INVESTIGATIONS ON THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC PARAMETERS

IV. SOLVATION EQUILIBRIA AND R_M vs. COMPOSITION RELATIONSHIPS OF SOME PHENOLS

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SUMMARY

R_M vs. composition relationships of a number of phenols have been determined for three systems of the type electron donor solvent + diluting solvent–water. Approximately linear relationships were obtained when the composition of the mixed solvent was plotted on a logarithmic scale, which is in agreement with equations derived by applying the law of mass action to the formation of solvation complexes in the organic phase. The slope of the lines was found to depend on the number of hydroxyl groups in the molecule of the solute.

INTRODUCTION

The R_M concept permits information to be obtained from partition chromatography data about the molecular structure of chromatographed solutes[1-4]. "Group selective" systems especially provide easily interpreted data[5]; good results are sometimes obtained by plotting R_F values determined for two group-selective systems as an R_F–R_F correlation[6]. In order to improve selectivity and group separations, molecular complex formation has been extensively utilized[7]; thus, olefins and aromatic hydrocarbons selectively form π-complexes with silver ions (*e.g.*, ref. 8); *vic.*-diols form complexes with borates[9,10], tungstates, molybdates, etc. The presence of groups capable of ionization in acidic or alkaline media can be ascertained by the use of buffered aqueous phase[11], etc.
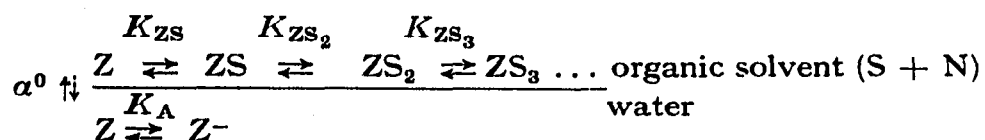
One of the techniques of structural investigation consists in interpretation of the relationships between the chromatographic parameters and the composition of mixed solvents in a family of analogous systems[1]. Such investigations are facilitated when one of the components of the mixed phase is capable of forming specific molecular complexes with the molecules of the chromatographed solute. The composition of these complexes depends on the number of functional groups interacting strongly with the active solvent (*e.g.*, by H-bonding); also the values of the stepwise formation

constants (which determine the R_M vs. composition relationships) depend on the functional groups, their steric situation, inductive effects, etc. If the formation and breaking of these complexes is sufficiently rapid, the spreading of the zone is limited[12] and the formation equilibria can be described by the law of mass action[7,13]. Composition effects have been made use of widely in the chemistry of complex compounds in aqueous solutions[14,15] and in investigations on the extraction of metals by liquid ion exchangers[16]; the formation of π-complexes between metal ions and unsaturated organic compounds has also been investigated[8]. The use of these effects in gas–liquid chromatography has recently been discussed by KARGER[17] (cf. also PURNELL[18]); on the other hand, H-bonding between organic compounds has attracted less attention (e.g., refs. 19, 20).

In the present paper R_M vs. composition relationships are reported for a number of mono-, di- and trihydroxy derivatives of benzene and naphthalene chromatographed in three systems of the type electron donor solvent + cyclohexane–water. As electron donor solvents (class B after PIMENTEL AND McCLELLAN[21]) cyclohexanone, tri-n-butyl phosphate and tri-n-butylamine were used. The molecular interactions in the organic phase are relatively simple in these cases, since the solvents are not associated; and in view of the low concentrations of the phenols, it can be presumed that stronger interactions are limited to H-bonding in the solvation complexes (OH $\cdots\cdots$ O and OH $\cdots\cdots$ N). The diluting solvent, cyclohexane, was found to be a weak extractant of the phenols (except for the two naphthols) so that it could be presumed that in the system cyclohexane–water the dominating effects are the hydration of the phenols and squeezing from the aqueous phase[1]. The R_F values of the di- and trihydroxy compounds only increased at higher concentrations of the electron donor solvent.

