INVESTIGATIONS ON THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC PARAMETERS

I. TWO HOMOLOGOUS SERIES OF PYRIDINE DERIVATIVES SUITABLE AS REFERENCE COMPOUNDS IN LIQUID–LIQUID PARTITION CHROMATOGRAPHY

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SUMMARY

R_M values of several 4-pyridyl alkyl ketones and 4-pyridyl alkyl alcohols determined for a number of aqueous and non-aqueous liquid–liquid partition chromatographic systems, were found to be linearly dependent on the number of carbon atoms in the alkyl chain. The R_M values of the ketones or alcohols varied in the range of optimal accuracy of chromatographic data; the compounds are thus proposed as reference solutes for liquid–liquid partition chromatography, using a method analogous to that of the KOVATS' indices employed in gas–liquid partition chromatography.

INTRODUCTION

In recent years, the interpretation of structural effects in chromatography has developed rapidly, mostly due to the R_M concept; work in this field up to 1965 has been summed up in an excellent review by BUSH[1]; shorter reviews have been written by GREEN AND MARCINKIEWICZ[2], GREEN AND MCHALE[3], LEDERER[4,5] and PROCHAZKA[6]. In liquid–liquid partition chromatography, oxygen-containing organic compounds have been investigated mostly, whereas nitrogen compounds have been avoided, presumably in view of the complicating effect of ionization equilibria on chromatographic behaviour (see, for instance, PARDEE[7] and MOORE AND BAKER[8] for chromatography of peptides and MACEK AND VEJDĚLEK[9] for alkaloids). Because a high proportion of drugs and substances of biochemical importance are organic nitrogen compounds, investigations on the chromatographic behaviour of organic nitrogen bases have been started in this laboratory; the investigations are related to our earlier work on some theoretical problems of liquid–liquid partition chromatography (see, for instance, refs. 10–12). In addition to the theoretical significance of this problem, it was worth considering that among the parameters determining the action of drugs

and their migration kinetics in the body, their solubility and basicity play an important part[13] as they do in liquid–liquid partition chromatography. In other words, if the body is regarded as an extremely complex chromatographic system, in which the blood plays the role of the developing solvent, a certain parallelism can be expected between the behaviour of drugs and their chromatographic parameters in common "simple" partition systems.

In the course of the investigations two homologous series of pyridine derivatives have been obtained
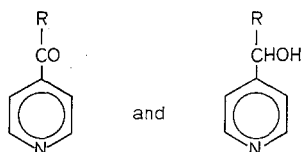
where R is an alkyl of 1–5 carbon atoms. It was found that the compounds are suitable as references for liquid–liquid partition chromatography, since in numerous solvent systems the elongation of the alkyl chain entails a variation of the R_M values in the range of optimal accuracy (−0.5 to +1.0, which corresponds to R_F values 0.75 and 0.1, respectively). This is due to the fact that the first members of the two series, the methyl derivatives, contain two hydrophilic centers in relatively small molecules: the heterocyclic nitrogen and the keto or hydroxyl group. Further advantages of the compounds are that they can be easily prepared, are stable and can be detected easily with Dragendorff's reagent. In the numerous systems investigated (see below) the R_M value was found to be linearly dependent on the number of carbon atoms in the alkyl chain; the two homologous series can thus serve as reference compounds for the determination of the chromatographic parameters of other substances in a manner analogous to the "KOVATS' indices" in gas–liquid partition chromatography[14,15], where n-alkanes are used as the reference series and the retention times are expressed by the (fractional) number of carbon atoms of a hypothetical alkane emerging from the column simultaneously with the solute. KOVATS' indices are now extensively employed in gas chromatography since they eliminate to a large extent the irreproducibility of conditions of gas chromatographic analysis. The difficulties in improving the reproducibility of liquid chromatography were extensively discussed at the 3rd Líblice Symposium (1967)[16]; in adsorption chromatography on alumina and silica gel the situation has been improved as a result of introduction of polycyclic aromatic hydrocarbons as reference solutes by SNYDER[17], but the problem seems to remain an open question in liquid–liquid partition chromatography. However, the development of automated equipment for the latter technique provides a stimulus for attempts to solve this problem.

