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QUALITATIVE ION-PAIR REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The chromatographic properties of 132 compounds were investigated in two ion-pair systems on reversed-phase thin-layer plates. The systems consisted of tetrabutylammonium and tetramethylammonium halides for the anionic mode and of sodium heptyl and methyl sulphonates for the cationic mode.

Carboxylic and sulphonic acids yielded significant positive ΔR_F values in the anionic system. In the cationic system the ΔR_F values of most of the carboxylic acids were not significantly different from zero, but the sulphonic acids yielded negative ΔR_F values significantly different from zero.

For the carboxylic acids the magnitude of ΔR_F in the anionic system depended on the nature of the substituent and also of the substitution pattern. In effect, the magnitude of ΔR_F appeared to be directly related to the extent to which the ionogenic group contributed to the hydrophilic character of the molecule.

Aromatic amines yielded no ΔR_F values significantly different from zero in either mode. Of the aliphatic amines the ΔR_F values in the cationic system were positive, their magnitude increasing from primary toward tertiary amines. These amines, except for some primary ones, trailed in the anionic mode.

It is concluded that qualitative ion-pair thin-layer chromatography can be helpful in the identification of functional groups of unknown compounds.

INTRODUCTION

In the past, more or less successful efforts have been made to correlate the chromatographic behaviour of organic substances with their chemical characteristics. Martin, in 1950[1], demonstrated a linear relationship between the R_M values of homologous series, R_M being defined as

$$R_M = \log \frac{1}{R_F} - 1$$

Reichl[2], elaborating on Martin's work, introduced the concept of ΔR_M as the difference between the R_M values of a compound in two solvent systems. He demonstrated that ΔR_M was constant for a specific functional group, in this case the carboxylic

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group, provided that the chromatographic properties of the rest of the molecule were similar in the two solvent systems. Reichl obtained solvent pairs meeting this condition by the addition of acetic acid to a solvent mixture and chromatographing with or without the acid. Reichl[2] also demonstrated that ΔR_M was a direct function of the number of carboxylic groups in a molecule, and that an amino group offsets the effect of a carboxylic group. He also found, not unexpectedly, that the method did not work with strongly ionized acids; the condition of chromatography in, alternately, the ionized and the non-ionized state, is not fulfilled[3].

At about the same time, Lederer and Kertes[4] demonstrated the usefulness of paper impregnated with ion exchanger both for the separation of ionogenic compounds and for estimating the valence of ionogenic groups. Since then many papers have been published about the use of ion-exchange or ion-pair chromatography[5–10] on paper or thin layers. These works, however, were mainly aimed at improving the separation characteristics of chromatographic systems.

In the present paper we have adapted Reichl's ΔR_M method for use in reversed-phase ion-pair chromatography. In our choice of pairs of chromatographic systems we have utilised the experimental fact that compounds capable of forming ion pairs, when chromatographed on reversed-phase plates impregnated with counter-ions, are retained longer the larger the alkyl chain of these counter-ions.

In such systems the magnitude of ΔR_F for a compound depends on the extent to which the compound's ionogenic group renders it hydrophilic. In our reversed-phase systems we chose tetramethylammonium (N1) and tetrabutylammonium bromide (N4) as the pair of impregnating agents for the anionic, and the sodium salts of methyl- (S1) and heptyl-sulphonic acid (S7) for the cationic system.

Taking into account that the method is not able to affect only the ionic group, we used ΔR_F approach instead of the ΔR_M because the former seems less sensitive to small structural differences in the non-ionic part of the molecule in our systems.

EXPERIMENTAL

Chemicals and reagents

Tetramethylammonium bromide (N1) and tetrabutylammonium bromide (N4) were obtained from Fluka (Buchs, Switzerland), and sodium methylsulphonate (S1) and sodium heptanesulphonate (S7) from Eastman-Kodak (Rochester, NY, U.S.A.).

Impregnation of the plates

Silica gel Si 60 F_{254} silanized thin-layer plates (art. 5747; Merck, Darmstadt, G.F.R.) were impregnated with N1, N4, S1 or S7 detergent by dipping them into a 0.05 *M* methanolic solution of either detergent. Then the plates were air-dried in a vertical position and stored in a dust-free, dark place. Under these conditions the plates were stable for at least three months.

Preparation and application of the test-substance solutions

The test compounds were dissolved in acetonitrile–water (1:1) in a concentration of *ca.* 0.01 *M*. Some ammonia was added for the chromatography of α-amino acids. Then 10 μl of these solutions were spotted onto each of the four differently

impregnated plates. The solvent was evaporated under a gentle stream of nitrogen. As a reference compound, 10 μl of a 0.01 M acetanilide solution were spotted onto each plate.

