CHROMATOGRAPHIC BEHAVIOUR AND CHEMICAL STRUCTURE

I. SOME NATURALLY OCCURRING PHENOLIC SUBSTANCES

by

E. C. BATE-SMITH AND R. G. WESTALL

Low Temperature Research Station, Cambridge (England)

Close connection between R_F value and structure is to be expected from theoretical considerations put forward by CONSDEN, GORDON, AND MARTIN[1] and more recently by MARTIN[2]. In the former paper the relation

$R_F = \frac{A_L}{A_L + \alpha A_S}$ was derived in which

$R_F = \frac{\text{movement of band of substance under investigation}}{\text{movement of the advancing front of liquid}}$,

A_L = cross sectional area of solvent phase,

A_S = cross sectional area of water phase,

α = partition coefficient of substance between the water and the solvent phase.

This relation can be expressed

$$\alpha = \frac{A_L}{A_S}\left(\frac{1}{R_F} - 1\right) \qquad 1$$

Since $\frac{A_L}{A_S}$ is equal to the ratio of the volume of solvent and water phase in the chromatogram, it can be taken as an arbitrary constant K for a given solvent and a given paper at a given temperature; or if desired it can be determined from the ratio of the weight of paper used to that of the developed chromatogram.

It was shown by these authors that for six amino-acids, the partition coefficient calculated by means of the above relation agreed well with the partition coefficient directly measured by ENGLAND AND COHN[3].

In his more recent paper, MARTIN considers the factors likely to determine the distribution of a substance between two immiscible solvents. For ideal solutions he deduces that the partition coefficient of a substance A between two phases S and M is related to the free energy required to transport one mole of A from S to M by the expression

$$\ln \alpha = \frac{\Delta \mu_A}{RT} \qquad 2$$

Further, he shows that the addition of a group X to the substance A should change the partition coefficient by a given factor depending on the nature of the group and on

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the pair of phases employed but not on the rest of the molecule. Thus, we can expect where the original molecule of A is substituted by n groups X, m groups Y, etc.

$$RT \ln\alpha = \Delta\mu_A + n\Delta\mu_X + m\Delta\mu_Y + \text{etc.} \qquad 3$$

Substituting $K\left(\frac{1}{R_F} - 1\right)$ for α from equation 1

$$RT \ln K\left(\frac{1}{R_F} - 1\right) = \Delta\mu_A + n\Delta\mu_X + m\Delta\mu_Y + \text{etc.} \qquad 4$$

ENGLAND AND COHN[3] have shown that the partition coefficients of a homologous series of amino-acids change regularly with each additional carbon atom in the chain, which would imply that the R_F values should also change with a corresponding degree of regularity. As CONSDEN, GORDON, AND MARTIN[1] pointed out, a rational change in R_F value with constitution of the amino-acids can be discerned, but the number of members of homologous series among the amino-acids is too small for the principles governing this regularity to be firmly established.

An opportunity to test MARTIN'S deduction has arisen in the course of an investigation of the chromatographic behaviour on filter paper of the naturally occurring C_{15} compounds belonging to the classes: anthocyanins, flavones, chalkones, etc.; and certain hydroxy-, methoxy-, and carboxy-derivatives of benzene which are or may be formed by degradation of the C_{15} molecule. The expectation of a close relationship between R_F value and chemical constitution, especially in respect of the nature and number of these particular substituent groups, has been amply borne out.

METHODS

The apparatus used for determining the R_F values of the substances studied was in general the same as that originally described by CONSDEN, GORDON, AND MARTIN[1] but in detail the procedure followed more closely that of PARTRIDGE[4]. Whatman No. 1 filter-paper sheets have been used almost exclusively, and, since the main object has been to catalogue accurate R_F values, the chromatograms were run on strips in one direction only. The solutions of the test substances were made up in approximately 0.1% (w/v) *n*-butanol made acid with conc. HCl so as to contain 1% HCl (w/v), excepting the anthocyanins which were made up in similar concentration in 1% aqueous HCl. 3–4 μl of each solution were applied as a circular spot on the filter paper by means of a small pipette. Usually 5 or 6 substances were run in parallel on each paper strip, the spots being applied at 2 cm intervals along a horizontal line drawn 8 cm from the top of the paper.

Trial runs carried out with a number of solvent mixtures already mentioned in the literature revealed that the butanol-acetic acid-water mixture (40–10–50) by volume recommended by PARTRIDGE[4] for sugars was especially suitable for the classes of substances now being studied. A search for a second solvent mixture which would vary the order of the R_F values has not been entirely successful; various basic mixtures were tried but either the R_F values were excessively low or the substances failed to run as discrete spots. However, *m*-cresol-acetic acid-water (50–2–48) by volume has proved quite useful for two reasons. First, although the R_F values of the substances having different degrees of hydroxylation were preserved in the same order as in butanol-

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acetic acid, some variation occurred between compounds of the same degree of hydroxylation. Secondly, substances having R_F values in butanol-acetic acid between 0.5 and 1.0, in *m*-cresol-acetic acid have R_F values between 0 and 1.0; differences between closely related substances are therefore more marked.

A number of chromatograms run at room temperature indicated that the R_F value of a single substance could vary appreciably from day to day when run in the same solvent system. Variation in temperature is an important factor affecting the R_F value. Temperature variation alone, however, was by no means the only factor contributing to this erratic behaviour. Equilibria in these ternary mixtures are sensitive to temperature fluctuations but it is also possible for slow esterification to take place, thereby adding an ester to the system and subtracting acid and alcohol from it. An experiment carried out with this point in mind indicated that consistent R_F values could be obtained for a period of at least 14 days, after allowing the butanol-acetic acid-water mixture to stand for 3 days at constant temperature, providing that the running conditions, enumerated below, were observed. It is also important that the cover of the vessel in which the chromatograms are run should fit well, preferably sealed with vaseline, in order to prevent differential evaporation of the components of the solvent mixture. To summarize the procedure, in order to obtain accurate and reproducible R_F values, it has been found necessary to take the following precautions:

1. Chromatography is carried out at a temperature which is constant to within $\pm$ 0.5° (all R_F values quoted in this paper were determined at 20°).

