INVESTIGATIONS ON THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC PARAMETERS

III. PARTITION OF PYRIDYL ALKYL KETONES BETWEEN ORGANIC SOLVENTS AND HYDROCHLORIC ACID

MARIA BIEGANOWSKA, EDWARD SOCZEWIŃSKI AND STEFANIA BIEGANOWSKA

Department of Inorganic and Analytical Chemistry, Medical Academy, Lublin (Poland)

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SUMMARY

R_M vs. H_0 relationships of two homologous series of pyridyl alkyl ketones were determined for systems of the type organic solvent/aqueous hydrochloric acid. In accordance with theoretical anticipations, linear R_M vs. H_0 plots were obtained for H_0 values in the range -1, $+1$. R_M values for methylene groups were found to be constant in the systems studied so that the homologous series, especially 2-pyridyl alkyl ketones, can be used as reference compounds for the chromatography of weak lipophilic organic bases in strongly acidic liquid-liquid partition systems.

INTRODUCTION

In investigations on the structural effects in liquid–liquid partition chromatography, relatively little notice has been paid to simple derivatives of pyridine. There were some reports on the separation of pyridine bases in the form of N-oxides or N-methyl iodides; on the other hand, few authors separated free pyridine bases or their salts. A review of papers concerning the chromatographic seperation of pyridines has recently been given by NEUHÄUSER AND WOLF[1].

In the first paper in this series[2], we have reported the R_M values of several homologous 4-pyridyl alkyl ketones and 4-pyridyl alkyl alcohols in liquid–liquid partition systems, the polar phase being MacIlvaine's buffer solution, water, formamide, ethylene glycol or dimethylformamide. For stronger solvents such as chloroform, high R_F values of the ketones have been obtained even for the lower extreme of the MacIlvaine's buffer solutions (pH 2); therefore, in the present investigations we have extended the range of acidity by employing moderately concentrated solutions of hydrochloric acid (0.2–10.0%, which corresponds to Hammett's H_0 values of $+1$ and -1, respectively[3]) as polar phase. The use of such systems proved to give good results in the chromatography of weak hydrophobic bases[4]. As the developing liquid, ten nonpolar or weakly polar solvents with various donor–acceptor properties were used.
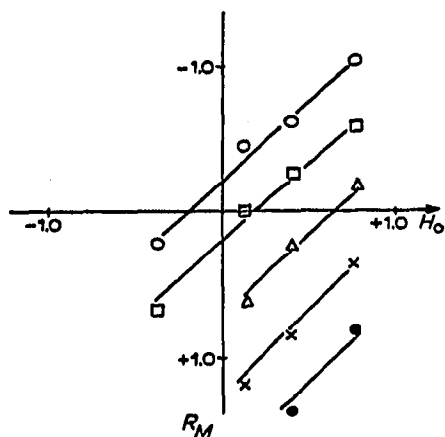
Fig. 1. R_M vs. H_0 relationships of five 2-pyridyl alkyl ketones. Mobile phase: cyclohexane. The alkyl group is denoted as follows: ● = Me; × = Et; △ = Pr; □ = Bu; ○ = Am.