THEORETICAL DISCUSSION OF THE PARTITION MECHANISM

If the chromatographed solute (phenol) is denoted by Z and the active (electron donor) solvent by S, the partition mechanism can be represented in a simplified form as follows:

$$\alpha^0 \updownarrow \frac{Z \underset{}{\overset{K_{ZS}}{\rightleftarrows}} ZS \underset{}{\overset{K_{ZS_2}}{\rightleftarrows}} ZS_2 \underset{}{\overset{K_{ZS_3}}{\rightleftarrows}} ZS_3 \ldots}{Z \underset{}{\overset{K_A}{\rightleftarrows}} Z^-} \quad \begin{array}{l} \text{organic solvent (S + N)} \\ \hline \text{water} \end{array}$$

The diagram represents the partition of free molecules of the solute between the organic solvent and water, the formation of solvation complexes ZS, ZS_2 and ZS_3 in the organic phase, and dissociation of the phenol in the aqueous phase. In view of the very weak acidic properties of the phenols ($pK_A \doteq$ 10, except for gallic acid) the last effect can be neglected for acidic and neutral aqueous solutions.

Assuming that the H-bonding groups of the solute are equivalent (i.e., the stepwise formation constants are independent of the order of formation of the complexes ZS, ZS_2 and ZS_3), relatively simple R_M vs. composition relationships can be obtained; the partition and solvation can be described by the following constants (neglecting the activity coefficients; index "w" denotes concentration in the aqueous phase, lack of index—organic phase; cf. ref. 20, where $k = 1/\alpha$):

$$\alpha^0 = [Z]_w/[Z]\,;\, K_{ZS} = [ZS]/[Z][S]\,;\, K_{ZS_2} = [ZS_2]/[ZS][S]\,;\, K_{ZS_3} = [ZS_3]/[ZS_2][S]$$
$$\alpha = [Z]_w/\Sigma_i[ZS_i] = [Z]_w/[Z]\,(1 + K_{ZS}[S] + K_{ZS}K_{ZS_2}[S]^2 + K_{ZS}K_{ZS_2}K_{ZS_3}[S]^3) \quad (1)$$
$$R_M = \log \alpha + \log v_w/v_{org} = \log \alpha^0 + \log v_w/v_{org} - \log (1 + K_{ZS}[S] +$$
$$+ K_{ZS}K_{ZS_2}[S]^2 + K_{ZS}K_{ZS_2}K_{ZS_3}[S]^3) \quad (2)$$

We can still introduce $R_M{}^0$, the R_M value for the system diluting solvent–water, where the solute is present in the organic phase in the free form only:

$$R_M{}^0 = \log \alpha^0 + \log v_w/v_{org} \quad (3)$$

The shape of the R_M vs. log [S] relationship is determined by the stepwise formation constants (strictly speaking, their proportion[15,22]). For example, in Fig. 1 such relationships are represented for the case when the stepwise formation constants are in the ratio 1 : 10 : 100. The three curves represent a monofunctional solute which can form only 1:1 solvation complexes, a bi-functional solute (ZS and ZS_2) and a tri-functional solute (ZS, ZS_2 and ZS_3, depending on the concentration of the active solvent S). It can be seen that in certain concentration ranges approximately linear relationships can be obtained, the slopes of which (absolute values) indicate the average compositions of the solvation complexes which predominate in the given concentration ranges. The diagram is analogous to R_M vs. pH relationships of organic acids and bases, since protonization can be considered a special case of complex formation (cf. refs. 1, 23). Analogous relationships can also be obtained in certain cases of adsorption chromatography[24] (PURNELL's case A II).

Only fragments of such relationships can be obtained from paper chromatographic data (reliable R_M values in the range -0.5 to $+1.0$, which corresponds to $0.7 > R_F > 0.1$). Thus, it is only in exceptional cases that full information on complexation equilibria can be obtained from PC data (see below, Fig. 5). Fuller and more precise information can be obtained from static experiments or from column liquid–liquid partition chromatography.