EXPERIMENTAL

The first three ketones were obtained by Claisen synthesis[18] from ethyl isonicotinate and ethyl esters of acetic, propionic and butyric acid, respectively. The remaining two ketones, i.e., pyridyl-butyl ketone and pyridyl-amyl ketone were obtained by reaction of sodium salt of ethyl isonicotinylacetate with propyl bromide

and butyl bromide, respectively[19]. The respective pyridyl alcohols were prepared by reduction of the ketones with sodium borohydride[20].

The compounds were analysed by partition chromatography. Whatman No. 4 paper was cut into strips 7×23 cm at right angles to the machine direction and impregnated with water or MacIlvaine's buffer solution ($0.2\ M$ $Na_2HPO_4 + 0.1\ M$ citric acid), blotted between two sheets of filter paper and dried in air until the moisture content dropped to 0.5 g of aqueous phase per 1.0 g of dry paper and then transferred immediately to chromatographic tanks, $33 \times 5 \times 7$ cm. The developing solvents possessed various H-bonding properties, thus, cyclohexane, heptane, benzene and carbon tetrachloride belonged to class N (ref. 21), chloroform to class A, di-n-butyl ether to class B. The chromatograms were developed to a distance of 16 cm and the spots detected by immersion in a modified Dragendorff's reagent (0.2 g of bismuth subnitrate dissolved in 1 ml of conc. HCl added to 100 ml of 3 w/v % KI). In the second series of experiments less volatile liquids were used as the stationary phase. Whatman No. 4 paper strips were immersed twice, with a 5 min interval, in *ca.* 20 v/v % acetone solutions of formamide, dimethylformamide or ethylene glycol, the excess solution being immediately blotted off between two sheets of filter paper so that the degree of impregnation amounted to 0.5 g of the nonvolatile liquid per 1 g of dry paper.

The experiments were carried out at $22 \pm 1°$.

The results presented in Figs. 1–8 are averages from at least three experiments.

RESULTS AND DISCUSSION

Aqueous systems

In Fig. 1 the R_F and R_M values of the solutes are plotted against the number of carbon atoms in the alkyl chain n_C, for systems in which pure water was used as the stationary phase. Since R_M values calculated from R_F coefficients beyond the range 0.1 to 0.7 are less accurate, the R_M *vs.* n_C relationships beyond the range $+1$ to -0.5 are represented by dashed lines. The more commonly employed definition of R_M proposed by BATE-SMITH AND WESTALL is used ($R_M = \log (1 - R_F)/R_F$); however, the R_M axis is directed towards negative (see Fig. 1), as if the REICHL definition were used, so that the R_M and R_F values both increase.

It can be seen from Fig. 1a that the homologous series of alcohols give for aqueous systems regular, linear relationships between the R_M value and number of carbon atoms in the alkyl chain, which provides a further proof of the constancy of the ΔR_M (CH_2) increment for a homologous series. The increment for a single methylene group equals *ca.* -0.60 when cyclohexane, benzene and di-n-butyl ether are used as developing solvents which is in excellent agreement with PIEROTTI's data (see Fig. 1 in ref. 1). For the system chloroform–water the R_M *vs.* n_C line is somewhat less steep, the ΔR_M (CH_2) value amounting to *ca.* -0.50.

