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CORRELATION STUDIES BETWEEN PAPER CHROMATOGRAPHIC MOBILITY AND THE CHEMICAL STRUCTURE OF CERTAIN BIO-ORGANIC SUBSTANCES AND THEIR SIGNIFICANCE FOR IDENTIFICATION

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SUMMARY

For approximately 1100 compounds, mainly belonging to the natural product series, the paper chromatographic mobility data in six solvent systems are reviewed The most characteristic paper chromatographic mobility patterns were illustrated and compiled with respect to their irregular mobility (according to the definition in the text) in these solvent systems. These irregularities in the mobility served as a good indication for the presence of several chemical entities, *e.g.* 1,3- and 1,4-dihydric phenols, alkaloids, amines and hydroxyindoles, judged by the mobility only. The close structural relationship of these compounds and the type of paper chromatographic mobility is discussed. A few examples are also given for the compounds (so far investigated) which might interfere with this classification.

I would like to review some of the findings with respect to the paper chromatographic (PC) mobility (*i.e.* R_F values) and the chemical structure of several types of compounds which have been investigated. About 600 compounds belonging to the phenolic group of substances of plant and animal origin, as well as secondary metabolites and metabolic products, biogenic amines, amino acid derivatives, alkaloids, hippuric acids, indole derivatives, etc. have each been run in six selected PC solvent systems and their R_F values are recorded[1–4]. In addition to this enormous pool of information, similar data for another 500 compounds have been collected[5–7] from synthetic organic substances, which were in some way related to the earlier mentioned natural products, in order to cover and demonstrate the PC mobility changes caused by substituents in the ring-structures investigated other than those which occurred in nature.

When arranging these solvents in a special order (by decreasing hydrophility and polarity, combined with pH changes) and presenting the R_F values for a single compound in a diagram, a great number of chromatographic spectra were obtained. These spectra very often showed interesting shifts in R_F values from one solvent to another, compared to the regular patterns, which at least for neutral compounds had a tendency to decrease their R_F values uniformly.

The significance of this work, although started some 15 years ago, was not fully exploited until quite recently as there were difficulties in obtaining some of the key compounds, for instance in the indole series, where some compounds have only become commercially available in recent years.

This system for visual comparison by means of standard R_F patterns was originally designed to demonstrate the presence of 1,3- and 1,4-dihydric phenol derivatives (both acidic and neutral) in different mould culture media in the presence of other substances, some of which were known earlier as mould metabolic products. Even at this stage, with only a limited number of compounds having been investigated, several irregularities in the R_F value patterns for this type of compound attracted attention and later proved to be a useful indication of the occurrence of the above-mentioned phenol derivatives in different species with a high degree of accuracy.

Gradually this systematisation of PC data was expanded to cover many other types of compounds and was later extended into the field of human metabolites in health and in different diseases. This alone brought about an extensive coverage of indolic compounds and some of the drugs (and their metabolites) used in treatment of mental diseases. This in turn added new data concerning the irregular but rather group-specific R_F value distribution patterns, which will be discussed in this paper.

In general, it was found that these PC solvent systems were fairly selective and gave satisfactory correlations for the identification of 1,3- and 1,4-dihydric phenol derivatives, hydroxylated indoles, alkaloids, N-containing heterocyclics, aromatic pyruvic acid derivatives, natural products of the 2-pyrone, 4-pyrone and tetronic acid type—all of which constitute a broad spectrum of substances of natural origin.

It also became clear at an early stage of this composite study that the six selected PC solvents were not exactly ideal, although they had been proved to possess quite remarkable resolving powers, and that it would be necessary to formulate and add a few more solvent systems in the future, especially when certain less common types of compounds are studied. The decision to keep the number of solvents at a maximum of six was mainly dictated by practical reasons, and the disadvantage of this low number of solvents was to some extent compensated by the introduction of a set of twelve to fifteen standard detection reagents, which made it possible to operate with such a low number of solvents and still obtain useful data concerning the chemical structure of the substances involved. The details about the various possible detection procedures are omitted. It is assumed that any of the compounds found in Figs. 1–17 could be detected by means of nonspecific reagents and indications for the structure are based solely on studies of the R_F value patterns, together with any necessary calculations.

Furthermore, the irregular R_F changes, presented in Figs. 4–17, are very striking and any contribution from normal fluctuations of R_F values can in most cases be neglected.