2. The solvent mixture is thoroughly shaken and is allowed to stand for 3 days at the temperature at which it is to be used.

3. The paper strip, with test spots applied, is equilibrated for 24 hours with the vapour of the aqueous phase of the solvent-water mixture before irrigation commences (ISHERWOOD AND JERMYN)[5].

4. A control substance, with an R_F value between 0.2 and 0.8, is run with every chromatogram and the solvent discarded as soon as this begins to show incorrect values. Papers on which, for any reason, the control substance differs by more than $\pm$ 0.02 from the standard value are disregarded. It is not as a rule permissible to adjust the R_F value of an unknown substance by a proportional correction for any deviation from the standard value of the control, since different substances are affected to different extents by changes in conditions.

5. Irrigation of the paper strip with the solvent is allowed to continue until the leading edge has travelled a distance of 30–35 cm from the line upon which the spots are applied. Butanol-acetic acid takes about 16 hours at 20° C whilst *m*-cresol -acetic acid requires 24 hours or more.

It is emphasized that these precautions have been taken for the purpose of determining the R_F values of a large number of substances under precisely similar conditions with the object of correlating these values with the chemical constitution of the compounds. It is not implied that such closely controlled conditions need to be observed in the day to day routine chromatography which is now carried out in many laboratories; obviously, nevertheless, such work will gain in value by reason of the increased accuracy which can be attained by observing these precautions.

DEVELOPING REAGENTS

The position of the spots on the dried paper chromatograms was revealed, in the

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TABLE I

Substance	Butanol-acetic acid		*m*-Cresol-acetic acid		Ammoniacal Silver Nitrate (in the cold)	Ferric Chloride spray reagent
	R_F	R_M	R_F	R_M		
Benzoic acid	0.92	—1.08	0.93	—1.12	—	—
Catechol	0.91	—1.02	0.74	—0.46	++	black
Cinnamic acid	0.94	—1.24	0.92	—1.08	—	yellow
o-Coumaric acid	0.94	—1.24	0.82	—0.66	+	orange
Gallic acid	0.68	—0.33	0.08	1.08	++	dark grey
m-Hydroxybenzoic acid	0.91	—1.02	0.72	—0.42	—	pale yellow
p-Hydroxybenzoic acid	0.90	—0.95	0.72	—0.42	—	dark yellow
Orcinol	0.91	—1.02	0.75	—0.48	+	grey
Phloroglucinol	0.76	—0.50	0.16	0.72	+	grey
Phloroglucinol carboxylic acid	0.55	—0.09	0.06	1.24	—	grey
Protocatechuic acid	0.85	—0.76	0.35	0.27	++	bottle green
Pyrogallol	0.77	—0.53	0.38	0.21	++	reddish brown
Quinol	0.88	—0.86	0.69	—0.36	+	grey
Resorcinol	0.91	—1.02	0.63	—0.23	+	grey
β-Resorcylic acid	0.93	—1.12	0.54	—0.07	—	purple
Salicylic acid	0.95	—1.30	0.84	—0.73	—	purple
Vanillic acid	0.92	—1.08	0.81	—0.64	—	buff

case of the anthocyanins and of the majority of the flavones and flavonols, by the natural pigmentation of the substances. By treating the papers with NH_3 vapour, the yellow colours of anthoxanthin pigments are greatly intensified. A number of substances, especially the anthocyanins, change colour in NH_3 which affords valuable evidence in characterizing unknown compounds. The use of ultraviolet light, both in presence and absence of NH_3 vapour, gives further useful information, and in some cases the spots could only be revealed by this means. The source of U.V. light used has been a G.E.C. Osira lamp which gives some emission in the visible region. Two reagents have been used for revealing the positions on the paper strips occupied by the colourless polyphenols and hydroxy- or methoxy-benzoic acids studied. Most of these substances reduce PARTRIDGE's ammoniacal silver nitrate reagent in the cold. The number of hydroxyl groups and their position determine the rate at which the reduced silver spots develop, and this can be used as further diagnostic evidence. The reagent is prepared by mixing equal volumes of silver nitrate (0.1 N) and ammonia solution (5 N). The majority of the C_6 compounds studied give a characteristic coloured spot after chromatography when sprayed with a 2% aqueous solution of ferric chloride. A stock 10% aqueous solution was made up and diluted as required. The R_F and R_M values of the C_6 compounds studied in butanol-acetic and *m*-cresol-acetic acid together with the reactions given by the spraying reagents are given in Table I*.

PREPARATION OF MATERIAL

Of the relatively large number of C_{15} compounds which have been studied chromatographically many were not available through the normal channels, and the authors are indebted to other workers for gifts of samples of these.

In a few cases the substances required have been prepared from the original plant material. For instance, diosmin was isolated from dried hyssop by the method of

* R_F values of C_{15} compounds are given by BATE-SMITH in *Biochemical Symposium no.* 3 — *Partition Chromatography* (in the press).

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OESTERLE AND WANDER[6] and diosmetin was obtained by hydrolysing the glycoside with 5% H_2SO_4 in 50% aqueous ethanol in an autoclave at 120° C for 4 hours. Tectoridin was obtained by extracting roots of *Iris tectoris* with ethanol following the method of BAKER[7] for iridin. Tectorigenin was produced by hydrolysis using a similar method to that outlined above for diosmetin. Apigenin was obtained from apiin in the same way, and phloretin from phloridzin after mild hydrolysis with mineral acid.