Development of the chromatograms

The chromatograms were developed vertically in vapour-saturated tanks containing methanol–water (6:4) solution, in which 0.05 M of the corresponding detergent was dissolved to prevent gradient elution[5]. The running distance was fixed at 15 cm. Thus the elution time was *ca.* 2 h at room temperature. Afterwards the plates were air-dried.

Detection

Spots of compounds with adequate UV light absorption properties were located by fluorescence quenching at 254 nm. The following colouring reagents were used as alternative methods: (a) ninhydrin for primary and secondary amines and amino acids; (b) iodo-platinate for tertiary amines; (c) aniline-xylose for carboxylic acids. The procedures were as described by Smith[11].

R_F normalisation

The R_F values were normalised with reference to acetanilide using the following formula:

$$\frac{R'_F\,(\mathrm{X})}{R'_F\,(\mathrm{ref})} \cdot 0.6 = R_F(\mathrm{X})\ (\mathrm{norm}) \qquad (1)$$

where $R'_F(\mathrm{X}) = R_F$ of unknown component on a plate; $R'_F(\mathrm{ref}) = R_F$ of acetanilide on the same plate; 0.6 = a constant $\approx$ mean R_F of acetanilide on all the plates used in this study.

RESULTS AND DISCUSSION

We screened both the anionic and cationic functions of 132 compounds, five times over. The results for the different classes of compounds tested are given in Tables I–V. The structures of compounds indicated by their generic names are given in Table VI. Fig. 1 is a graphical presentation of the results in these tables.

In view of our experimental data we held a mean ΔR_F of over ± 0.03 to be a significant score. The dotted lines in Fig. 1 indicate the borders of significance.

Anion screening (N1/N4-system)

Almost all the carboxylic and sulphonic acids, as well as the sulphuric acid ester, show a significantly positive ΔR_F value; of the phenols only the stronger acidic ones score significantly.

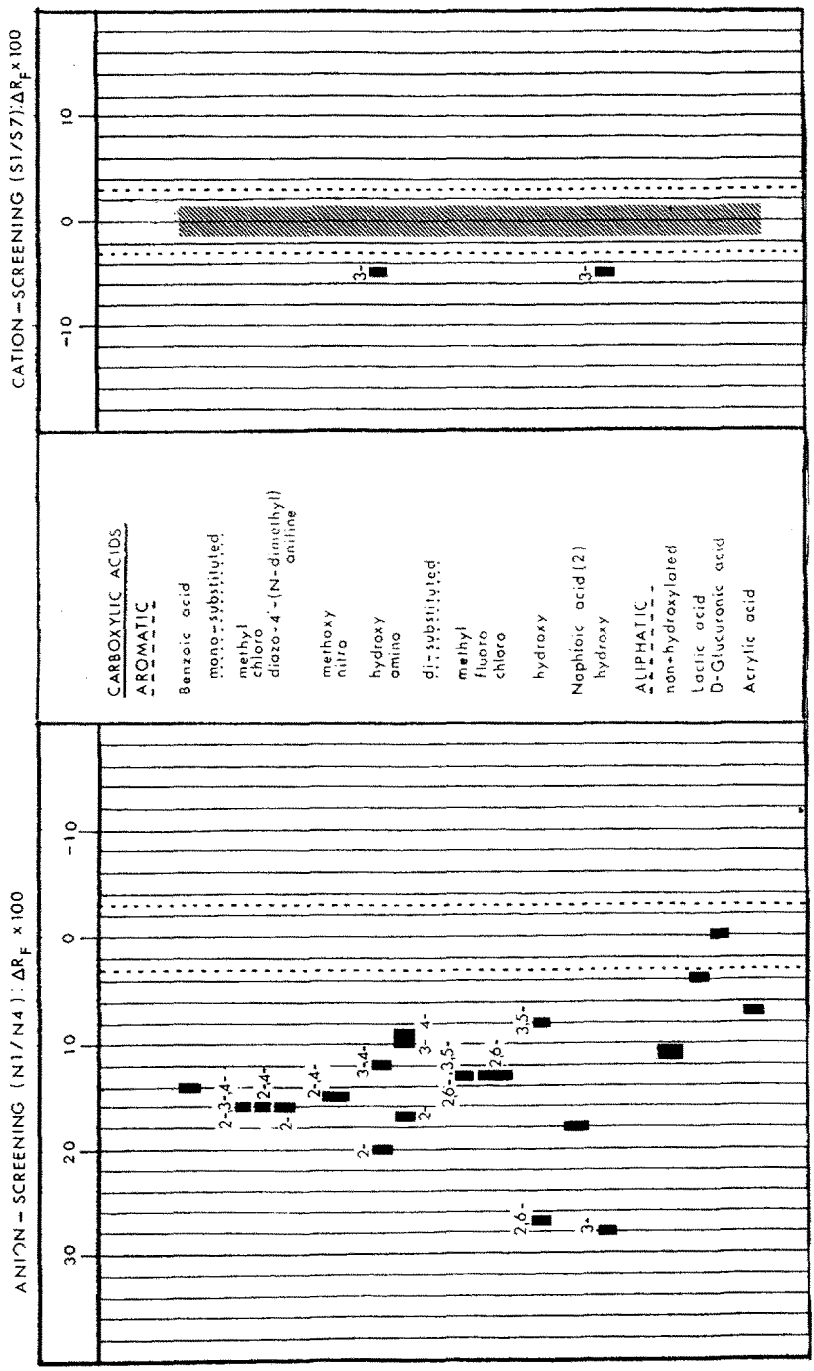
With the aromatic acids the values of ΔR_F vary with the substituent, while for each substituent they vary with the substitution pattern. The magnitude of ΔR_F appears to be directly related to the extent to which the ionogenic group contributes to the hydrophilic character of the molecule. This is illustrated by benzoic acid in comparison to 3- and 4-hydroxy-, 3- and 4-amino- and 3,5-dihydroxybenzoic acids.