EXPERIMENTAL

The "moist paper" technique was employed. Whatman No. 4 paper strips, 5 × 23.5 cm, were soaked in water, blotted between two sheets of filter paper and the phenols spotted ($\sim 10\ \mu l$ of 1% solutions in benzene or methanol). After the weight of the strip decreased to a value corresponding to 0.5 ml water per 1 g of dry paper, the strip was immediately transferred to a chromatographic tank (5 × 9 × 24 cm) for descending development. The spots were detected by coupling with bis-diazotized benzidine[25].

For practical reasons, the experimental data are represented as R_M vs. volume composition relationships, although in a strict approach it is preferable to plot the logarithms of the rational partition coefficients against mole fractions of the electron donor solvent[20]. The alteration of the coordinate scales does not cause any marked deformation of the plots, except for high concentrations of the active solvent, since the molar volume of cyclohexanone is comparable to that of cyclohexane, and tributylphosphate and tributylamine have much higher molar volumes[20]. Molar concentrations of the active solvent are proportional to % v/v composition and are thus shifted by a constant value in the logarithmic scale.
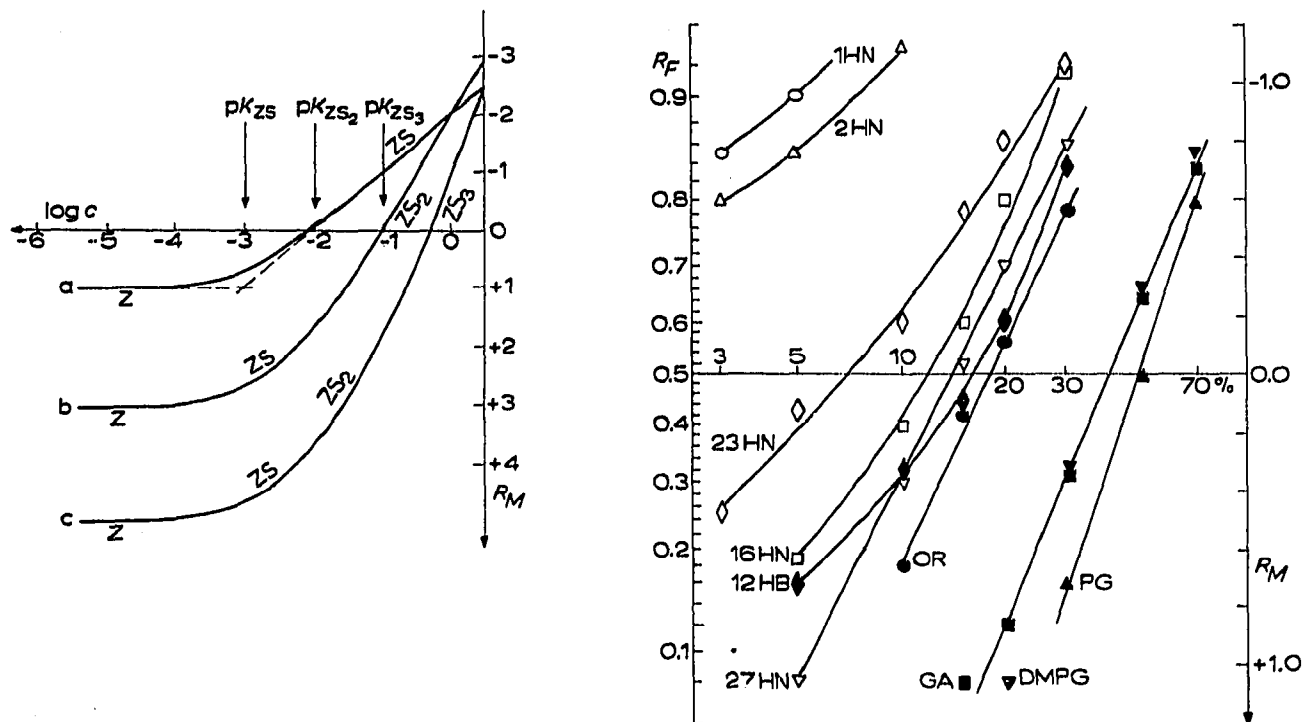
Fig. 1. Theoretical R_M vs. log c relationships of three hypothetical solutes: (a) $K_{ZS} = 1000$; $K_{ZS_2} = K_{ZS_3} = 0$; $R_M{}^0 = +1.0$. (b) $K_{ZS} = 1000$; $K_{ZS_2} = 100$; $K_{ZS_3} = 0$; $R_M{}^0 = +3.0$. (c) $K_{ZS} = 1000$; $K_{ZS_2} = 100$; $K_{ZS_3} = 10$; $R_M{}^0 = +5.0$. Predominating forms of the solutes are indicated on the curves. The shapes of the curves (b, c) are general for the 10:1 ratio of stepwise formation constants; the vertical positions of the curves are determined by $R_M{}^0$ values, and horizontal positions—by numerical values of formation constants.