For strong and moderate extractants (chloroform, benzene), the variation of R_M values with the length of the alkyl chain occurs in a suitable range of R_M values but with nonpolar solvents (cyclohexane) measurable R_M values are only obtained for the higher members of the series. Thus, in order to avoid the extension of the series, the respective 4-pyridylketones are more suitable as reference compounds for nonpolar extractants (see Fig. 1b). In this case the value of ΔR_M (CH_2) is again *ca.* -0.60.
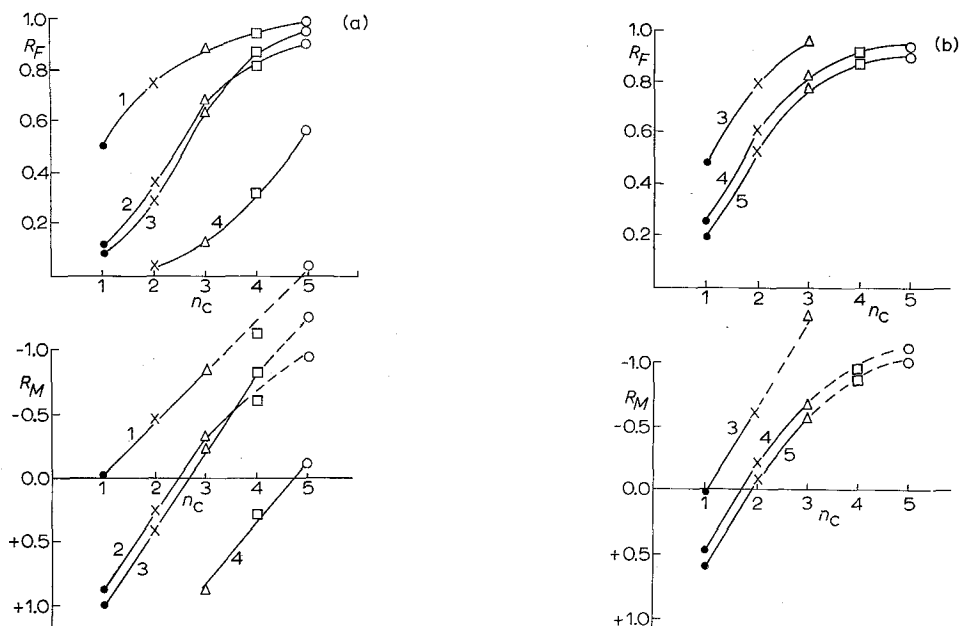
Fig. 1. R_F vs. n_C and R_M vs. n_C relationships of 4-pyridylalkyl alcohols (a) and the corresponding ketones (b). Stationary phase: water. Mobile phase: chloroform (1), benzene (2), di-n-butyl ether (3) cyclohexane (4) or heptane (5).
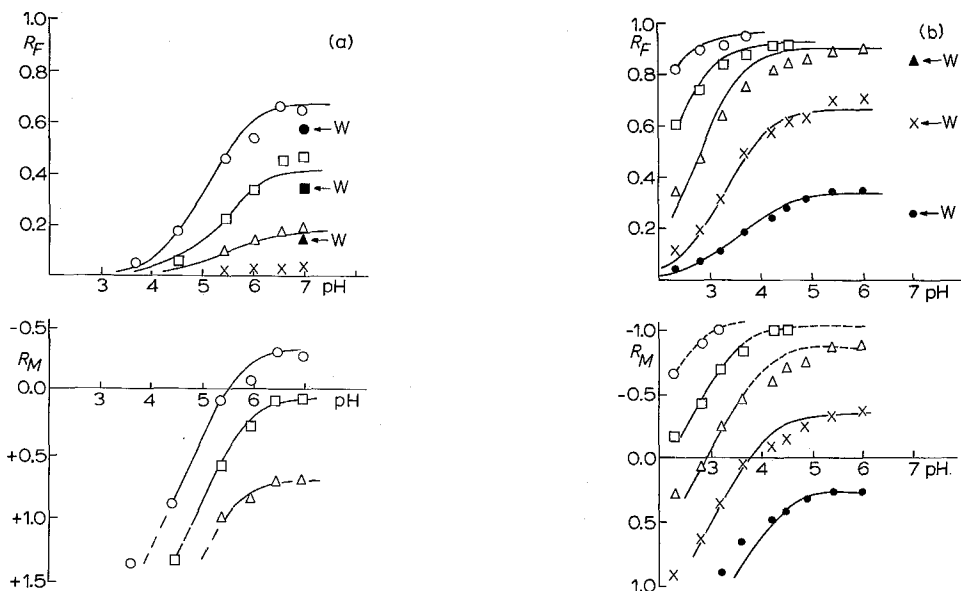