TABLE I

QUALITATIVE ION-PAIR SCREENING RESULTS OF THE CARBOXYLIC ACIDS

No.	*Compound*	$R_F \times 100$						*No.*	*Compound*	$R_F \times 100$					
		N1	*N4*	*Δ*	*S1*	*S7*	*Δ*			*N1*	*N4*	*Δ*	*S1*	*S7*	*Δ*
	Mono-aromatic acids								*Mono-aliphatic acids*						
1	Benzoic acid	76	62	14	72	71	1	33	Acetic acid	80	68	12	75	76	−1
	Mono-substituted							34	Propionic acid	82	70	12	77	78	−1
2	2-Methyl	77	61	16	76	75	1	35	Butyric acid	77	66	11	77	75	2
3	3-Methyl	75	59	16	71	69	2	36	Valeric acid	73	62	11	69	68	1
4	4-Methyl	75	59	16	72	71	1	37	Caprionic acid	68	57	11	–	65	
5	2-Chloro	77	61	16	77	77	0	38	Caprylic acid	54	44	10	55	56	−1
6	4-Chloro	75	59	16	72	72	0	39	Capric acid	39	29	10	38	40	−2
7	2-Diazo-4′-(N-dimethyl aniline)	56	40	16	55	55	0	40	Acrylic acid	80	73	7	82	83	−1
8	2-Methoxy	78	63	15	74	73	1	41	Lactic acid	78	74	4	80	81	−1
9	4-Methoxy	76	61	15	72	71	1	42	D-Glucuronic acid	83	83	0	82	80	2
10	2-Nitro	77	62	15	72	74	−2	43	Phenolphthalein-D-glucuronide	76	65	11	79	85	−6
11	4-Nitro	75	60	15	73	76	−3								
12	2-Hydroxy	75	55	20	73	74	−1		Phenylacetic acid						
13	3-Hydroxy	79	67	12	76	81	−5		*Mono-substituted*						
14	4-Hydroxy	81	69	12	75	76	−1	44	4-Methoxy	80	67	13	78	77	1
15	2-Amino	78	61	17	73	72	1	45	2-Hydroxy	74	56	18	77	78	−1
16	3-Amino	79	69	10	79	81	−2	46	4-Hydroxy	79	66	13	80	81	−1
17	4-Amino	80	71	9	76	78	−2	47	2-Pyridylacetic acid	89	70	19	79	78	1