The anthocyanidins were prepared by hydrolysing the respective anthocyanins for 15 min at 100° with 5.5 N HCl. An unexpected difficulty arose when attempts were made to determine the R_F values of the anthocyanidins in the two solvent mixtures mentioned above. The solutions were applied to the paper as coloured spots but during irrigation with the solvent the pigment colour gradually faded, and when the chromatograms were finally dried it was not possible to discern the position of the aglucones with certainty. The probable explanation is that the anthocyanidin, which is applied to the paper as the oxonium chloride, ionizes under the conditions of the run, and that the free base travelling faster than the Cl^- gradually forms a colourless compound. The colour however, can be preserved by using as the flowing solvent a mixture of butanol and 2 N HCl in equal volumes. As usual, the butanol layer is placed in the trough and the aqueous layer is put in the bottom of the tank. After irrigation with the solvent and subsequent drying of the paper (the oven temperature should not exceed 80° C to avoid charring of the paper) the HCl acid front can be seen (in U.V. light) some distance behind the solvent front occupying a position represented by an R_F value of between 0.8–0.9. The aglucone spots tend to be elliptical, and this solvent mixture is not recommended for C_6 or C_{15} compounds in general.

RESULTS

CHROMATOGRAPHY OF SIMPLER POLYPHENOLS

The close correlation between structure and R_F values of the C_{15} compounds which will be discussed later suggested that a study of the phenolic compounds derived from benzene might yield results of value in analytical chemistry. Results with a limited but representative range of such compounds, obtained under conditions already defined, are plotted in Fig. 1*. The two spraying reagents previously described have proved adequate for developing the spots of all the compounds quoted with the exception of benzoic acid and *m*-hydroxybenzoic acid, the positions of which were indicated by spraying with a solution of brom-cresol green in ethanol. Aromatic compounds having two hydroxylic substituent groups in the nucleus can be developed on paper with ammoniacal silver nitrate. Those in which the two hydroxyls are *ortho-* and *para-* with respect to one another reduce vigorously in the cold. This general rule is obeyed by all the C_6 phenolic substances examined. Resorcinol, orcinol and phloroglucinol reduce slowly and only slightly. A property of phloroglucinol which is likely to be of great diagnostic value is its distinctive blue fluorescence under the U.V. lamp in presence of ammonia. *o*-Coumaric acid has an exceedingly brilliant greenish fluorescence both in presence and absence of ammonia.

* A reputed sample of syringic acid was found to contain two constituents, one of which preponderated. This substance had an R_F value 0.91 in butanol-acetic acid and 0.55 in *m*-cresol-acetic acid. The latter value would suggest a content of at least two free hydroxyl groups. Although the specimen had correct m.p. and p_K values it must be provisionally considered unlikely that these are the correct chromatographic constants for this substance.

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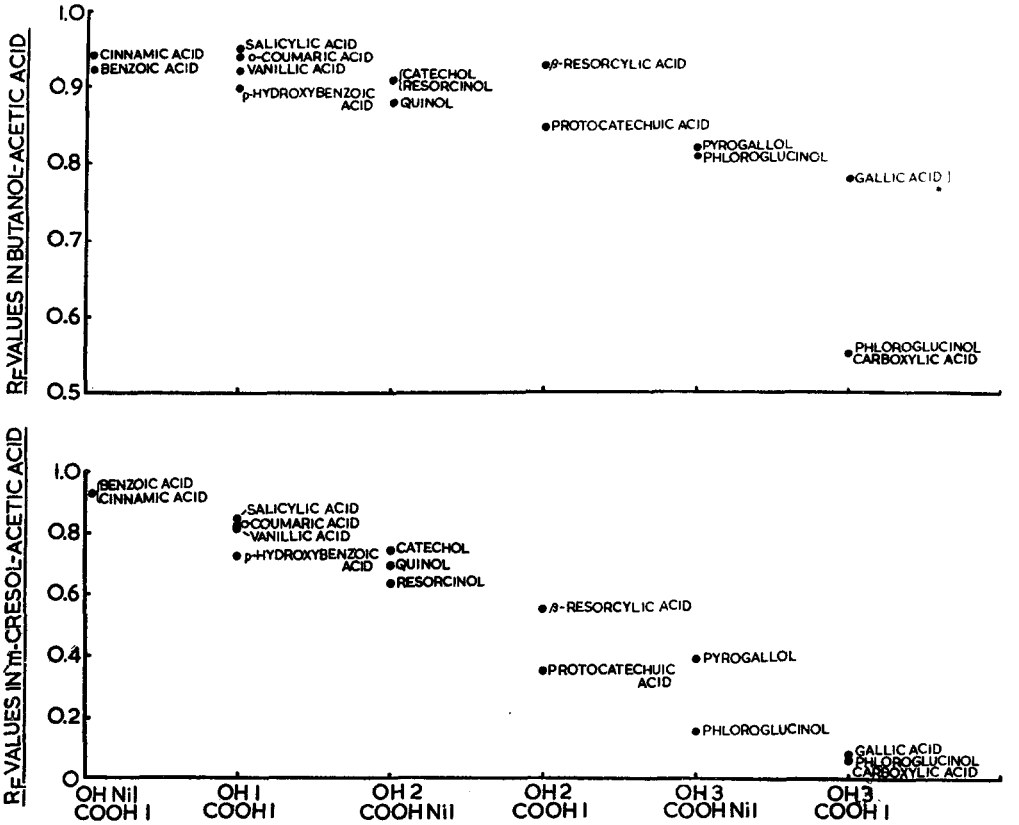

Fig. 1. R_F values of benzene derivatives in butanol-acetic acid and *m*-cresol-acetic acid

In considering the data in Fig. 1, from the systematic point of view, it is apparent that the degree of hydroxylation of the compounds has a pronounced effect upon the R_F value. This fact is especially striking in the *m*-cresol-acetic acid graph where the wider spread of R_F values demonstrates the value of this solvent mixture. The R_F values of substances differing only in the number of hydroxyl groups depend primarily on the number and only secondarily on the position of such groups. Methylation of hydroxyl groups causes an increase in R_F much less than would be caused by an additional hydroxyl group.