In view of the low mutual solubility of the phases, the partition systems employed were expected to give better selectivities[5] than the solvent systems employed by NEUHÄUSER AND WOLF[1], who chromatographed some homologues of pyridine in systems of the type lower alcohols–hydrochloric accid–water. Furthermore, the increasing interest in olumn liquid–liquid chromatography[6] is a stimulus to investigate par-
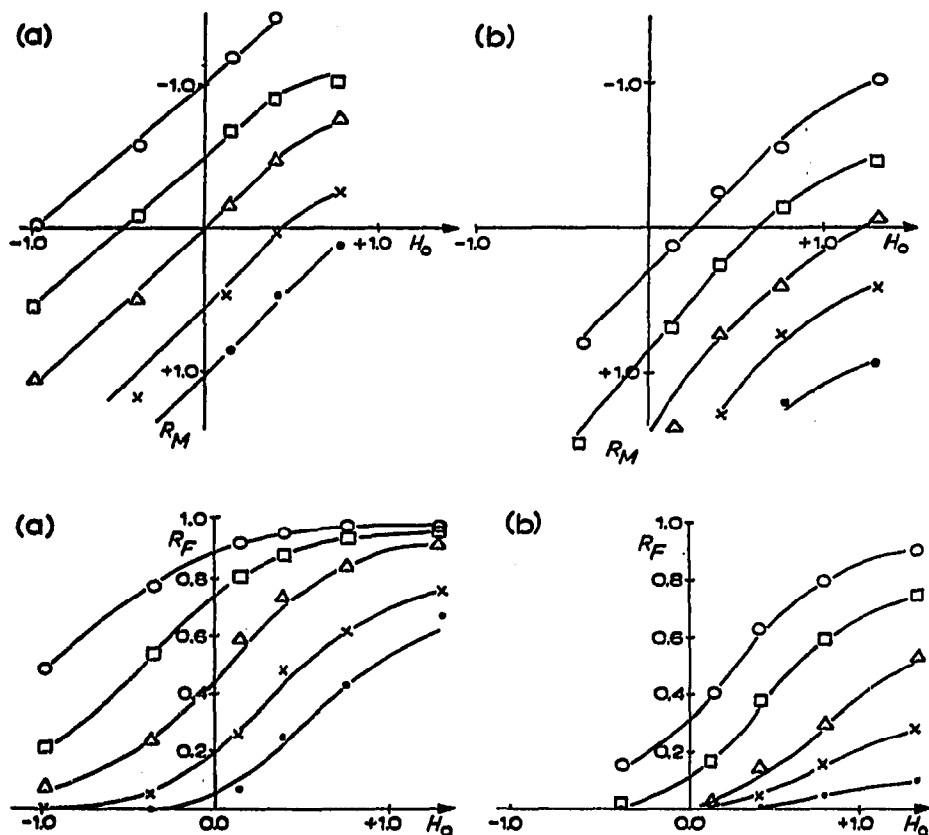
Fig. 2. Upper plots: R_M vs: H_0 relationships of 2-pyridyl alkyl ketones (a) and 4-pyridyl alkyl ketones (b). Lower plots: corresponding R_F vs. H_0 relationships. Mobile phase: benzene.
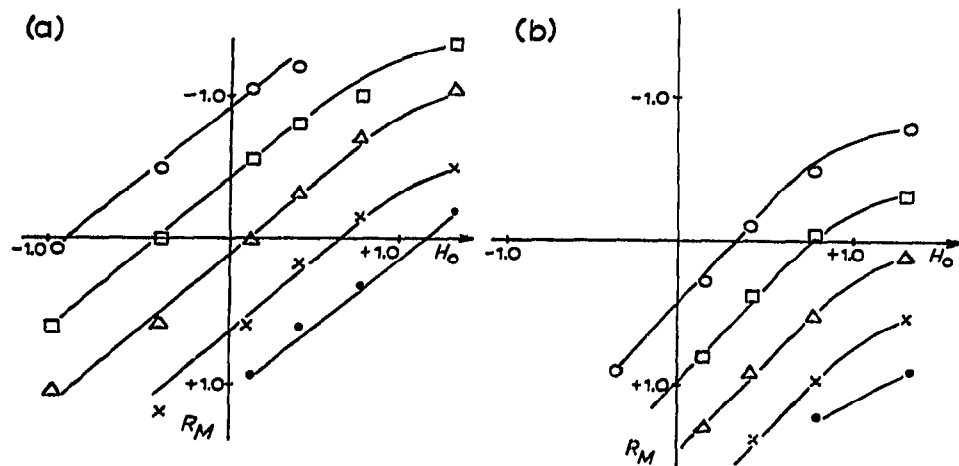
Fig. 3. R_M vs. H_0 relationships of 2-pyridyl alkyl ketones (a) and 4-pyridyl alkyl ketones (b). Mobile phase: toluene.
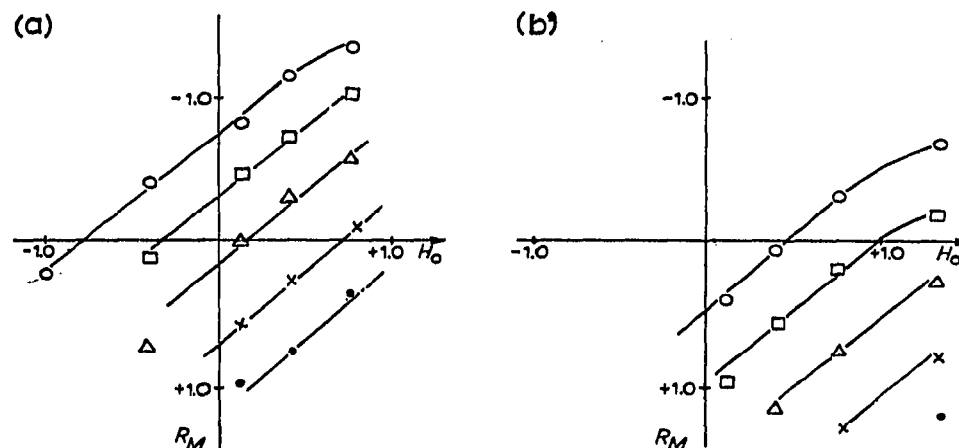