	Di-substituted						
18	2,6-Dimethyl	72	59	13	70	70	0
19	3,5-Dimethyl	67	54	13	66	66	0
20	2,6-Difluoro	81	67	14	78	75	−2
21	2,6-Dichloro	73	59	14	73	72	−1
22	2,6-Dihydroxy	76	49	27	73	76	−3
23	3,5-Dihydroxy	80	72	8	79	82	−3
24	Naphthoic acid (2)	65	47	18	67	66	1
	Mono-substituted						
25	3-Hydroxy	68	40	28	69	74	−5
	Bis-aromatic acids						
26	5,5′-Methylene disalicylic acid	77	44	33	79	85	−6
27	6,6′-Methylene di-(3-hydroxy)benzoic acid	78	57	21	80	85	−5
28	3,3′-Methylene di-(2-hydroxy,1-naphthoic acid)	75	33	42	81	87	−6
29	3,3′-Methylene di-(2,6-dihydroxy)benzoic acid	78	42	36	83	88	−5
	Benzenedicarboxylic acids						
30	Phthalic acid	79	63	16	76	80	−4
31	Isophthalic acid	80	68	12	74	71	+3
32	Terephthalic acid	78	68	10	82	87	−5
48	3-Pyridylacetic acid	81	70	11	81	81	0
49	Phenoxyacetic acid	78	58	20	75	76	−1
	Mono-substituted						
50	2-Hydroxy	77	57	20	77	78	−1
51	2-Methyl hippuric acid	73	60	13	73	74	−1
52	4-Methyl hippuric acid	73	61	12	74	75	−1
53	Cinnamic acid	70	56	14	70	70	0
54	Cloximate acid	64	51	13	68	70	−2
55	5-Carboxyl-4′-(trifluoromethyl)phenyl valerophenone	57	48	9	62	64	−2
	Aliphatic diacids						
56	Malinic acid	78	64	14	77	75	2
57	Succinic acid	80	64	16	73	71	2
58	Glutaric acid	77	61	16	71	69	2
59	Pimelic acid	81	65	16	74	75	−1
60	*n*-Octylmalonic acid	52	36	16	53	54	−1
61	Malic acid	80	74	6	79	77	2
62	Tartaric acid	80	64	16	73	72	1
63	1,5-Dihydroxypimelic acid	81	67	13	77	77	0
64	4-Phenylenediacetic acid	81	67	14	81	79	3
65	Maleic acid	80	70	10	79	85	−6
66	Fumaric acid	83	75	8	85	90	−5
	Aliphatic triacids						
67	Citric acid	80	72	8	81	79	2


ANION – SCREENING (N1/N4): ΔR_F x 100
30
20
10
0
-10
CARBOXYLIC ACIDS
AROMATIC
Benzoic acid
mono-substituted
methyl
chloro
diazo-4-(N-dimethyl) aniline
methoxy
nitro
hydroxy
amino
di-substituted
methyl
fluoro
chloro
hydroxy
Naphtoic acid (2)
hydroxy
ALIPHATIC
non-hydroxylated
Lactic acid
D-Glucuronic acid
Acrylic acid
2-3-4-
2-4-
2-
2-4-
2-
3-4-
3-
2-
3-4-
2-
2-6-3,5-
2-6-
3,5-
2-6-
3-
CATION – SCREENING (S1/S7): ΔR_F x 100
10
0
-10
3-
3-