Fig. 2. R_F vs. pH and R_M vs. pH relationships of homologous 4-pyridylalkyl alcohols (a) and ketones (b). The lines in upper Fig. 2a are theoretical R_F vs. pH lines calculated for $pK_A = 5.5$ and $kr = 0.2$, 0.7 and 2.0, respectively. The three lower curves in upper Fig. 2b are theoretical curves for $pK_A = 3.8$ and $kr = 0.5$, 2.0 and 10.0, respectively. W represents R_F values obtained for the system cyclohexane–pure water. The alkyl group is denoted as follows: ● = Me; × = Et; △ = Pr; □ = Bu; ○ = Am.

The points of the butyl and amyl derivatives deviate from the straight line; however, they fall beyond the range of optimal accuracy and this is presumably the cause of this deviation. The series of pyridyl ketones is thus an excellent auxiliary set of reference compounds, the transformation of the =CHOH group into =CO group giving rise to a change of R_M of $ca.$ —1.6 units (calculated for the system cyclohexane–water, Fig. 1a, b); which corresponds to $ca.$ 3 methylene groups. Fig. 1a visualizes very clearly how the peculiar sigmoidal shape of the R_F vs. R_M relationship (see ref. 10, Fig. 4) is responsible for the S-shaped R_F vs. n_C relationship (or parts of the letter S: concave for higher values of R_F and convex for lower values).

In the next series of experiments, R_F vs. pH relationships were determined for both series of pyridine derivatives using cyclohexane as the developing solvent. For alcohols, low sigmoidal curves were obtained (Fig. 2) which for increasing pH exceed the values obtained with the system cyclohexane–water; this increase of R_F is presumably due to the salting-out effect of the increasing concentration of Na_2HPO_4. The position of the curves seems to indicate[22] that the pK_A value of the pyridyl alcohols is $ca.$ 5.5. To obtain measurable R_F values in lower ranges of pH, it would be necessary to increase the alkyl chain by several methylene units. On the other hand, the pyridyl ketones show higher R_F values (Fig. 2b) and are suitable for the calculation of retention indices in the range of pH 2.0 to 4.0 in which the R_M values are linearly dependent on pH. The pK_A values of the ketones estimated from the R_F vs. pH curves are somewhat lower than in the case of alcohols and amount to $ca.$ 3.7. In addition, in this case the R_F vs. pH lines at suppressed ionization (pH >5)
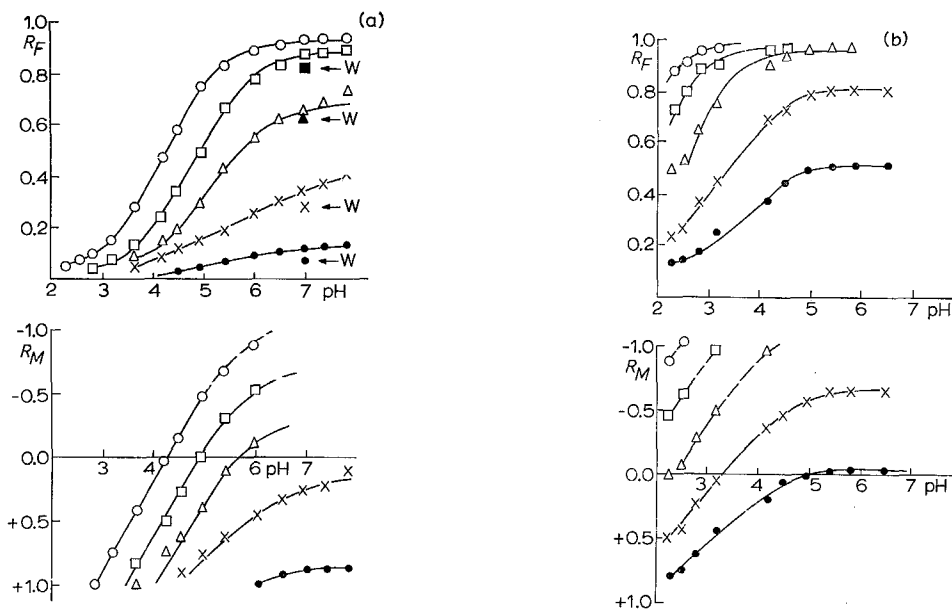