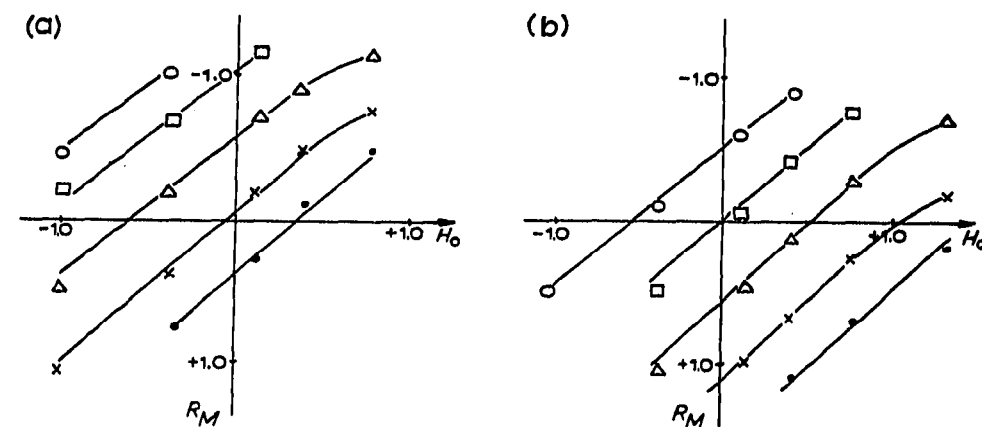Fig. 4. As in Fig. 3. Mobile phase: carbon tetrachloride.