The composition of the solvents was (in the order of their appearance in the diagrams):

F = ethyl methyl ketone–acetone–formic acid–water (40:2:1:6);

E = ethyl methyl ketone–diethylamine–water (921:2:77);

A = methyl isobutyl ketone–formic acid–water (10 parts ketone saturated with 1 part 4% formic acid);

B = chloroform–methanol–formic acid–water (10 parts of chloroform saturated

TABLE I

GENERAL R_F VALUE CHANGES IN THE SOLVENTS

Regular pattern (R)		
F > E > A > B > C > D	for neutral compounds = RN	Fig. 1
F < E > A > B > C > D	for bases (amines) = RB	Fig. 2
F > E < A > B > C > D	for acids = RA	Fig. 3
Irregular pattern		
A[a] < B > C D	in conjunction with RN, RB and RA	Figs. 4–13
A[a] B < C > D	only in conjunction with RN and RA	Figs. 14–17
A B C < D	observed, but found not significant	
A < B < C D	observed only once, occurs with RB pattern	

[a] These first two irregular patterns, in conjunction with the corresponding regular patterns are discussed in greater detail and examples of each of these observed changes will be presented in the figures. The R_F value changes and minor alternations at levels over R_F 0.90 are, in most cases, not considered significant and are omitted from this discussion.

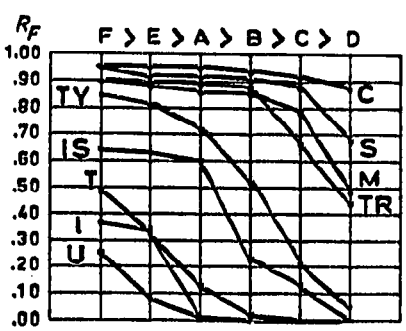
with a mixture of 1 part methanol and 1 part 4% formic acid);

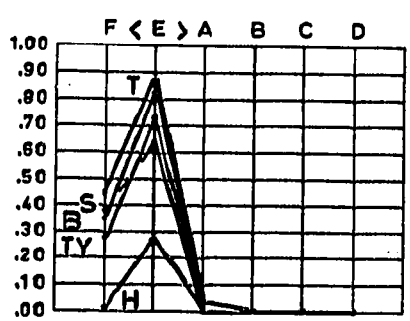
C = benzene–ethyl methyl ketone–formic acid–water (a mixture of 9 parts benzene and 1 part ketone saturated with 1 part 2% formic acid);

D = benzene–formic acid–water (10 parts benzene saturated with 1 part 2% formic acid).

Table I gives the general outline for the changes in the regular patterns of R_F values for neutral (RN), acidic (RA) and basic (RB) types of compounds. These patterns are predictable knowing the solvent composition and that the solvent E contains diethylamine.

The irregularity based on a sudden elevation of R_F values for a change from solvent A to B is observed in connection with all regular patterns. The second irregularity, that is elevation of the R_F for a change from solvent B to C, is only noticed in connection with RN and RA patterns. The third irregular pattern is not considered significant, since it is in the order of 0.01–0.02 R_F units. The fourth possibility, which





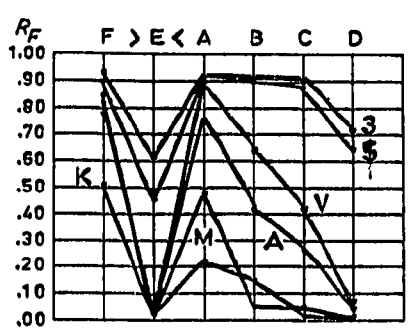


Fig. 1. Regular pattern for neutral compounds. C = *p*-cresol; S = salicylaldoxime; M = 5-methoxy-3-indolealdehyde; TR = N-acetyltryptamine; TY = N-acetyltyramine; IS = isoniazid; T = thiourea; I = indican; U = urea.

Fig. 2. Regular pattern for amines. T = tryptamine; S = serotonine; B = bufotenine; TY = tyramine; H = histamine.

Fig. 3. Regular pattern for acids. 3 = 3-methylsalicylic acid; 5 = 5-methylsalicylic acid; V = vanillic acid; A = *p*-aminobenzoic acid; M = 4-hydroxy-3-methoxymandelic acid; K = kojic acid.

only occurs with the RB pattern, has only been observed once and involves a 2-step elevation of R_F values in the solvents, thus A < B < C, which is unique. The compound is the drug Dromoran = *dl*-3-hydroxy-N-methylmorphinan.