Little experience has, as yet, been gained in using the method for detecting benzene derivatives in complex mixtures but the following instances will serve to demonstrate its possibilities. Free phloroglucinol has been shown to be present in the petals of garden varieties of *Pelargonium*, and gallic acid has been detected after mild hydrolysis of extracts from the same source whilst the presence of protocatechuic acid as a degradation product of cyanin after alkali fission can be demonstrated with ease.

CHROMATOGRAPHY OF NATURAL AND SYNTHETIC C_{15} COMPOUNDS

1. *The aglucones*

Scrutiny of the results as the pure specimens were collected had indicated that the more highly hydroxylated substances had in general lower R_F values than the less

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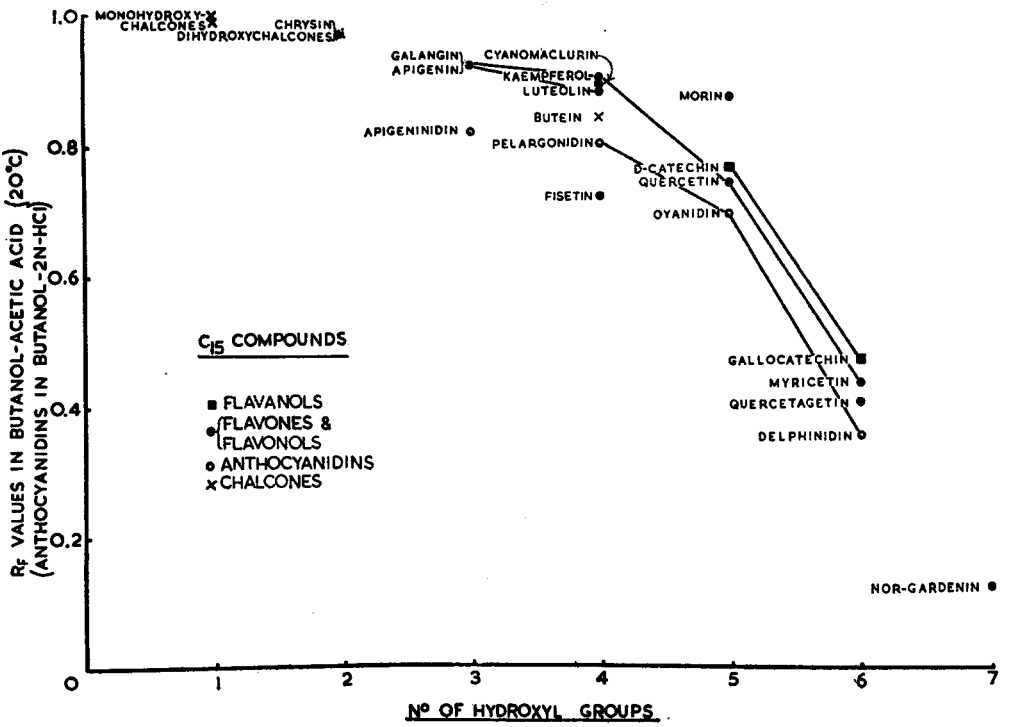

Fig. 2. R_F values of simple C_{15} compounds in acid butanol

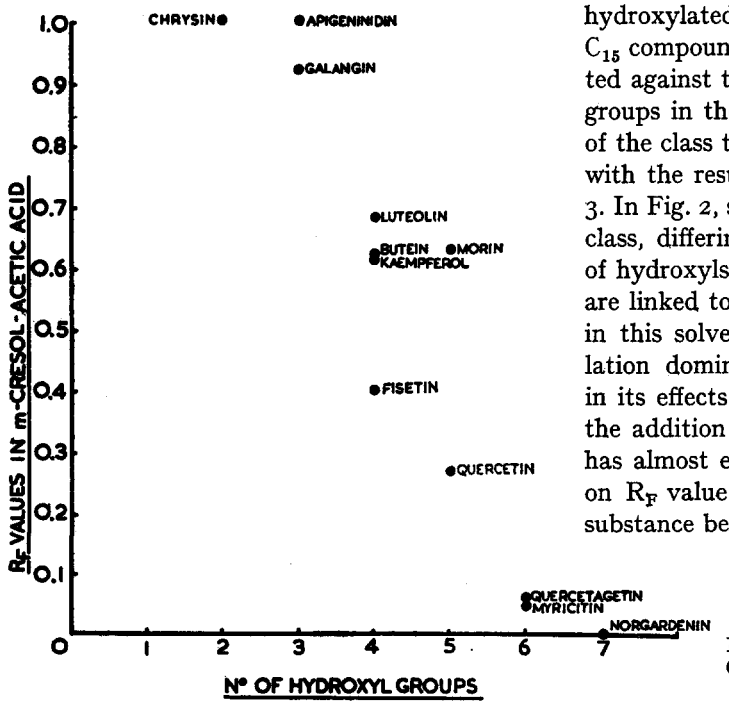

Fig. 3. R_F values of simple C_{15} compounds in *m*-cresol-acetic acid

hydroxylated. The R_F values of the C_{15} compounds were therefore plotted against the number of hydroxyl groups in the molecule, irrespective of the class to which they belonged, with the result shown in Figs 2 and 3. In Fig. 2, substances in the same class, differing only in the number of hydroxyls in the right-hand ring, are linked together. It is clear that in this solvent, weight of hydroxylation dominates all other factors in its effects on R_F value; and that the addition of one hydroxyl group has almost exactly the same effect on R_F value to whatever class the substance belongs.