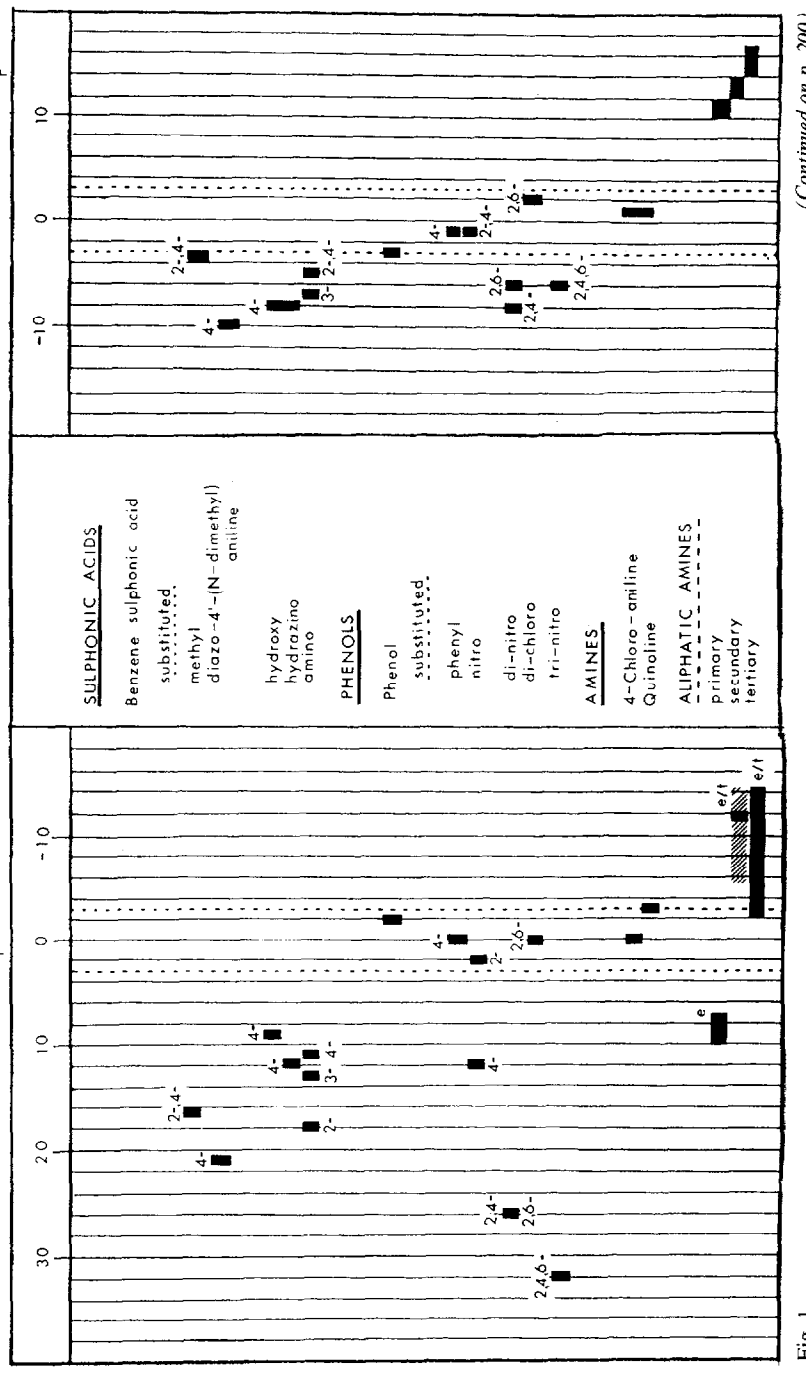


Fig. 1.

(Continued on p. 200)

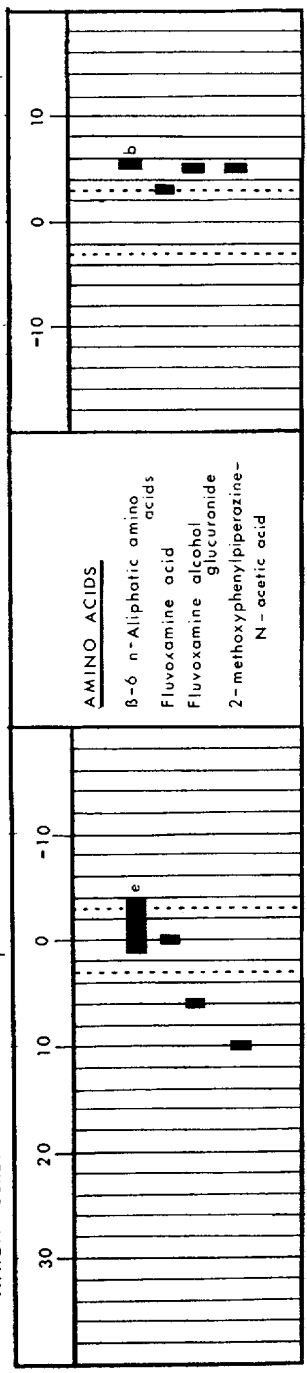


Fig. 1. Correlation chart ΔR_F *versus* structure. The numbers in the diagram represent the substitution site, the letters indicate the appearance of the spot on the plate (e = elongated, b = broad, t = trail). The dotted lines are the borders of significance.

TABLE II

QUALITATIVE ION-PAIR SCREENING RESULTS OF THE SULPHONIC ACIDS AND A SULPHATE

No.	*Compound*	$R_F \times 100$					
		N1	*N4*	*Δ*	*S1*	*S7*	*Δ*
	Mono-sulphonic acids						
	Benzenesulphonic acid						
	Mono-substituted						
68	2-Methyl	78	61	17	74	77	− 3
69	4-Methyl	77	61	16	76	80	− 4
70	4-Hydroxy	78	69	9	77	85	− 8
71	2-Amino	82	64	18	77	82	− 5
72	3-Amino	83	70	13	78	83	− 5
73	4-Amino	84	73	11	78	83	− 5
74	4-Hydrazin	83	70	12	84	93	− 8
75	4-Diazo-N′-dimethylaniline	56	35	21	58	68	−10
	Di-sulphonic acids						
76	1,3-Benzenedisulphonic acid	82	67	15	84	89	− 5
77	Phenol-2,4-disulphonic acid	75	51	24	83	89	− 6
78	Ponceau-3R-red	89	53	36	95	99	− 4
79	E 111	77	54	23	84	96	−12
80	E 122	72	37	35	80	94	−14
81	E 125	75	39	36	80	94	−14
82	Phenolphthalein disulphate	83	49	34	83	90	− 7
	Tri-sulphonic acids						
83	E 123	85	58	27	89	99	−10
	Tetra-sulphonic acids						
84	E 126	90	52	38	90	99	− 9
85	E 151	82	50	32	88	99	−11
86	E 152	84	57	27	79	97	−18