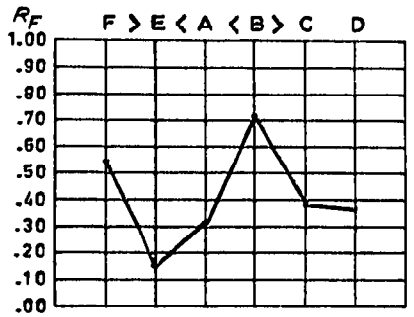
In Figs. 1–3, the three regular patterns are exemplified. Fig. 1 shows the mobility of neutral compounds and slightly acidic compounds (without a free carboxyl group); a characteristic of this distribution pattern is a gradual decrease of R_F values from solvent F to solvent D. The solvents were arranged in order of decreasing hydrophility, solvents F and A to D being slightly acidic with variable contents of formic acid. The second solvent in the series (E) is a basic solvent containing diethylamine.

Aromatic aldehydes (and ketones), carboxylic acid esters, and amines with their amino group blocked (by acetylation, formylation) give this type of pattern, although they are much more depressed in solvent D compared to the monohydroxyphenols or naphthols. For acetylated amines the depression of the R_F starts even in solvents B and C. Compounds with a free amino group, which is not balanced by any carboxyl group, show typical maxima for the R_F values in solvent E. For simple aromatic amines with the amino group in the side chain, Fig. 2 gives some examples; for complicated basic substances, secondary and tertiary amines, and amino substituted benzenes and heterocyclic derivatives, Figs. 6–13 should be consulted.

Urea and indican, which are usually present in large amounts in extracts of animal origin, have a sufficiently low mobility in solvents F and E (Fig. 1) not to interfere with the separation of other compounds. In this region of low mobility are also found all aliphatic and aromatic amino acids, many purines and pyrimidines, practically all of which only move in solvents F and E, with an R_F below 0.50.

Fig. 3 shows a regular pattern for acids, and all those compounds with at least one free carboxyl group show a minimum R_F value in solvent E due to its basic character. This lowering can be slight (only 5–10 $R_F \times 100$ units), when aromatic carboxylic acids are involved, and also if the hydroxy substitution is in the *ortho* position with respect to the carboxyl thus providing hydrogen bonding. *m*- and *p*-hydroxybenzoic acids cause a pronounced fall in R_F values, which decrease close to zero. Compounds with at least three free hydroxy groups per benzene ring (*e.g.* pyrogallol and to some extent phloroglucinol) show a lowering of the R_F values in solvent E, thus behaving like a strong acid. A few 4-pyrone derivatives, without a carboxyl group, are also acidic (kojic acid).

Fig. 4 illustrates the irregular mobility pattern, in solvents A and B where the R_F shows an increase, by comparison with the RA pattern. 5- and 6-membered non-aromatic rings (with one hetero oxygen atom) of the 2- and 4-pyrone and tetronic acid type are a common feature of these compounds, which could be considered as lactones of aliphatic ketoacids; they are acidic, some of them contain no carboxyl group and are known to be natural products of mould and vegetable origin. Pyruvic acid and diacetyl show a similar shift in their R_F values due to the keto and diketo character of these aliphatic compounds. There is a slight but definite indication that coumarin and its not too heavily substituted derivatives belong by mobility to this series, since they are all derivatives of 2-benzopyrone. The benzene ring fused to the pyrone has a tendency to diminish the shift A < B and to bring it close to A = B, with a difference of only 1–2 $R_F \times 100$ units. Only few 4-pyrone derivatives of the chromone, flavone and xanthone series have been investigated. A number of these natural products are extensively hydroxylated and the R_F-elevating effect in



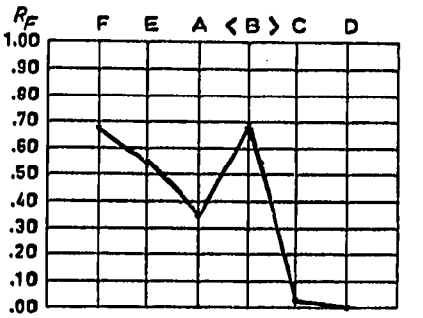


Fig. 4. Irregular mobility of compounds without nitrogen (mostly natural products). *Carolic acid* illustrated. Other examples: carlic acid, terrestric acid, terrein, 2,6-dimethyl-4-pyrone, maltol (= 3-hydroxy-2-methyl-4-pyrone), 4-pyrone-5-carboxylic acid, laevulinic acid, 2-pyrone-5-carboxylic acid, pyruvic acid, diacetyl, and pinonic acid. Change of R_F values according to the criteria F > E > A (regular pattern) and A < B > C. In cases where a free carboxyl group is present or acidic compounds are involved, the regular pattern change for acids is indicated by F > E < A, irregularity in A < B > C remains.

