough and is much less than the increase required to classify these compounds as alkaloids. Some examples of purines and pyrimidines with typical double-peak values in solvents E and B (alkaloid pattern) are 6-furfurylaminopurine (Table VIII), 1,3-dimethyluracil (Table VII) and the drug trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine] (Table X). 1,3,7-Trimethylxanthine (caffeine), which has been investigated earlier[5] (Table XI in ref. 5), does not give a clean alkaloid mobility pattern.

Table VI includes mobility data for two basic compounds, nicotinamide and N'-methylnicotinamide. It can be seen that the R_F value peak in solvent B is intact (A < B > C), but the basic effect is too weak to show another increase in solvent E. Instead, there is a slow but steady decrease in the R_F values in the order F > E > A.

Returning to aromatic aldoximes and ketoximes (Tables II and III) it was mentioned in the introduction that several aldehydes and ketones can be converted into their corresponding oxime derivatives, which have mobility patterns that are useful for the identification of the parent compounds.

The *m*- and *p*-hydroxybenzaldoximes showed similar R_F patterns, which were recorded earlier for 1,3- and 1,4-dihydric phenol derivatives. The main characteristics occurred in solvent C, with a sudden increase in the R_F value instead of a regular pattern, following the sequence F > E > A > B < C > D. The same regularity was observed with *m*- and *p*-hydroxyacetophenone oximes. The corresponding *ortho* derivatives did not show this increase in R_F values in solvent C, which is in accordance with a similar finding for 1,2-dihydric phenols. It is interesting to note that this shift in R_F values, A > B < C > D, occurred also with 2,4-, 2,5- and 3,4-dihydroxybenzaldoximes. A similar change with 1,2,3- and 1,3,5-trihydric phenols has not been observed. The corresponding model substances of the 1,2,4-, 1,2,5- and 1,3,4-trihydric phenol types are not available. However, it seems reasonable to predict, on the basis of the results obtained by the analysis of the oxime mobilities, that the increase in R_F values in solvent C will occur with these derivatives also.

For several oximes, their possible *trans* and *cis* isomers separated only in the two solvents C and D. N-methylpyrrole-2-aldoxime (Table II) gave two distinctly different spots, with the same colour reactions, whereas for acetoin oxime (Table III) the separation occurred in all the solvents.

This remarkable pattern F > E > A > B < C > D, with an increase in R_F values in solvent C, was also recorded for dimethylglyoxime, acetoin oxime (one of the two analogues), 2,3-pentanedione oxime (but not for 2,4-pentanedione or 2,5-hexanedione oxime), and 2,4-dihydroxyacetophenone oxime (Table III). This phenomenon is obviously connected with the interaction of the molecule of the compound by hydrogen bonding with methyl ethyl ketone, which is one of the components

in solvent C in addition to the non-polar benzene. On the other hand, methyl ethyl ketone in solvents that contained a higher proportion of water, such as solvents F and A (methyl isobutyl ketone), does not distinguish between 1,2-, 1,3- and 1,4-dihydric phenols or hydroxy oximes.

In conclusion, it could be mentioned that the use of oximes for improving the rather high and uniform R_F values for some of the biologically occurring parent aldehydes and ketones, gives a potential route for obtaining these compounds as separable units, with interesting new mobility characteristics for selective and comparative purposes, facilitating the final identification.

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