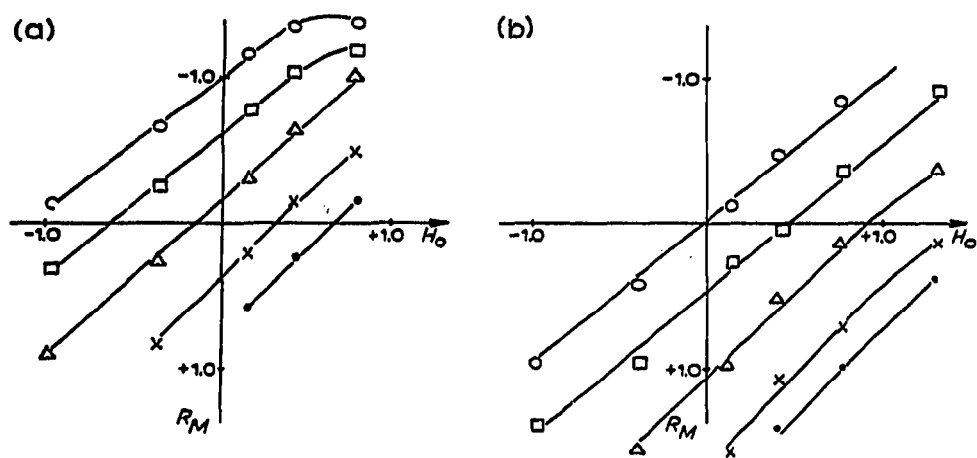Fig. 5. As in Fig. 3. Mobile phase: chloroform.

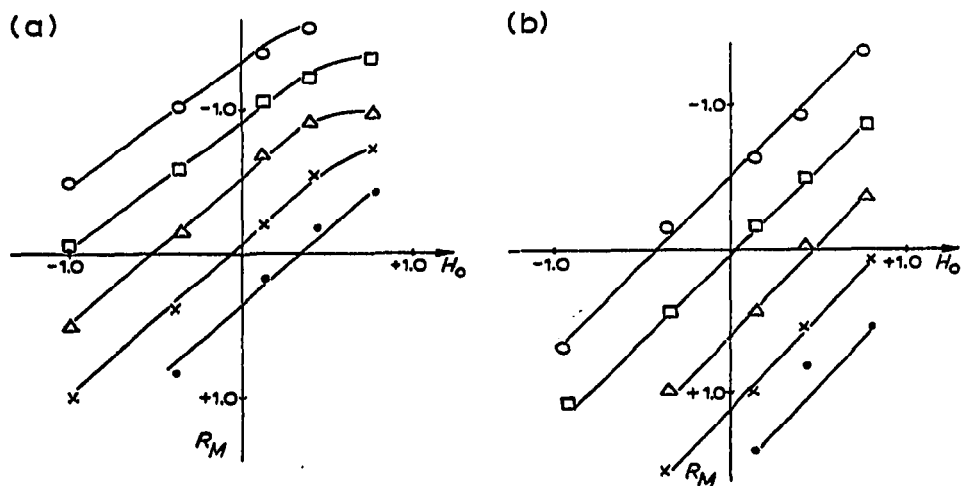Fig. 6. As in Fig. 3. Mobile phase: 1,2-dichloroethane.

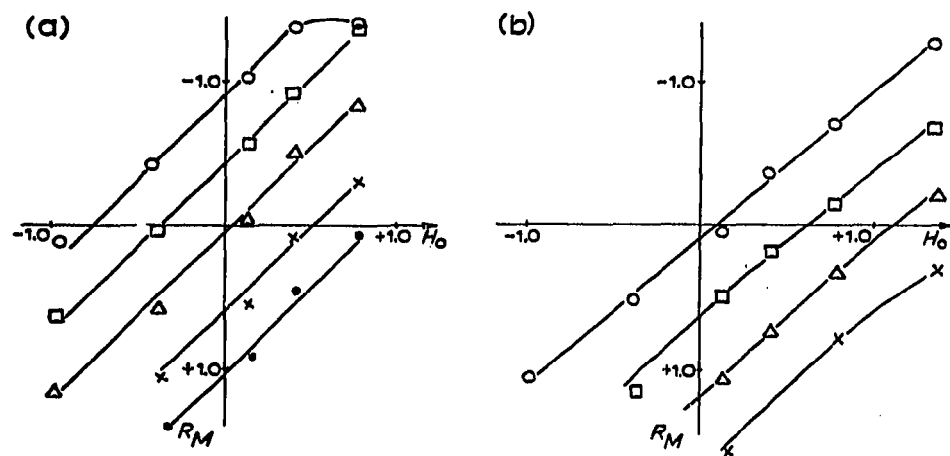Fig. 7. As in Fig. 3. Mobile phase: tetrachloroethane.