Fig. 5. Irregular mobility of compounds with mainly N-heterocyclic hydroxy derivatives, except hydroxyindoles, and a few aminobenzene derivatives. *4-Hydroxyquinaldine* illustrated. Other examples: 2- and 4-hydroxyquinoline, 2- and 4-hydroxypyridine, 2,6-dihydroxypyridine, 2- and 4-mercaptopyridine, 3-pyridineacetic acid, 2-, 3- and 4-pyridinecarboxylic acid, 6-methylpyridine-2-carboxylic acid, 2- and 6-quinolinecarboxylic acid, 4-hydroxy-6-methoxyquinoline, 4-hydroxy-N-methyl-2-quinolone, 6-methoxy-4-quinolinecarboxylic acid, 4-hydroxy-8-methoxy-3-quinolinecarboxylic acid, 2-hydroxy-5-methylaniline, N-methyl- and 3,5-dimethylanthranilic acid, *m*-aminobenzaldehyde, aceturic acid, phenoxyaceturic acid, *o*-methoxyphenaceturic acid, *o*-methoxyhippuric acid, *o*-aminohippuric acid methyl ester, 3-indolylacetaldehyde, 3-indolylacetic acid methyl ester, 2-methyl-5-methoxy-3-indolylacetamide, melantonin, 2,3-dihydroxyquinoxaline, *dl*-desthiobiotin, pyocyanine chloride, adenine, dehydrouracil, pyridoxine, phenothiazine-5-sulphoxide, and levomepromazine-5-sulphoxide. For further explanation, see legend to Fig. 4.

solvent B is thus cancelled out (naringenin, Fig. 15). More natural products of this type are the pyrylium derivatives, with a tetravalent oxygen in the 4-pyrone ring (no examples available) and flavylium salts (cyanidine chloride, Fig. 15).

In Fig. 5 the irregular shift, A < B, in the solvents together with RN pattern is described. A few pyridine- and quinolinecarboxylic acids overlap with RA pattern (Fig. 4). The most characteristic feature of this group of compounds seems to be that they all contain N. A majority of these are N-heterocyclic derivatives of pyridine and quinoline. If these compounds are hydroxylated, this type of pattern is only given when the hydroxy group is in the 2- or 4-position. Other possible hydroxylations result in an entirely different mobility pattern (Figs. 10–13). This is quite remarkable, and also accounts for the behaviour of the corresponding mercapto derivatives. The most likely explanation seems to be that 2- and 4-hydroxy substituents occur in the solvents in their corresponding keto form, 2-pyridone, 4-pyridone, 2-quinolone and 4-quinolone, respectively. In fact, 2-quinolone is a lactam of *o*-aminocinnamic acid and therefore seemingly correlated with lactones (Fig. 4) by the solubility. Since this group of substances, with irregular shifts in A < B, contain both weakly acidic and basic compounds, the differences in this respect will only be seen in solvent E as a lowering of the R_F value (compared to solvent A) or as the maintenance of the gradual decrease through solvents F to A, similar to neutral compounds. Some other groups of compounds (Fig. 5) with a generally similar pattern, but all individually different, are: four indole derivatives (out of a 100 investigated), including melatonin and 3-indolylacetaldehyde (both natural products); five glycine conjugates (out of 40) of

the aceturic and hippuric acid type; four aminobenzene derivatives and adenine and dehydrouracil. The 5-sulphoxide metabolites of the phenothiazine drug series are also found here, but the drugs in themselves have another pattern, where the basicity of the substituted heterocyclic ring determines the mobility (Fig. 11).

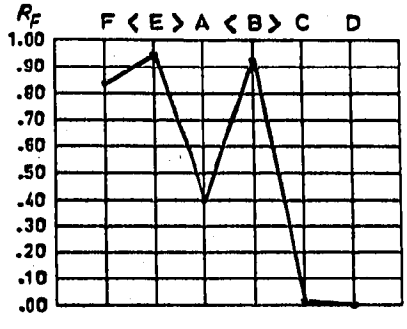
Irregular mobility patterns in the solvents, with changes in the R_F values according to the criteria F < E > A (regular pattern for bases) and A < B > C, are presented in Figs. 6–9. When R_F values for the alkaloids and closely related biogenic amines are plotted in a diagram, two R_F maxima, one in solvent E, the other in solvent B, become clearly visible. This double peak pattern has been found to be highly significant for this type of compounds of natural origin, however, there are synthetic bases which might be confused with these compounds. Hence the divergences in mobility are shown in greater detail for comparison in Figs. 10–13.