TABLE III

QUALITATIVE ION-PAIR SCREENING RESULTS OF PHENOLS

No.	*Compound*	$R_F \times 100$					
		N1	*N4*	*Δ*	*S1*	*S7*	*Δ*
87	Phenol	56	58	−2	63	66	−3
	Mono-substituted						
88	4-Phenyl	39	40	0	41	42	−1
89	2-Nitro	51	49	2	50	51	−1
90	4-Nitro	61	49	12	51	52	−1
	Poly-substituted						
91	2,6-Dichloro	31	31	0	45	43	2
92	2,6-Dinitro	70	44	26	72	78	−6
93	2,4-Dinitro	70	44	26	71	79	−8
94	2,4,6-Trinitro	65	33	32	70	76	−6

TABLE IV

QUALITATIVE ION-PAIR SCREENING RESULTS OF AMINES

The letter in the table characterise the spot on the plate (b = broad; e = elongated; t = trail).

No.	*Compound*	$R_F \times 100$					
		N1	*N4*	*Δ*	*S1*	*S7*	*Δ*
	Aromatic amines						
95	4-Chloroaniline	55	55	0	57	56	1
	N-Heterocylic compounds						
96	Quinoline	44	47	− 3	46	45	1
	Aliphatic amines (mono)						
	Primary						
97	*n*-Butylamine	56e	49e	7	66	55	11
98	*n*-Hexylamine	47e	37e	10	58	46	12
99	*n*-Octylamine	25e	17e	8	43	33	10
100	Fluvoxamine	20e	10e	10	38	26	12
101	N-Naphthylene ethyldiamine	22b	9e	13	47	39	11
102	α-(2-Amino ethane)pyridine	48e	24e	24	60	49	11
103	Benzylamine	48b	10b	38	53	44	10
	Secondary						
104	Diethylamine	63e	–	–	74	60	14
105	Diethanolamine	–	48e	–	69	64	5
106	Ritodrine	70e	84e	−14	76	64	12
107	Isoxsuprine	t	t	–	64	51	13
108	Betahistine	58e	69e	−12	67	67	13
	Tertiary						
109	Triethanolamine	85e	81e	4	77	71	6
110	Tripropylamino	67e	69e	− 2	68	54	14
111	Mebeverine	65e	76e	−11	66	49	17
112	Mebeverine alcohol	63e	77e	−14	64	49	15
113	Secoverine	t	t	–	43	28	15
114	2-Methoxy-N-phenylpiperazine	t	t	–	52e	45e	7
	Diamines						
115	Diaminoethane	0 →	30t	–	0 →	30t	–
116	Diaminopropane	0 →	30t	–	0 →	30t	–
117	Diethylamino-N-ethylamine	20 →	50t	–	20 →	50t	–
118	Diethylamino-N-propylamine	20 →	50t	–	20 →	50t	–
119	2-Methoxyphenylpiperazine-N-ethylamine	22	11	11	42	34	8
	Tetraamines						
120	Triethylenetetraamine	0 →	30t	–	0 →	30t	–
121	Hexamethylenetetraamine	73	72	1	72	67	5

However, both 2-hydroxy- and 2,6-dihydroxybenzoic acids deviate grossly from this pattern. Evidently hydrophilic substituents adjacent to the aromatic acidic group function as one hydrophilic entity with respect to the counter-ion.

For the aliphatic acids as a group, ΔR_F is smaller than for the aromatic carboxylic acids. The lipophilic interaction of the aliphatic part of the molecule probably contributes more to the retention mechanism than an aromatic moiety does.

The difference in ΔR_F between 2- and 4-nitrophenol cannot be explained by

TABLE V

QUALITATIVE ION-PAIR SCREENING RESULTS OF AMINO ACIDS

The letters in the table characterise the spot on the plate (b = broad; e = elongated; t = trail).

No.	*Compound*	$R_F \times 100$					
		N1	*N4*	*Δ*	*S1*	*S7*	*Δ*
	α-Amino acids						
122	Tyrosine	t	t	–	t	t	–
123	Aspartic acid	77	69	8	74	72	2
124	Glutamic acid	78	68	10	75	72	3
125	Lysine	t	t	–	t	t	–
	Amino acids (β, γ etc.)						
126	β-Alanine	78e	77e	1	79	73b	6
127	γ-Aminobutyric acid	78e	77e	1	80	74b	6
128	5-Aminovaleric acid	77e	80e	−3	80	75	5
129	6-Aminocaprionic acid	77e	81	−4	80	75	5
130	Fluvoxamine acid	51	49	2	51	47	4
131	Fluvoxamine alcohol glucuronide	63	57	6	59	54	5
132	2-Methoxyphenylpiperazine-N-acetic acid	77	67	10	69	64	5

different K_a values; at the pH of the chromatographic system both phenols are dissociated to a similar extent. Since 2-nitrophenol is indifferent to the discrimination mechanism of the counter-ions, it probably forms no ion-pair because of strong intramolecular hydrogen-bonding, in contrast to 4-nitrophenol.