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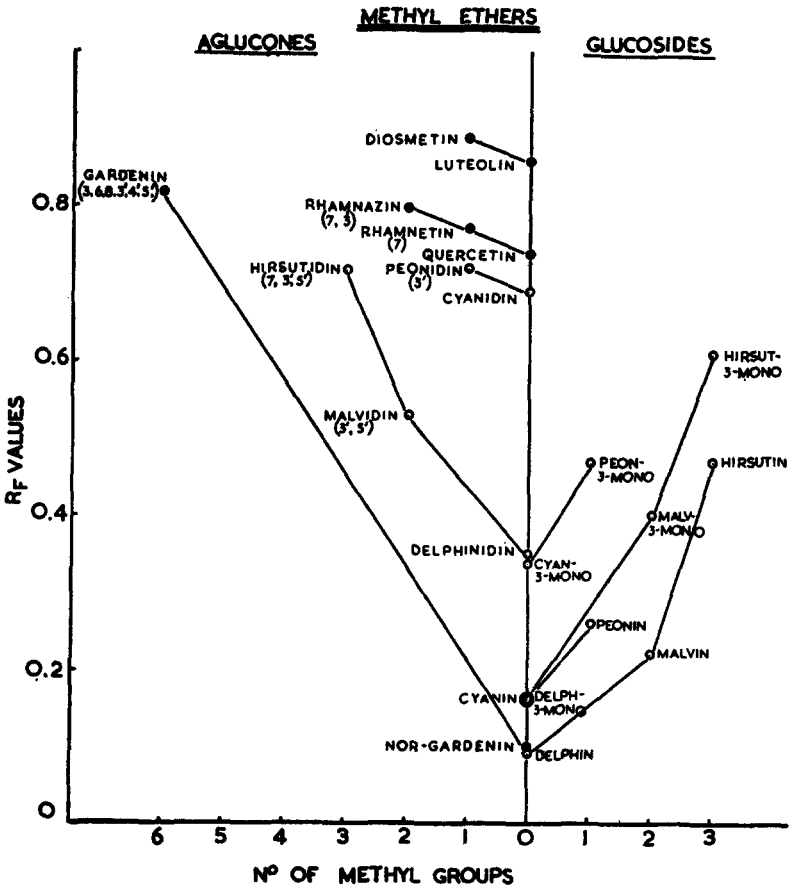

Fig. 4. R_F values of methyl ethers of C_{15} compounds in butanol-acetic acid

2. *Methylation*

The R_F values of the methyl ethers of a number of anthocyanins, flavones, etc. are plotted in Fig. 4, together with those of the parent non-methylated substances. The R_F value is seen to rise with successive methylation of hydroxyl groups, the extent of the rise depending little, apparently, upon the position of the methylated hydroxyls, but depending upon the degree of hydroxylation of the parent substance.

As a rule, the rise of R_F value per unit hydroxyl methylated appears to be only about one-third as large as would result from the complete removal of the hydroxyl group methylated.

3. *Glycosidation*

Glycosidation of hydroxyl groups with glucose, in whatever position, usually causes a large decrease in R_F value (Fig. 5). Glycosidation of a second hydroxyl group has as large, or almost as large an effect as that of the first, but the attachment of a second sugar group to the first (*cf.* mekocyanin, apiin) has a much less effect on R_F value.

Glycosidation with rhamnose has an irregular effect on R_F value, which may be either increased or decreased. In all but one of the five instances shown in Fig. 5, the

References p. 440.

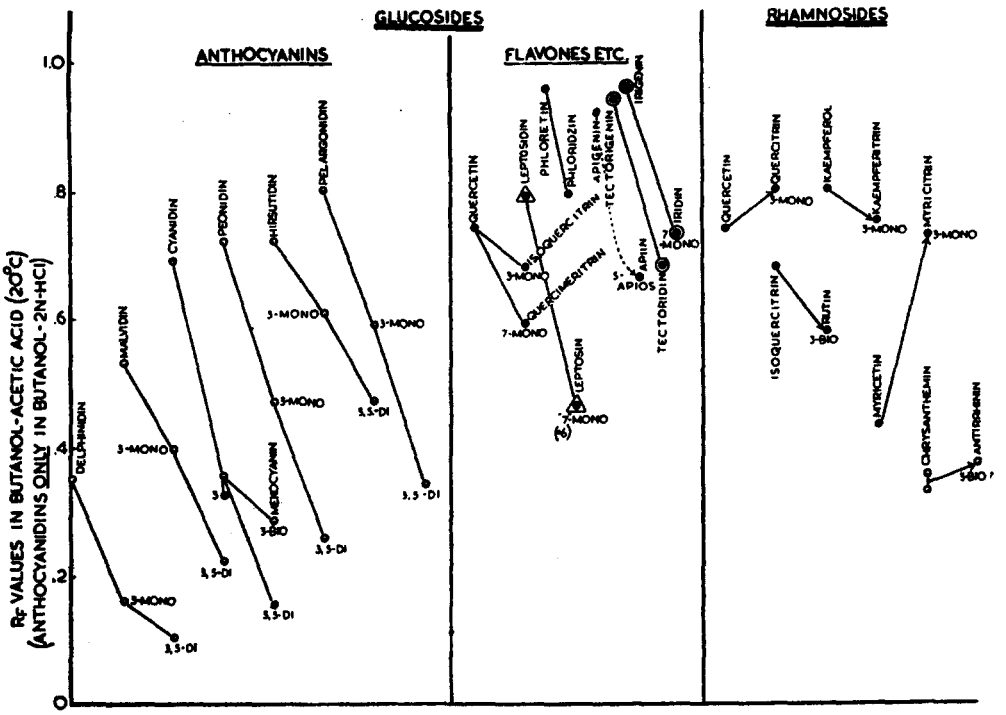

Fig. 5. R_F values of glycosides in acid butanol

effect is small. Thus in the case of rhamnose, the decrease in R_F value to be expected as a result of the attachment of a sugar residue is counterbalanced, or more than counterbalanced, by the effect of the terminal methyl group in increasing the R_F.