Fig. 3. R_F vs. pH and R_M vs. pH relationships for the two homologous series chromatographed in the system di-n-butyl ether–buffer solution. W represents R_F values obtained for the system cyclohexane–pure water. The alkyl group is denoted as follows: ● = Me; × = Et; △ = Pr; □ = Bu; ○ = Am.

are not horizontal but have positive slopes which indicates that in the theoretical equation[10]:

$$R_F = \frac{kr}{kr + 1 + 10^{\,pK_A - pH}}$$

also the partition coefficient (k) of unionized base (and perhaps its pK_A as well) is a function of the concentration of Na_2HPO_4 in the buffer phase (salting-out effect). Linear, parallel and regularly spaced R_M vs. pH relationships are obtained for the three higher ketones in the range of pH 2.0 to 4.0. Since the carbonyl group shields the pyridine ring from the inductive effect of the alkyl group, the pK_A values of the members of the series are probably similar so that Fig. 2b illustrates a family of R_F

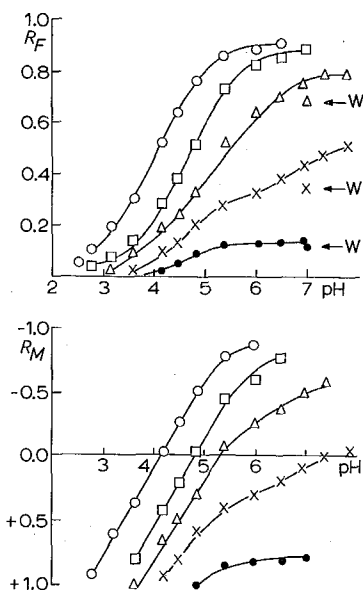Fig. 4.   Fig. 5

Fig. 4. R_F vs. pH and R_M vs. pH relationships for the series of alcohols chromatographed in the system benzene–buffer solution. W represents R_F values obtained for the system cyclohexane–pure water. The alkyl group is denoted as follows: ● = Me; × = Et; △ = Pr; □ = Bu; ○ = Am.
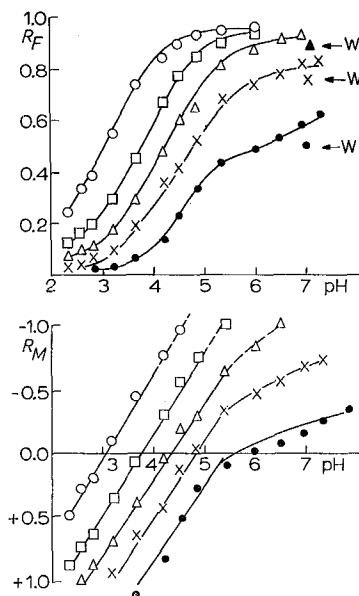
Fig. 5. As in the preceding figure; mobile phase: chloroform.

vs. pH curves for various values of the partition coefficient k and a constant value of pK_A (see ref. 10, Fig. 22 and Fig. 19). Analogous plots for di-n-butyl ether as the developing solvent are presented in Fig. 3; the curves are higher due to the better extraction of the compounds by butyl ether. At suppressed ionization the ΔR_M (CHOH → CO) is lower as in the case of cyclohexane and amounts to ca. -1.0; this is probably due to the specific interaction of the hydroxyl group with the ether oxygen of the organic solvent.