In the anion screening, primary amines yielded a positive ΔR_F value. With the secondary and tertiary amines, trailing invariably occurred in the range of negative ΔR_F values.

Compounds having both an acidic and basic function have small and often insignificant ΔR_F values. In the latter case intramolecular ion-pairing probably prevails.

Cation screening (S1/S7 system)

Fig. 1 shows that only the aliphatic amines yielded significantly positive ΔR_F values. Moreover, a clear distinction was obtained between primary, secondary and tertiary amines.

The amino acids have much lower ΔR_F values (like amine substituents with strong hydrophilic groups); with fluvoxamine acid the value is low enough to be insignificant, again probably owing to intramolecular ion-pairing.

No significant ΔR_F values were obtained with 4-chloroaniline or with quinoline. It therefore seems justifiable to consider weakly basic nitrogen substituents merely as hydrophilic substituents in the anion-screening discussion.

The significantly negative ΔR_F values of the sulphonic acids clearly distinguish them from the carboxylic acids.

Dependence of ΔR_F on the number of ionic groups

Table VII is a survey of the ΔR_F values in the N1/N4 system of the carboxylic

TABLE VI

STRUCTURES OF COMPOUNDS INDICATED BY THEIR GENERIC NAMES

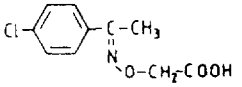
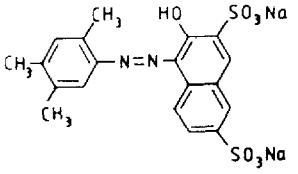
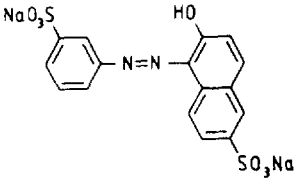
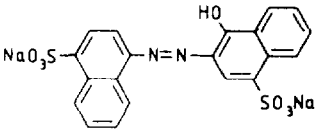
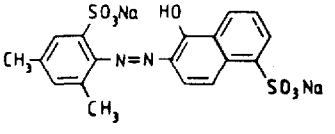
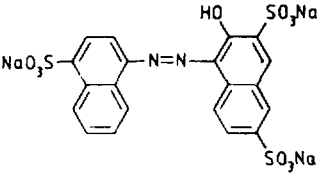
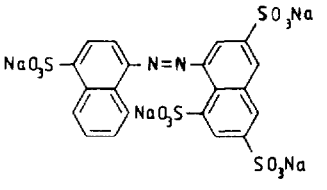
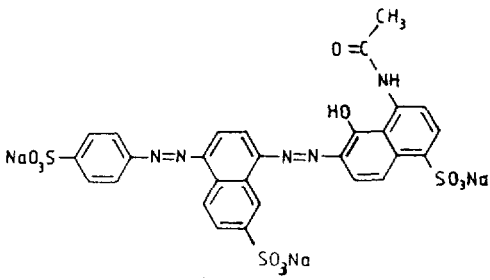
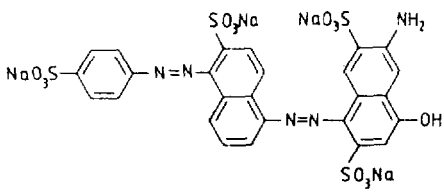
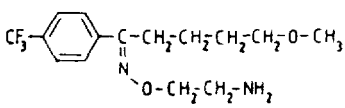
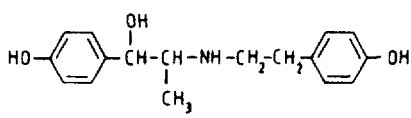
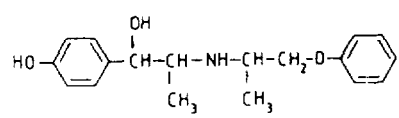
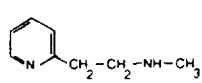
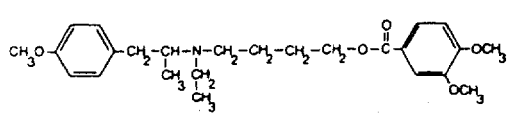
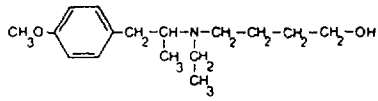
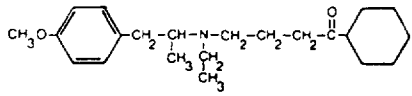
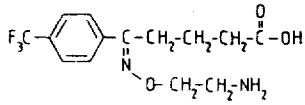
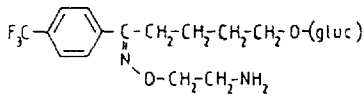
No.	*Generic name*	*Structure*
54	Cloximate acid	
78	Ponceau-3R-red	
79	E 111	
80	E 122	
81	E 125	
83	E 123	
84	E 126	