DISCUSSION

The correlation now demonstrated in various classes of aromatic compounds between changes in R_F values and increase in substituent groups fulfils the expectation outlined in the introduction to this paper. There are departures from regularity in behaviour; this is no more than would be expected from the behaviour of the sugars, where chromatographic separation is readily achieved of molecules differing only in the geometrical arrangement of the hydroxyl groups and hydrogen atoms. These departures are more marked in the lighter C_6 derivatives than in the C_{15} compounds. In some cases reasons can be suggested for the irregularities observed, and these will be discussed in due course.

As at est of the validity of equation (4), $\log\left(\frac{1}{R_F} - 1\right)$ plotted against n, while $\Delta\mu_A$, m, etc. remain constant, should give a straight line. The results for a number of substances have been so plotted in Figs 6, 7 and 8. In Figs 6 and 7, the points joined together relate to substances differing only in the numbers of hydroxyl groups attached to the molecule. In Fig. 8, the points joined refer to substances differing only in the numbers of glucose groups attached to hydroxyl groups in the molecule, the number of hydroxyl groups otherwise remaining constant. These selected results show that in a

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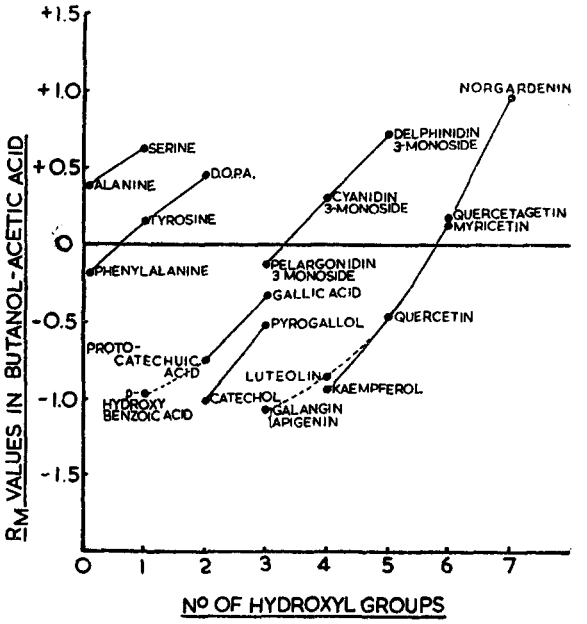
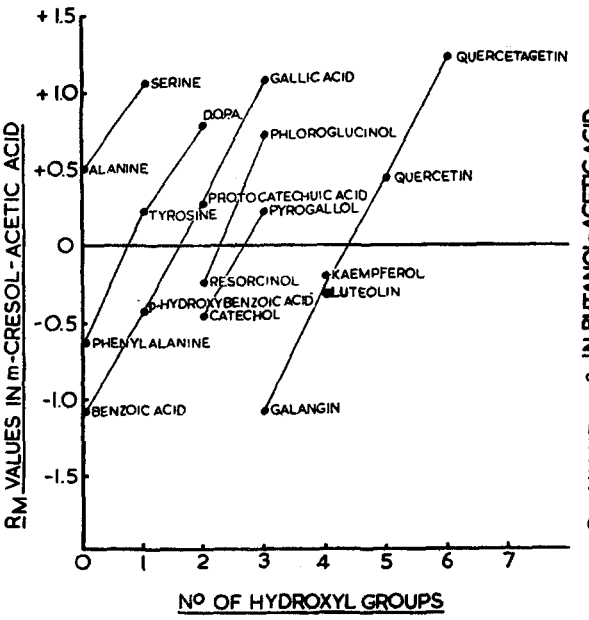
large number of instances the rule is obeyed and, from the parallelism of the straight lines, that the $\Delta\mu$ terms for a particular substituent group are similar in magnitude in each solvent, irrespective of the nature of the molecule in which substitution occurs.



The values of $\log\left(\frac{1}{R_F} - 1\right)$, because they vary directly with the partition coefficient and also, in many cases, change by equal increments with each successive addition of a particular substituent group, are likely to be more useful for many purposes than the R_F values. It is proposed that these values should be termed R_M values*.

A particularly useful property of the R_M value, as contrasted with the R_F value, is that it is a simple function of temperature and of the relative volumes of the solvent phases. One consequence of this is that when a reference substance is used as a check upon the running conditions it is the R_M values of the known and unknown substances



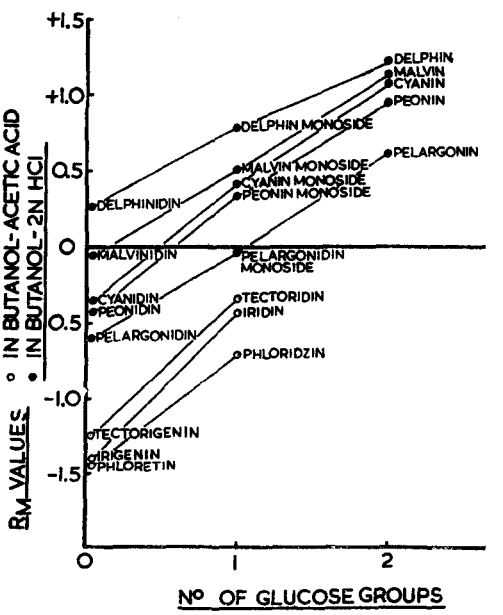


Fig. 6, 7, and 8. $R_M\left(= \log\left(\frac{1}{R_F} - 1\right)\right)$ values in acid butanol

* The subscript M is selected as unambiguous, and more convenient for typographic purposes than the Greek μ.