Fig. 2. Experimental R_M vs. log% v/v $C_6H_{10}O$ plot for the system cyclohexanone + cyclohexane–water. For notation of the solutes, see Table I.

Experiment R_F values were plotted directly on H-shaped diagrams with two parallel ordinate axes: a proportional R_M scale and a subordinate R_F scale (see Fig. 2). Such diagrams were found to be very convenient in practice.

RESULTS AND DISCUSSION

The experimental data obtained for the system cyclohexanone + cyclohexane–water are presented in Fig. 2. It can be seen that regular R_M vs. log % v/v relationships are obtained, the lines being mostly straight or slightly curved, apparently fragments of theoretical curves of the type illustrated in Fig. 1. Analogous relationships obtained for the tributylphosphate system are given in Fig. 3, and for the tributylamine system in Fig. 4.

Cyclohexanone (Fig. 2) is a relatively weak extractant and the more hydrophilic solutes, gallic acid, phloroglucinol and dimethylphloroglucinol are extracted only at higher concentrations of cyclohexanone in the mixed organic phase. In accordance with expectation, the highest R_F values are obtained for the monohydroxy compounds, and intermediate R_F values for dihydroxy compounds, the two benzene derivatives, pyrocatechol and orcinol (5-methylresorcinol) giving lower R_F values in view of their lower molecular volumes. The slopes of the lines also depend on the number of proton
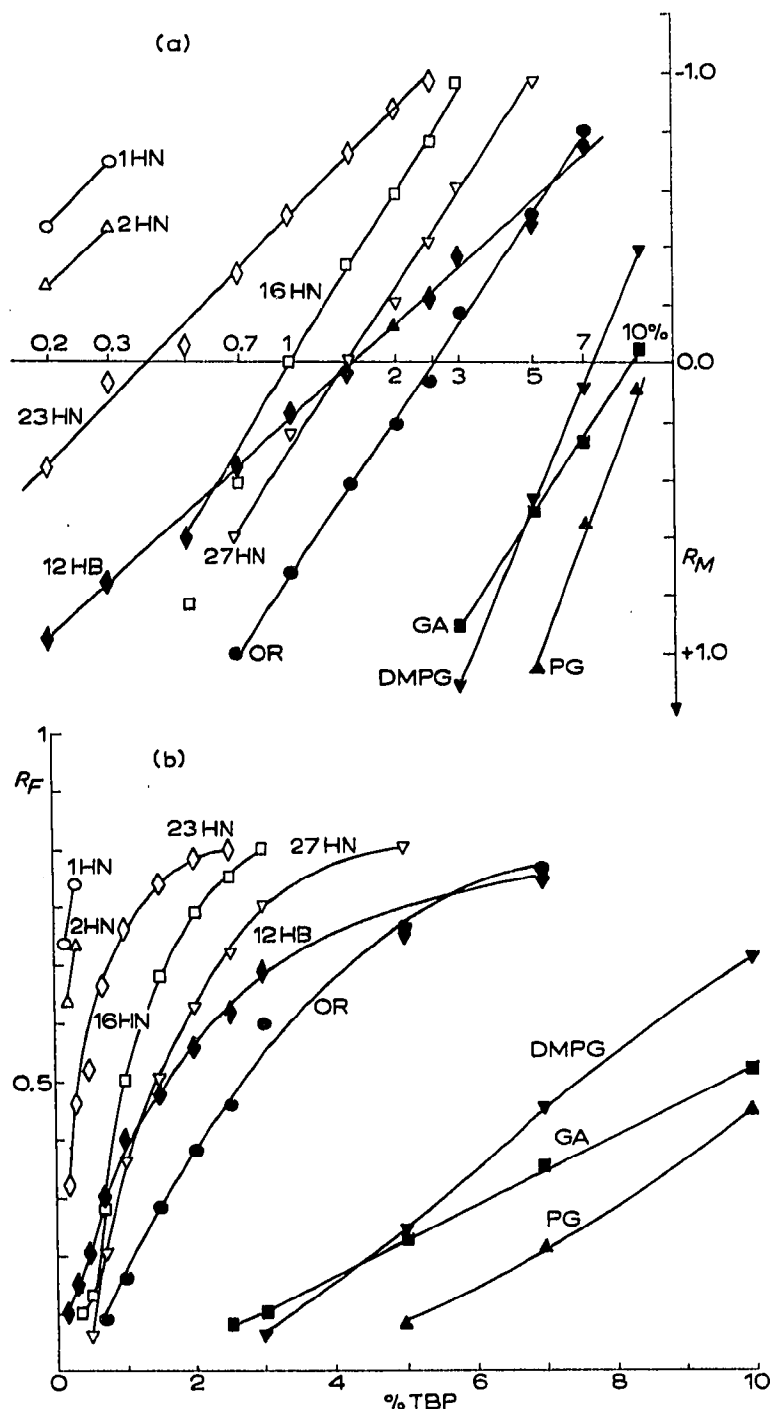
Fig. 3. (a) R_M vs. log % v/v TBP plot for the system tri-n-butyl phosphate + cyclohexane–water. (b) Same data, R_F vs. % TBP plot.