The general pattern (Series A, Figs. 6–9), obtained by investigation of about 40 alkaloids, can be divided for identification purposes into 4 separate sub-patterns, based on the slight but characteristic differences in the mobility in solvents F and A, and to some extent in solvents C and D. Figs. 6–9 show these standard patterns and below each diagram there is a list of the compounds belonging to each of these subdivisions.

The first compound (in italics) only is illustrated in the diagram. The others follow the pattern but are still very individual. The values of the R_F, under or over 0.50, particularly in the most critical solvents (F and A) are chosen as the limits for the subdivisions. A few compounds that exhibit some extra divergences in their R_F values are specially indicated.

The alkaloids were divided into 4 series (IA–IVA). Series IA (Fig. 6) is characterised by high R_F values in solvent F (over 0.50), by moderate values in solvent A (under 0.50 but not less than 0.10), and by values in C usually under 0.10 and those in D close to zero. This includes the data from quinoline and indole alkaloids of more complicated structure. Series IIA (Fig. 7), which includes even more complex indole alkaloids, is differentiated by the R_F values in F (less than 0.50) and R_F values in A (less than 0.10). The mobility in C and D being of the same order as for series IA.

In series IIIA (Fig. 8) the R_F values in solvent F are over 0.50, the values in



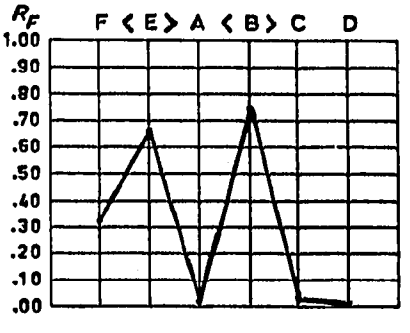
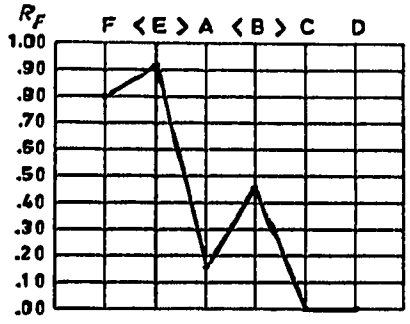


Fig. 6. Irregular mobility of alkaloids and biogenic amines (Series A) in solvents. Change of R_F values according to the criteria F < E > A (regular pattern for amines) and A < B > C. Series IA: *aconitine* illustrated. Other examples: ajmaline, ibogaine, papaverine (C and D < 50), quinidine, quinine, *d*-bromolysergic acid diethylamide (D < 10), lysergic acid diethylamide, and heroin.

Fig. 7. Irregular mobility of Series A compounds (*cf.* Fig. 6). Series IIA: *brucine* illustrated. Other examples: reserpine (C < 15), strychnine, and phenylalanine methyl ester.



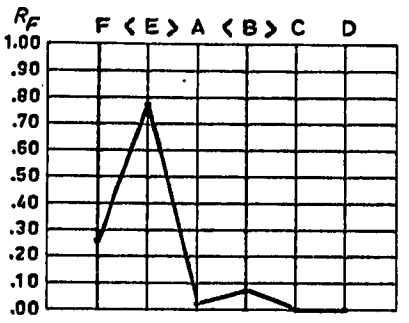


Fig. 8. Irregular mobility of Series A compounds (*cf.* Fig. 6). Series IIIA: *ergotamine* illustrated. Other examples: boldine, corynanthine, harman, harmine, harmol, isomescaline, laudanosine (C < 10), 3-methoxy-4,5-methylenedioxyamphetamine, 6-methoxytetrahydroharman, 2-methyl-N,N-dimethyltryptamine (C and D < 10), norharman, and yohimbine.