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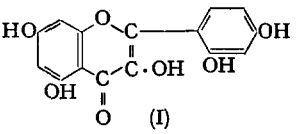
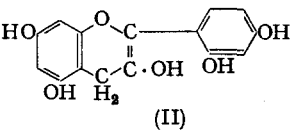
which are in direct proportion and not the R_F values. This property has already proved its usefulness in comparing chromatograms of petal extracts run at different temperatures; the chromatograms can be shown to coincide exactly if the corrections are made by simple proportionality in terms of R_M values but no simple factor based on R_F values can be found which will bring the chromatograms into exact coincidence.

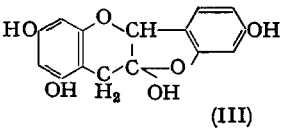
If the relations deduced by MARTIN[2] were universally valid, $\Delta\mu$ and therefore also ΔR_M, for any given substituent should be a constant. Several striking instances of such constancy can be mentioned. In the case of the glucosidation of position 5 of the anthocyanidin monoglucosides to form diglucosides the values of ΔR_M in butanol-acetic acid are: pelargonidin 0.43, cyanidin 0.42, peonidin 0.40, malvidin 0.38 and hirsutidin 0.40; and in butanol-2 NHCl, 0.65, 0.66, 0.62, 0.62 respectively (no value for hirsutidin). Substitution of hydroxyl ortho to hydroxyl in the benzene nucleus gives the following values; catechol 0.49, protocatechuic acid 0.43; while *p*-hydroxy benzoic acid, 0.19 and tyrosine, 0.29 give considerably lower values. There is a tendency for the value of ΔR_M for a particular type of substitution to decrease as R_M increases (cf. Figs 6, 7 and 8). Since a high R_M value is associated with a high degree of polar substitution and *vice versa*, this may mean that the change in chemical potential caused by a substituent group is less, the greater is the polar substitution already existing in the molecule*.

Morin. The position of morin is clearly anomalous. According to the accepted constitution of this substance (I) five hydroxyl groups are present, but it possesses the R_F value of one having only four hydroxyls. This is even more apparent when *m*-cresol is used as the flowing solvent (*cf.* Fig. 3). Now cyanomaclurin, with practically the same R_F value as morin, also has the 4', 6' hydroxyl substitution, and according to PERKIN[8] has the formula (II)


(I)


(II)

APPEL AND ROBINSON[9] concluded, however, that its constitution in acid and neutral solution is that of a semi-acetal (III) which, having only four hydroxyl groups, provides a satisfactory explanation of its R_F value. The same opportunity for semi-acetal formation occurs in morin (IV)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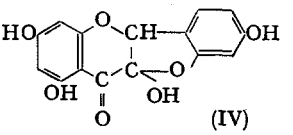
(IV)

and it would to be worthwhile considering whether this may not, in fact, be the form in which it exists in the slightly acid condition of the flowing chromatograms.

Fisetin. Two samples of this substances, one or possibly both, from *Quebracho*, agree in showing two spots in both butanol-acetic acid and *m*-cresol-acetic acid. The faster running spot (R_F 0.85, 0.73 respectively) occupies a position which would be expected from the hydroxyl content according to the accepted structure (V)

* An analogous instance, in which the change in free energy of ionization decreases with increase in number of polar substituent groups, is that of the chloroacetic acids, recently mentioned by SHORTER AND STUBBS[11].

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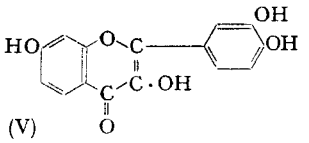
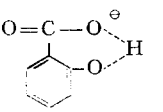

(V)

but it is the slower spot (R_F 0.72, 0.40) which gives the characteristic colour reactions of fisetin, especially the brilliant greenish yellow fluorescence in U.V. light. These R_F values are those more nearly to be expected from a pentahydroxy flavone (*cf.* quercetin 0.74, 0.27). It is difficult to envisage any likely transformation of the molecule which would result in the production of this necessary extra hydroxyl group. Pending further evidence, it is suggested that the behaviour of this substance may be due to the unusual freedom from substitution of carbon atom 5, and that the unusual fluorescence is also related to this feature of the molecule.

Quercetin and quercetin glucosides. Although the R_F values of quercetin are somewhat low in relation to its hydroxyl content, they are not conspicuously so. Isoquercetin and quercimeritrin do not, however, show so large a ΔR_M as would be expected of monoglucosides, and it seems likely, therefore, that this flavonol has configurational peculiarities which affect its chromatographic behaviour.

Phenols and phenolic acids. In general, a vicinal arrangement of substituents tends to cause the R_F value to be higher than a more separated arrangement, This is especially marked in the case of the *ortho*-hydroxycarboxylic acids in butanol-acetic acid and *m*-cresol-acetic acid: salicylic acid (R_F 0.95, 0.84) has higher R_F values than *meta*- (R_F 0.91, 0.72) and *para*- (0.91, 0.72) hydroxybenzoic acids; -resorcylic acid (0.93, 0.55) than protocatechuic acid (0.85, 0.35). An explanation of this may be found in the formation of a chelate ring, which BAKER[10] suggests as a reason for the considerably stronger acidity of the *ortho*- as compared with the *meta*- and *para*-hydroxybenzoic acids. The result of chelation would be to diminish the hydroxyl character of both the carboxylic and the phenolic group, and the behaviour of salicylic and β-resorcylic acids, is, in fact, approximately that to be expected of a substance having one less hydroxyl group.