donor groups in the molecule of the solute, the R_M vs. log % lines spreading fanwise, with slopes of monohydroxy compounds in the range o to 1, the dihydroxy compounds at ca. 2 and trihydroxy compounds at ca. 3 (see Table I).

In the case of 2,3-dihydroxynaphthalene and 1,2-dihydroxybenzene the effect of the adjacent positions of the two hydroxyl groups is apparent: at concentrations of
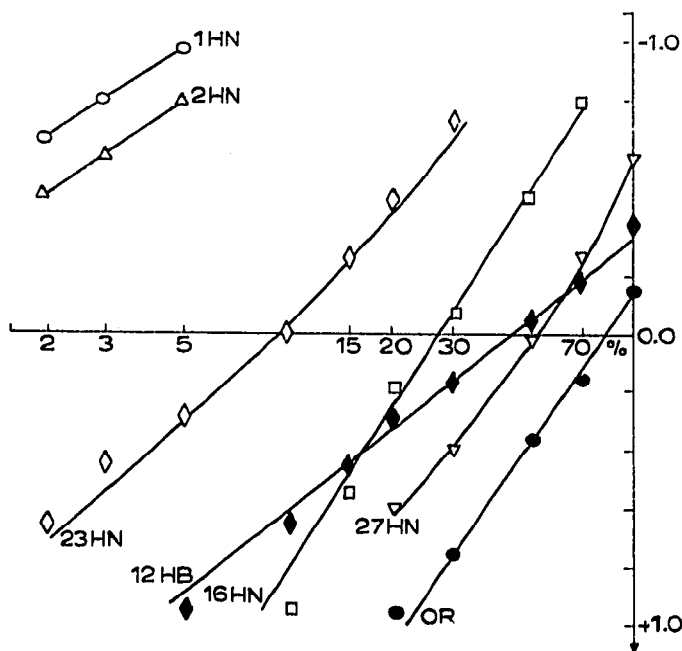
Fig. 4. R_M vs. log % v/v TBA relationships for the system tri-n-butylamine + cyclohexane–water.

cyclohexanone below 10% the two lines become less steep, the slope approaching 1.0 which indicates formation of complexes of an average ratio of 1:1. The formation of ZS_2 complexes is apparently hindered by the vicinity of the two hydroxyls (cf. also ref. 24). This effect is presumably also operative in the case of gallic acid, which theoretically could even form ZS_4 complexes, but seems to form only ZS_3 complexes like phloroglucinol.