For the benzene system, the R_F vs. pH curves are similar to those obtained for butyl ether (Fig. 4); for chloroform the curves are still higher so that accurate R_M values can only be obtained for the series of alcohols (Fig. 5).
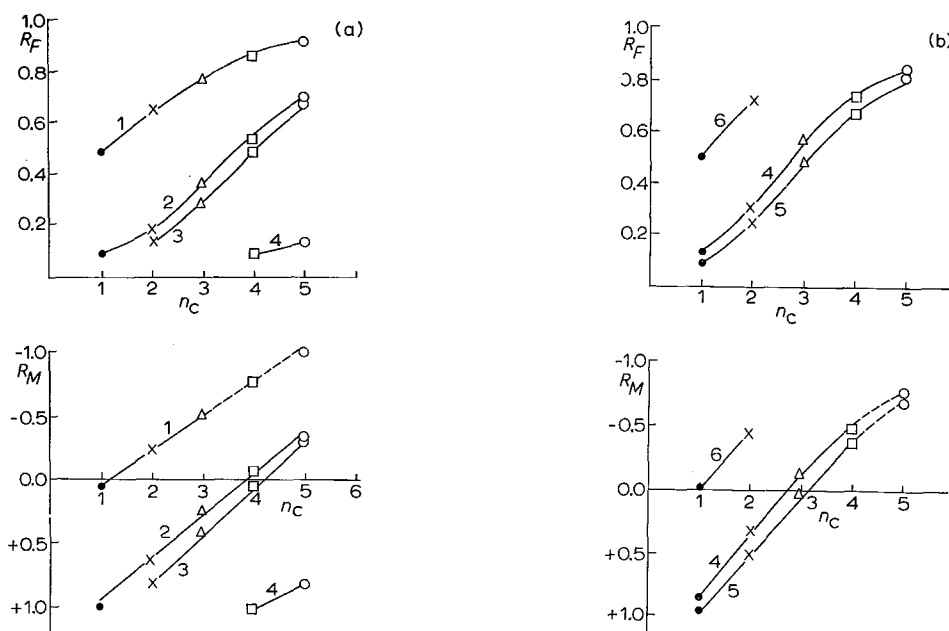
Fig. 6. R_F and R_M values as function of the number of carbon atoms in the alkyl group of the homologous alcohols (a) and ketones (b). Stationary phase: formamide. Mobile phase: chloroform (1), benzene (2), di-n-butyl ether (3), cyclohexane (4), heptane (5) and carbon tetrachloride (6). The alkyl group is denoted as follows: ● = Me; × = Et; △ = Pr; □ = Bu; ○ = Am.

Non-aqueous systems

Results obtained for formamide-impregnated paper are presented in Fig. 6. It can be seen that for strong and moderate extractants, R_M values are obtained for the alcohols in the suitable range (Fig. 6a); for weak solvents, the series of alcohols has to be extended by several additional methylene groups, or substituted by the ketones (Fig. 6b). The R_M values vary linearly in the range of optimal accuracy; the absolute value of ΔR_M (CH_2) is lower than in the case of aqueous systems and amounts to −0.45 to −0.50 for the nonpolar solvents (ketones, Fig. 6b) and −0.40 to −0.30 for mobile phases of moderate or strong solvent strength (alcohols, Fig. 6a).

Similar relationships were obtained employing ethylene glycol as the stationary phase (Fig. 7). The ΔR_M (CH_2) value is still lower and amounts to −0.30 R_M units.

When dimethylformamide (DMF) was used as the stationary phase, the R_F values of the alcohols were too low; therefore, R_F values were determined by using mixtures of cyclohexane and benzene as the mobile phase, (not exceeding 50 v/v % benzene in view of the miscibility of dimethylformamide with the nonpolar phase at higher concentrations of benzene[23]). Linear R_M *vs.* volume composition relationships were obtained in view of the absence of stronger interactions in the less-polar phase[10]. The ΔR_M (CH_2) value is *ca.* −0.2 in the series of alcohols and *ca.* −0.25 in the case of ketones. For the system 40% benzene + 60% cyclohexane/DMF, for which suitable R_F values are obtained for both ketones and alcohols, the difference in the R_M values of the methyl ketone and the methyl alcohol is *ca.* −0.60 R_M units and for the corresponding amyl derivatives *ca.* −0.7 units.