Catechol and Pyrogallol. In *m*-cresol-acetic acid the R_M values of catechol and pyrogallol (–0.46, 0.20 respectively) are lower than those of resorcinol and phloroglucinol (–0.22, 0.48); the latter pair having the values more in agreement with expectation than the former pair. The ΔR_M between catechol and pyrogallol (–0.24) is practically the same as that between resorcinol and phloroglucinol (–0.28); it would appear, therefore, that hydroxylation of phenol in the *ortho* position produces a much less effect on R_M, *i.e.*, is accompanied by a much smaller change in chemical potential, than hydroxylation in the *meta* position. This exceptional behaviour can be regarded as yet another example of the "*ortho*-effect" (*cf.* WATSON[12]).

The information which it now seems can be gained by accurate chromatography not only with regard to the identity of unknown substances but also with regard to some aspects of the molecular configuration of organic molecules clearly justifies the expenditure of effort towards the attainment of accuracy and reproducibility in the determination of R_F values beyond what is at present customary. As was deduced by MARTIN, the R_F value of a substance is predominantly a function of its partition coefficient between the solvents forming the mobile and the stationary phases. While the determination of the partition coefficient is likely to give more accurate and unequivocal data, the chromatographic method has three advantages: it is simple and rapid; it requires extremely little material; and that material does not need to be of a high degree of purity, because impurities are separated in the course of the determination.

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In fact, impurities are detectable by this method which might otherwise remain undetected.

It is surprising, in view of the suggestive results obtained by COHN and his collaborators with the amino-acids, that so little work has been done on the relationship between partition coefficients and structure. It is easy to see how the regularity in this relationship escaped recognition in earlier work on paper partition chromatography since this work was concerned with amino-acids and sugars where separation of substances on the paper is frequently achieved by virtue of "irregularities" due to the geometrical arrangement of polar groups on short carbon chains. Nevertheless, regularities were perceived and pointed out by CONSDEN, GORDON, AND MARTIN in the case of the amino-acids[1] and can also be clearly seen in the case of the sugars (ISHERWOOD AND JERMYN[13]). A case in which difference in R_F value can be explained in terms of the positional arrangement of hydroxyl groups is that of the catechins, discussed in the following note.

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SUMMARY

The R_F values, accurate and reproducible to $\pm$ 0.02, in butanol-acetic acid and *m*-cresol-acetic acid of a number of naturally-occurring flavones, anthocyanins, and related compounds have been determined; also the R_F values of a number of hydroxy-, methoxy-, and carboxy-derivatives of benzene.

It is shown that the R_F values are related to the nature and number of substituent groups in the C_{15} and C_6 skeleton, respectively, in such a way that in many instances a straight line is given when $\log\left(\frac{1}{R_F} - 1\right)$ is plotted against the number of substituent groups of any one kind. This relationship, predicted by MARTIN[2], follows from a relationship between constitution and partition coefficient of which the amino-acids provide a notable example (ENGLAND AND COHN[3]). The symbol R_M is suggested for the function $\log\left(\frac{1}{R_F} - 1\right)$.

Several instances of departure from regularity in the above respect are attributable to constitutional factors, and especially to *ortho* or vicinal arrangement of substituent groups.

RÉSUMÉ

Les valeurs de R_F, exactes et reproductibles à $\pm$ 0.02 dans l'acide acétique et butanol, et dans l'acide acétique et *m*-crésol, d'un certain nombre de flavones naturelles, ont été déterminées. Les valeurs des R_F d'un certain nombre de dérivés hydroxy-, méthoxy-, et carboxy-du benzène ont aussi été déterminées. On a montré que les valeurs des R_F sont liées à la nature et au nombre de substituants dans la structure en C_{15} et C_6, respectivement, de telle manière que dans plusieurs exemples on obtient une ligne droite en portant $\log\left(\frac{1}{R_F} - 1\right)$ en fonction du nombre de substituants de n'importe quelle nature. Cette relation, prévue par MARTIN[2] vient de la dépendance entre la constitution et le coefficient de partage, dont les acides aminés fournissent un exemple remarquable (ENGLAND ET COHN[3]). Le symbole R_M est proposé pour la fonction $\log\left(\frac{1}{R_F} - 1\right)$. Plusieurs exemples de manque de régularité dans le domaine précédent sont attribuables à des facteurs de constitution et spécialement à l'arrangement des substituants en position *ortho* ou vicinale.

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ZUSAMMENFASSUNG

Die R_F-Werte in Butaniol-Essigsäure und in *m*-Kresol-Essigsäure wurden für einige natürliche Flavone, Anthocyane und verwandte Verbindungen mit einer Genauigkeit und einer Reproduzierbarkeit von $\pm$ 0.02 bestimmt; ebenso wurden die R_F-Werte einer Anzahl von Hydroxy-, Methoxy- und Carboxyderivaten des Benzols bestimmt.

Es wird gezeigt, dass die R_F-Werte von der Natur und der Anzahl der Substituenten in der C_{15} und der C_6 Struktur abhängig sind und zwar so, dass man in vielen Fällen eine Gerade erhält, wenn man $\log\left(\frac{1}{R_F} - 1\right)$ gegen die Anzahl gleicher Substituenten aufträgt. Dieses Verhältnis, das von MARTIN vorausgesagt wurde, ist eine Folge der Abhängigkeit von Struktur und Verteilungskoeffizient, für welche die Aminosäuren ein ausgezeichnetes Beispiel liefern (ENGLAND UND COHN[3]). Das Symbol R_M wird für die Funktion $\log\left(\frac{1}{R_F} - 1\right)$ vorgeschlagen.

Verschiedene Abweichungen von der oben erwähnten Regelmässigkeit können der Struktur der Verbindung insbesondere *ortho*- oder Vicinalstellung von Substituenten zugeschrieben werden.

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