Tributylphosphate (Fig. 3), in spite of its higher molecular volume, is a stronger extractant than cyclohexanone; this is presumably due to the stronger electron donor

TABLE I

SLOPES OF R_M vs. log%v/v LINES (ABSOLUTE VALUES)

Symbol	Solute	Developing solvent			Number of OH groups
		$C_6H_{10}O$ + C_6H_{12}	TBP + C_6H_{12}	TBA + C_6H_{12}	
1HN	1-Naphthol	0.9	1.2	0.8	1
2HN	2-Naphthol	0.8–1.2	1.2	0.8	1
23HN	2,3-Dihydroxynaphthalene	1.2–1.8	1.3	1.0–1.4	2 (ortho)
16HN	1,6-Dihydroxynaphthalene	1.5–2.8	2.0	1.8	2
27HN	2,7-Dihydroxynaphthalene	2.4	1.9	1.4–2.2	2
12HB	1,2-Dihydroxybenzene	1.2–3.0	1.1	0.95	2 (ortho)
OR	O.cinol (3,5-dihydroxytoluene)	2.5	1.8–1.9	1.8	2
GA	Gallic acid	2.9	1.8	—	3 (ortho) + COOH
PG	Phloroglucinol	3.0	3.2	—	3
DMPG	Dimethyl phloroglucinol	3.6	2.8	—	3

properties of the PO group and perhaps also to its more favourable steric situation. Measurable R_M values are obtained in the range of lower concentrations of the active solvent. The sequence of solutes is similar to that of the previous case; however, the spread of the lines is more pronounced which indicates higher selectivities due to greater differences in the interaction forces. Phloroglucinol and its dimethyl derivative give parallel lines, whereas the line of gallic acid is less steep, presumably indicating the formation of ZS_2 complexes only.

In Fig. 3b, the R_F vs. % TBP relationships are represented to give a direct illustration of the optimization of chromatographic separations by the variation of the solvent composition. It can be seen that the relationships are more complex in this coordinate system and the interpretation of the solvation equilibria is less obvious.

Tri-n-butylamine (TBA, Fig. 4) in spite of its strongly basic properties (pK_A ca. 10) shows a relatively weak extraction power: even dihydroxy compounds are only extracted at high concentrations of TBA. This is presumably caused by the shielding of the nitrogen atom by the three alkyl groups and/or increased ionization of the solutes in the aqueous phase. Moreover, the increased electron donor properties of the active solvent cause greater spreading of the R_M vs. log % lines (higher selectivity). As in the former systems, the two $vic.$-diol compounds (12HB and 23HN) give less steep parallel relationships due to the mutual hindrance in H-bonding of two adjacent hydroxyl groups.

Among the phenols investigated, the two naphthols gave measurable R_M^0 values in the system cyclohexane–water. Because these two solutes can only form H-bonds with the electron donor solvents in the 1:1 ratio, their R_M vs. log % v/v relationships were determined for the TBA systems in a range of low concentrations of TBA. The results are presented in Fig. 5. The points are from the experimental data, but the lines are theoretical curves calculated from the equation

$$R_M = R_M^0 - \log (1 + K_{ZS}[S])$$

and fitted to the experimental points (cf. ref. 26). The asymptotes (dashed lines) cross at a concentration equal to the reciprocal of the formation constant of the ZS complex; this can be shown immediately by comparing the equations of the asymptotes:

$$R_M^0 = R_M^0 - \log K_{ZS} - \log [S]$$

Almost identical values of formation constants have been found for the complexes 1HN–TBA and 2HN–TBA; the composition of the organic phase corresponding to the intersection points is 0.022 mole/l so that $K_{ZS} = 45.5$ liter per mole.