Fig. 9. Irregular mobility of Series A compounds (*cf.* Fig. 6). Series IVA: *amphetamine* illustrated. Other examples: atropine (B < 15), cinchonine, codeine (B < 20), N,N-diethyltryptamine, N,N-dimethyltryptamine, ephedrine, gramine (F = 50), *p*-methoxyphenylethylamine (C = 5), nicotine (B < 20), hordenine, *β*-phenylethylamine, *l*-phenylalaninol, pilocarpine (B < 30), and physostigmine (B < 40).

A under 0.50 and the elevation of the R_F values in solvent B reach a maximum at 0.50; the mobility in C and D is also zero. Compounds of the type harman, harmine mostly show this pattern.

In series IVA (Fig. 9), the R_F value in solvent F is under 0.50 and the values in A are close to zero, the mobility maximum in solvent B for this type of compound was set at 0.10, but there are deviations because of the very heterogeneous character of the compounds involved. The values in solvents C and D are *ca.* zero, as seems to be general for alkaloids. In this last series, some more complicated biogenic amines (often N-methylated) are located as well as several alkaloids. This division into 4 series was intended to demonstrate the possibility of sorting the very divergent groups of compounds into chemically related units. At the present moment these arbitrary subdivisions serve practical purposes and as more data become available, the criteria for classifying might have to be changed. At this stage, it is important to show that these 4 series have a sufficiently selective character, in comparison to all other compounds of basic character, with similar double peak mobility.

In series IB to IVB (Figs. 10–13) the synthetic bases are compared with the previous alkaloid patterns with respect to their mobility, and several striking differences can be described.

In series IB (Fig. 10), all the R_F values are over 0.50, and the compounds are mostly methoxyaminobenzene derivatives, unsubstituted bases with 3 fused rings and surprisingly, a few of the hydroxyquinolines which did not satisfy the conditions set for Fig. 5.

For series IIB (Fig. 11), the main characteristic seems to be that the R_F values in 4 solvents (F, E, A, B) are over 0.50. In a few cases, a drop in the R_F value in solvent A to the 0.25 region can be noticed. Predominant compounds are diamino derivatives of diphenyl or naphthalene. Some drugs like methopromazine used in treatment of psychiatric disorders are also, from the mobility point of view, classified in this group, along with two hydroxyquinolines (substitution other than in the 2- or 4-position).

Series IIIB (Fig. 12), the only one which bears a resemblance to the previous

Fig. 10. Irregular mobility of aromatic amines, N-containing heterocyclic compounds (not indoles) and some hydroxy derivatives of pyridine, quinoline and isoquinoline (hydroxy substituent not in the 2- or 4-position). All of them are synthetic compounds, some of them are drugs, no natural products (Series B). Change of R_F values according to the criteria F < E > A (regular pattern for amines) and A < B > C. Series IB (all R_F values over 0.50): *acridine* illustrated. Other examples: 8-amino-6-methoxyquinoline, *o*-anisidine, *m*-anisidine, 8-aminoquinoline, 2,5-dimethoxyaniline, N,N-dimethylaniline, N-methylaniline, *o*-toluidine, 8-hydroxyquinoline, 8-hydroxyquinaldine, and 1,3-dihydroxyisoquinoline.

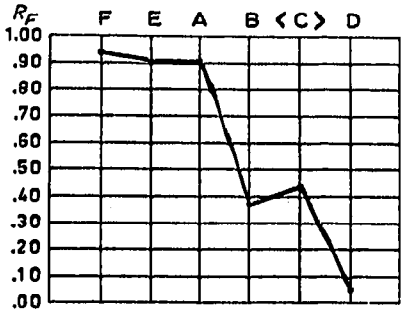
Fig. 11. Irregular mobility of Series B compounds (*cf.* Fig. 10). Series IIB (4 R_F values, in F, E, A and B, over 0.50): *aniline* illustrated. Other examples: *p*-anisidine, aminopyrine (A = 35), benzidine (A = 20), 1,5-diaminonaphthalene, 2,7-diaminonaphthalene (A = 25), *o*-dianisidine (C = 53), methopromazine, perphenazine, *o*-tolidine, *p*-tolidine (A = 50), 5-hydroxyquinoline, and 5-hydroxyisoquinoline (A = 29).

series IVA (amphetamine type of amines and alkaloids), deals exclusively with aminopyridine and phenylenediamine derivatives. Since all these are synthetic amines, it is not considered that they would seriously interfere with the patterns found by the natural products. A characteristic of the R_F value distribution in this group is either one value over 0.50 in solvent E or two values in solvents F and E. In the last pattern for synthetic bases (Series IVB, Fig. 13) of the imidazole type, the R_F values are all under 0.50, including the two hydroxypyridine derivatives.