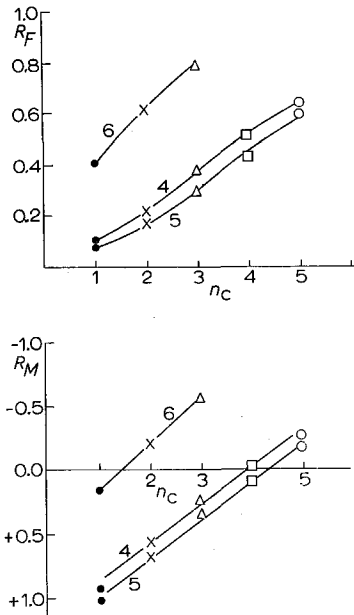Fig. 7.   Fig. 8

Fig. 7. R_F vs. n_C and R_M vs. n_C relationships of the ketone series in systems of the type: nonpolar solvent–ethylene glycol. Mobile phases denoted as in Fig. 6. The alkyl group is denoted as follows: ● = Me; × = Et; △ = Pr; □ = Bu; ○ = Am.
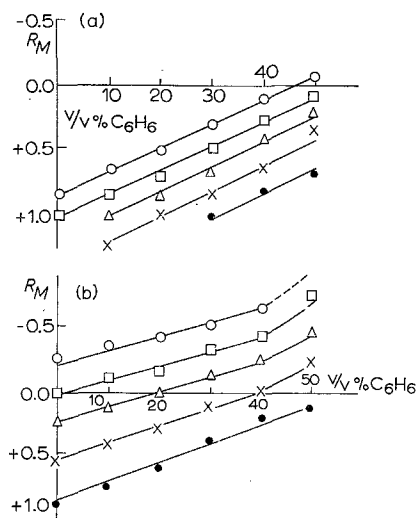
Fig. 8. R_M values of the five alcohols (a) and ketones (b) plotted as function of v/v % benzene in the mobile phase, composed of cyclohexane and benzene. Stationary phase: dimethylformamide. The alkyl group is denoted as follows: ● = Me; × = Et; △ = Pr; □ = Bu; ○ = Am.

CONCLUSIONS

The experiments demonstrate linear relationships between the R_M values of 4-pyridyl alkyl ketones and 4-pyridyl alkyl alcohols and the number of carbon atoms in the alkyl chain; deviations from linearity were observed only beyond the range of optimal accuracy (for $R_F > 0.70$) and thus were presumably due to gradient effects.

Suitable ranges of R_F values were obtained for weakly polar solvents of various solvent power and for water, formamide, dimethylformamide and ethylene glycol as the polar phase, which qualifies the homologous series investigated as reference solutes for the determination of chromatographic parameters of other solutes by the KOVATS' retention index method.

The change of R_M due to the reduction of =CO to a =CHOH group amounts to ca. -1.6 R_M units for cyclohexane–water and cyclohexane–formamide systems. This change is due to the stronger solvation of the hydroxyl group (and presumably to stronger solvation of the heterocyclic nitrogen in pyridyl alkyl alcohols in view of their higher basicity).

The use of retention indices eliminates or reduces a number of variables which cause irreproducibility of chromatographic data obtained in various laboratories. One such factors in impregnation methods is the variation of the degree of impregnation. Since the R_M values of various solutes vary linearly and are parallel when plotted

against the impregnation degree on a logarithmic scale[25], it can be expected that the use of a homologous series as a reference system will largely eliminate (or at least reduce) this effect, because a change of the ratio of volumes of the two phases should shift all R_M coefficients by a constant value.

On the other hand, when irreproducibility is caused by variation of composition of the solvent (or the pH of the aqueous phase[24]), the discrepancies can be expected to be reduced if the dependence of R_M upon the composition (or pH) is approximately parallel for the solutes investigated and for the reference solutes; thus, in systems with buffered aqueous phases the pyridyl alcohols will be useful in the range of pH 2 to 5 in which linear R_M vs. pH relationships are obtained. Other monoprotic bases strongly ionized within this range (i.e., having $pK_A > 5$) will most probably give linear R_M vs. pH relationships parallel to those of the reference compounds. If, however, the relationships of the reference substances are not parallel to those of the solutes which are being investigated, then it is at least possible to check on the basis of the R_F values of the reference solutes whether the conditions of the chromatographic experiments have been properly reproduced.

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