Since the organic solvent in the chromatographic systems is saturated with water, the solvation constants in the "dry" cyclohexane + TBA systems may deviate from the values determined from chromatographic data. Moreover, the contribution of the adsorption of the solutes on the liquid–liquid interface and by the cellulose is unknown; in the theoretical considerations liquid–liquid partition only has been assumed.

CONCLUSIONS

The experimental data indicate that R_M vs. composition relationships for suitably chosen solvent systems can provide information on the molecular structure
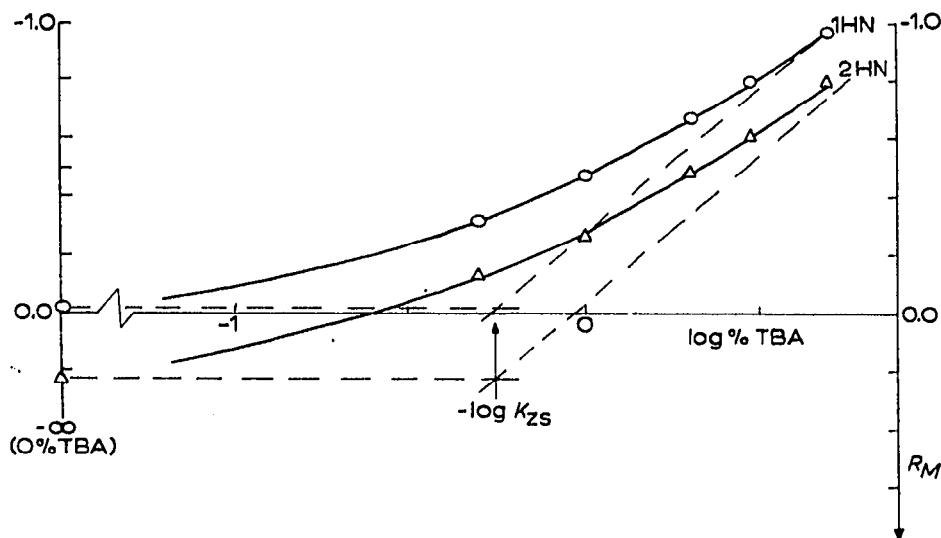
Fig. 5. Determination of the formation constants of HN–TBA complexes from paper chromatographic data.

of the solute, and especially about the presence and number of functional groups capable of H-bonding with the molecules of the active solvent. It is advantageous to plot chromatographic data in the coordinate system R_M–log concentration of the active solvent; in this system linear or slightly curved lines are usually obtained, whose slopes indicate the average composition of solvation complexes, and thus also, indirectly, the number of H-bonding groups. Steric effects due to the vicinal positions of H-bonding groups have been demonstrated; in aqueous systems, in contrast to non-aqueous ones[24], the volume of the solute molecule is also important (compare, for instance, 23HN and 12HB; see also ref. 27). R_M vs. composition relationships, obtained for a family of solvent systems, can be expected to give more reliable information than chromatographic data determined for a single solvent system only.

In view of the moderate accuracy and limited range of paper chromatographic data, it can be expected that more reliable information can be obtained from column liquid–liquid partition chromatography[28]. When an adequate accuracy and range is secured, chromatographic data could presumably be employed to study formation equilibria of solvation complexes, as in gas–liquid chromatography[8,17,18]; paper chromatography could then readily provide preliminary information. Complications may arise due to saturation of the weakly polar phase with the polar liquid (water in systems studied in this paper; formation of hydrates or mixed solvation complexes in the organic phase—the fact that the solubility of the polar liquid in the organic phase varies with its composition should be taken into account). Any contribution of adsorption effects should also be estimated if accurate interpretation of R_M vs. composition relationships is required[28].