With regard to the irregularity based on the R_F change in the solvents, B < C, as in conjunction with the RN pattern (Fig. 14), we find that 1,3- and 1,4-dihydric

Fig. 12. Irregular mobility of Series B compounds (*cf.* Fig. 10). Series IIIB (one or two R_F values in E and in F over 0.50): *2-aminopyridine* illustrated. Other examples: 3-aminopyridine, 4-amino-7-chloroquinoline (F > 50), atebrine, 3-hydroxy-2-hydroxymethylpyridine, *o*-phenylenediamine (F > 50), *m*-phenylenediamine, *p*-phenylenediamine, 4-aminopyridine, 2-amino-4-picoline and 2-amino-6-picoline.

Fig. 13. Irregular mobility of Series B compounds (*cf.* Fig. 10). Series IVB (all R_F values under 0.50): *imidazole* illustrated. Other examples: 3-hydroxypyridoxine (E = 57), and 4-desoxypyridoxine.



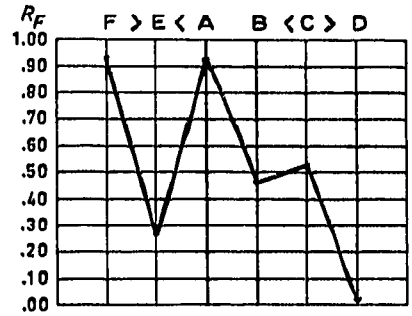


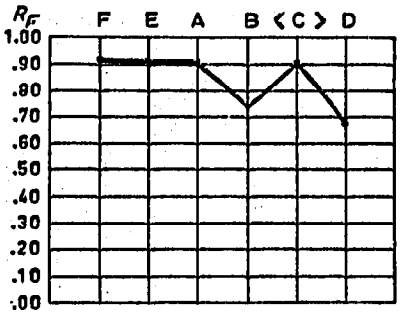
Fig. 14. Irregular mobility of neutral compounds. Change of R_F values according to the criteria F > E > A > B (regular pattern) and B < C > D. Pattern for 1,3-dihydric phenols. *Orcinol* illustrated. Other examples: 1,4-dihydric phenols, naphthalene-1,3-diol, -1,4-diol, -1,5-diol, 1,6-diol, -1,7-diol, and -2,7-diol (not -2,3-diol), phloretin, 3-hydroxybenzoic acid methyl ester, 3-indoleacetic acid hydrazide, 2-methyl-3-ethyl-5-aminoindole, and dimethylglyoxime.

Fig. 15. Irregular mobility of acidic compounds. Change of R_F values according to the criteria F > E > A > B (regular pattern) and B < C > D. In cases where a free carboxyl group is present, or three or more hydroxy groups, the regular pattern change for acids is indicated by F > E < A > B, irregularity in B < C > D remains. Pattern for 1,3-dihydric phenols + COOH. *Orsellinic acid* illustrated. Other examples: 1,4-dihydric phenols + COOH, 2,8-dihydroxy-3-naphthoic acid, phenylpyruvic acid, *p*-hydroxyphenylpyruvic acid, 3-indolepyruvic acid, 3-indoleglyoxylic acid, dehydracetic acid-5-carboxylic acid, spinulosin, naringenin, cyanidine chloride, 3,4-dinitro- and 2,5-dinitrobenzoic acid, 3- and 4-nitrosalicylic acid, 3,5-dichlorosalicylic acid, 3,5-diiodosalicylic acid, 5-formylsalicylic acid, and salicylic acid hydrazide.

phenol derivatives are predominant within this pattern, essentially independent of the presence of other substituents such as formyl, acetyl and amino groups. This is also true for all the naphthalene-diols, excepting the 2,3-diol (and probably the 1,2-diol), which is in accordance with similar exceptions in the benzene series (1,2-dihydric phenols). Only three nitrogen containing compounds are found in Fig. 14. The same irregularity as compared with the RA pattern (Fig. 15) is presented by an analogous distribution for the carboxylic acids of the 1,3- and 1,4-dihydric phenol series. Some of the natural products, which contain more than three hydroxy substituents per ring system, follow the RA pattern in solvents F, E and A. The shift to B < C is caused by the presence of either the 1,3- or 1,4-dihydroxy substitutions in the benzene part of the ring. A heterogeneous collection of synthetic substances of the nitro and dihalogeno salicylic acid type follow the same irregularity.