TABLE VI *(continued)*

No.	*Generic name*	*Structure*
85	E 151	
86	E 154	
100	Fluvoxamine	
106	Ritodrine	
107	Isoxsuprine	
108	Betahistine	
111	Mebeverine	

(Continued on p. 206)

TABLE VI *(continued)*

No.	*Generic name*	*Structure*
112	Mebeverine alcohol	
113	Secoverine	
130	Fluvoxamine acid	
131	Fluvoxamine alcohol glucuronide	

acids tested. The data are taken from Table I. The results show that, with certain exceptions, a relationship is valid between ΔR_F and the number of acidic groups (n) in the molecule. This empirical relationship was found to be

$$\Delta R_F(n) = \Delta R_F(n = 1)\ \{1 + 1/2\ (n - 1)\}$$

TABLE VII

THE RELATIONSHIP BETWEEN MONO- AND POLYACIDS WITHIN ANALOGUES

Monoacids	ΔR_F	*Polyacids*	ΔR_F *(calc.)*	ΔR_F *(exp.)*
Aliphatic acids (no. 33–39)	0.11	Aliphatic acids	0.16	0.16
2-Hydroxybenzoic acid (no. 12)	0.20	5,5′-Methylene-disalicylic acid (no. 26)	0.30	0.33
3-Hydroxy-2-naphthoic (no. 24)	0.28	3,3′-Methylene-di-(2-hydroxy-1-naphthoic acid) (no. 28)	0.42	0.42
2,6-Dihydroxybenzoic acid (no. 22)	0.27	3,3′-Methylene-di-(2,6-di-hydroxybenzoic acid) (no. 29)	0.40	0.36
Acrylic acid (no. 40)	0.07	Maleic acid (no. 65)	0.10	0.10
Lactic acid (no. 41)	0.04	Malic acid (no. 61)	0.06	0.06
		Citric acid (no. 67)	0.08	0.08

With regard to the exceptions, the phthalic acids (Table I, Nos. 30–32), fumaric acid (No. 66), 4-phenylenediacetic acid (No. 64) and benzene-1,3-disulphonic acid (Table II); No. 76), the chemical structures suggest this deviation is due to the

geometry of the molecules rendering impossible the simultaneous contact of the two acidic groups with the stationary phase. We did not include the monosulphonic acids in this survey because they did not have proper polysulphonic acid analogues.

Polyamines do not follow an analogous rule in the S1/S7 system. Under the screening conditions, *n*-alkane polyamines exhibited trailing on all plates from $R_F \approx$ 0–0.10 to $R_F \approx$ 0.30–0.60 (Table IV; Nos. 115–118 and 120). The cyclic polyamines reacted like amines with highly hydrophilic substituents.

Miscellaneous

α-Amino acids exhibited trailing from the start to $R_F \approx 0.80$ in both screening systems. The α-amino acids, which have an additional carboxylic group (Table V; Nos. 123 and 124), simply react like monoacids (as Reichl[2] found with his ΔR_M method) only the spot is broader, which indicates the presence of an α-amino acid with its poor chromatographic performance under these conditions. An additional amino group in the α-amino acid lysine (Table V; No. 125) yields the combination of chromatographic performance of an *n*-alkane polyamine and an α-amino acid; *i.e.* trailing from $R_F \approx$ 0–0.60.

CONCLUSIONS

Qualitative ion-pair chromatography is a useful tool for the identification of ionogenic groups in aromatic and aliphatic compounds. The system applied discriminates in general between carboxylic and sulphonic acids. When used with a set of reference compounds it also furnishes information about the nature of other than ionogenic substituents.

The method used is also indicative of the presence of primary, secondary and tertiary amino groups in aliphatic compounds.

In our laboratory we have applied quantitative ion-pair chromatography to the structural elucidation of biodegradation products of radioactive drugs. With the help of radiochromatogram scanning or scraping-off detection we could thus characterise drug metabolites in sub-microgram amounts. The technique also provided guidance in choosing appropriate derivatives for structural analysis of these metabolites by gas chromatography–mass spectrometry.

It is our experience that qualitative ion-pair TLC can provide a worthwhile and simple structural analysis, certainly in combination with other analytical methods such as infrared, ultraviolet, nuclear magnetic resonance spectroscopy or mass spectrometry, or even colouring reagents. Although the approach is somewhat different, in view of results we agree with Reichl when, in 1956, he foresaw that the idea of his "ΔR_M method" had a future.

ACKNOWLEDGEMENT

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