It is worthwhile considering the above relationships from the viewpoint of optimization of liquid–liquid partition systems. By variation of the composition it is possible to obtain suitable absolute values of R_M (for paper chromatography, in the proximity of zero) and to improve the selectivity; in view of the fanwise spreading of the R_M vs. log% lines, the selectivity (ΔR_M), generally speaking, increases with

dilution of the active solvent. The solvent B–water systems are less selective in view of partial counterbalancing of hydration effects in the aqueous phase and solvation effects in the organic phase[1]. However, differentiated slopes of the lines (e.g., for vic.-diols) can occasionally cause their intersection and then the optimal positions of the zones can be found from the plot. The approximately linear course of the relationships in the R_M vs. log % plots facilitates interpolation and (limited) extrapolation of experimental data.

The following ΔR_M values can be estimated from the experimental data; the corresponding pairs of solutes being indicated.

Nonpolar groups

DMPG–PG. The two methyl groups in dimethylphloroglucinol are between the three hydroxyl groups. ΔR_M (aryl-CH$_2$) was ca. −0.25 units for the TBP systems (Fig. 3); for cyclohexanone systems (Fig. 2) ΔR_M (α-CH$_2$) varied in the range −0.2 to −0.1, presumably due to hindrance of solvation by the methyl groups and to increasing mutual solubility of the phases at higher concentrations of cyclohexanone; the latter effect could also cause deviations from expected values of the slopes of R_M vs. log % relationships (see Table I).

Similar ΔR_M values were obtained for quinoline bases; for a number of systems of the type nonpolar solvent–water the $\Delta R_M{}^0$ values (corrected for the difference of pK_A values) of 6-methylquinoline and quinoline were found to be in the range −0.10 to −0.25 (cf. ref. 29, Table II).

23HN–12HB. ΔR_M (fused benzene ring, C$_4$H$_2$) was ca. −0.5 to −0.6 units in the range of low concentrations of cyclohexanone. For TBP systems the ΔR_M value tends to decrease in the range −0.70 to −0.75, and for TBA systems the ΔR_M varied from −0.65 to −0.9 with increasing concentration of the active solvent; apparently, in the TBP and TBA systems the additional aromatic ring markedly influences solvation of the two hydroxyl groups.

For the pair acridine–quinoline $\Delta R_M{}^0$ values from −1.1 to −1.3 were obtained for a number of aqueous systems[29]; however, there could be some contribution to these values from the steric hindrance between the nitrogen atom and the second benzene ring (see ref. 30, Table 11-3).

Polar groups

27HN–2HN; 16HN–1HN. Measurable ΔR_M (aryl-OH) was ca. +1.5 to +1.6 for the cyclohexanone systems; for TBP and TBA systems only rough estimates could be made by extrapolation of the lines of the dihydroxynaphthalenes. For TBP systems, the ΔR_M values estimated thus were ca. +1.8 and for TBA systems—ca. +2.3 (for low concentrations of the active solvent).

Vicinal effects (steric + inductive, cf. ref. 1, p. 409)

1HN–2HN; 16HN–27HN. For the pair 1HN–2HN the ΔR_M due to steric hindrance of solvation of the phenol group by the top of the adjacent benzene ring was −0.2 for all three systems (−0.25 for the system cyclohexane–water, Fig. 5). For 16HN–27HN the difference of R_M values was −0.2 for cyclohexanone systems and for the two remaining systems varied with concentration of the active solvents: for TBP, it was in the range −0.2 to −0.4 and for TBA it was in the range −0.4 to −0.5.

23HN–27HN. In view of the opposing effects of hydration in the aqueous phase and solvation in the organic phase, the ΔR_M due to vicinal β,β' positions of the two hydroxyl groups was found to vary with the concentration of the active solvent, in the range -0.55 to -0.45 for cyclohexanone systems and in the range -0.9 to -0.7 for TBP systems. For TBA systems the vicinal effect ΔR_M (β,β'), estimated for a narrow concentration range, was *ca.* -1.1.

It should be pointed out that from the viewpoint of the theoretical idealized R_M *vs.* log concentration relationship (eqn. 2) the ΔR_M values (and their dependence on composition) are determined by the a^0 values and the stepwise formation constants of the solvation complexes (see Fig. 1). In a strict approach, the variation of the activity coefficients with composition should be taken into account[8,15,17,18], especially at higher concentrations of the active solvent.

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