Finally, an interesting similar shift is obtained with aromatic and indolepyruvic acids (ketoacids in the more general sense). The unusual shift A < B, which was noticed earlier for pyruvic acid (Fig. 4) is carried over to B < C by substitution of pyruvic acid with a benzene or indole ring.

Figs. 16 and 17 illustrate the selective shift B < C for monohydroxyindoles in addition to the RN pattern. The prerequisite for this change seems to be that the hydroxylation is in the benzene part of the indole molecule. Addition of another neutral substituent (methyl group) to the pyrrole part of the indole produces similar changes in the same order, but shifted towards higher R_F values. If one remembers that practically none of the compounds with a similar shift (Fig. 14) gives a reaction with Ehrlich reagent (only two synthetic indole derivatives are listed, these yielding a yellow Ehrlich reaction), this gives a high selectivity for the demonstration of the presence and identification of hydroxyindoles based on the B < C irregularity. The



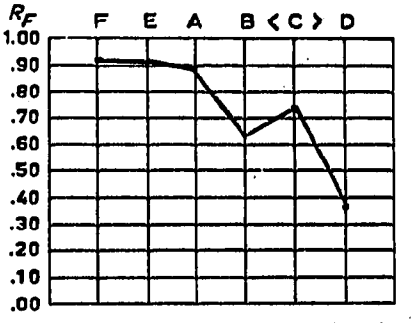


Fig. 16. Irregular mobility of monohydroxy indoles. Change of R_F values according to the criteria F > E > A > B (regular pattern for neutral compounds) and B < C > D. Pattern for hydroxyskatoles. *4-Hydroxyskatole* illustrated. Other examples: 5-, 6-, and 7-hydroxyskatole (2-hydroxyskatole no shift).

Fig. 17. Irregular mobility of monohydroxy indoles. Change of R_F values according to the criteria F > E > A > B (regular pattern for neutral compounds) and B < C > D. Pattern for hydroxyindoles. *4-Hydroxyindole* illustrated. Other examples: 5-, 6-, and 7-hydroxyindole (2- and 3-hydroxyindole no shift).

location of the substituent can be instantly reconfirmed by Ehrlich colour, although it is also fairly selectively predicted solely on the basis of mobility. A considerable interest in metabolic studies of these and other indolic compounds has recently developed as a result of attempting to find explanations for the biochemical background of mental diseases.

In conclusion I would like to point out that despite the fact that we have observed many interesting correlations for these PC solvent systems (and more will probably be found), they are only based on studies on the mobility of complicated chemical structures within the general limits of the compounds investigated. We still need some confirmation by means of selective reagents, in order to rule out several possibilities, in order to obtain a final identification. The solid basic information concerning the mobility of the different compounds within the limits of the scope and the number of solvents used seems, on the other hand, to provide a valuable guide for the selection of further purification and instrumental confirmation methods, whenever they become necessary.

Some of the correlations were better than one might have expected, in view of the variety of substances encountered. In order to bypass the large number of detection reagents needed, the number of solvents could indeed be increased and some exotic solvents might be formulated and tried out. This all brings us to the point where handling data of this enormous size becomes impossible from the practical point of view and automation in some way or other has to be used.

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DISCUSSION

NEHER: Do you get marked changes in the various chromatographic spectra if the products to be analysed are contaminated, *e.g.* with salts, buffers or other substances?

REIO: The answer is no. By using preferentially organic solvent extracts (*e.g.* ethyl acetate, ether, chloroform) of crude preparations, the salts (as well as peptides or sugars) are essentially eliminated. Concerning the irregular R_F value shifts (by sudden elevation of the R_F value pattern, instead of a gradual decrease, according to the definition in the text) in certain solvents for specific compounds—these are very pronounced (in the order of about 0.10–0.30 on the R_F scale) and in the presence of other moving compounds these spectra are not markedly affected or distorted.

NEHER: A useful additional parameter would be the electrophoretic behaviour at various pH values.

REIO: Yes, it would be useful in some cases. For phenolic compounds we have used several parameters, for instance, by esterifying the free hydroxy groups with [^{35}S]sulphate (by an enzymatic procedure) and separating these phenolic sulphate esters in two-dimensional PC systems and/or electrophoresis followed by autoradiography. This applies either directly to the aliquots or to the eluates from the selected R_F regions, which have been previously separated in solvents F $\rightarrow$ D.