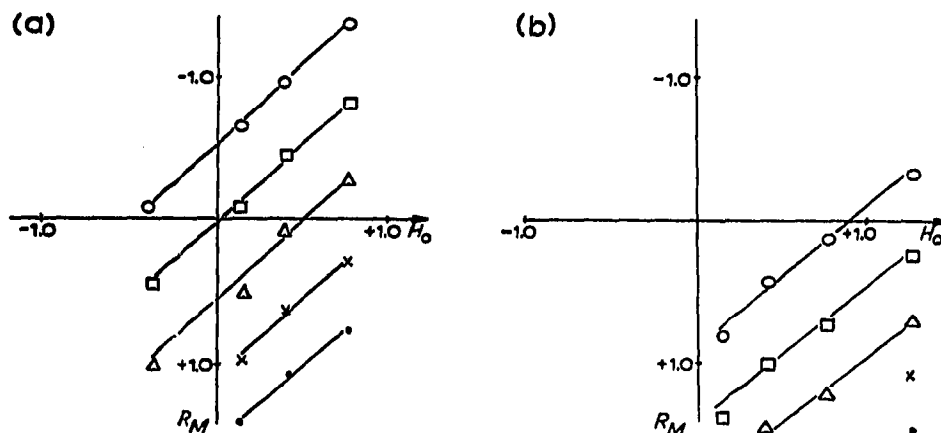Fig. 8. As in Fig. 3. Mobile phase: anisole.

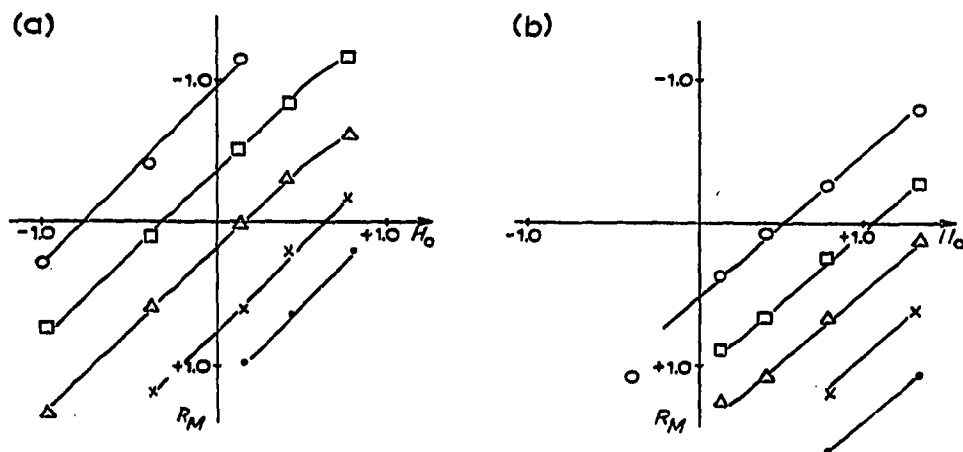Fig. 9. As in Fig. 3. Mobile phase: di-n-butyl ether.

Fig. 10. As in Fig. 3. Mobile phase: diethyl ether.

tition systems of minimal mutual solubility of the two phases, since the commonly employed onephase systems, or systems of marked mutual solubility, introduce serious complications in the technical problems of column chromatography.

The use of acidic aqueous phases permits the application of liquid–liquid partition chromatography to be extended to solutes which are strongly hydrophobic and/or have very weak basic properties and are thus strongly extracted into the organic phase in the usual pH range of buffer solutions.

EXPERIMENTAL

The pyridyl alkyl ketones were obtained by methods described in the first part of this series[2], the 2-pyridyl alkyl ketones being synthetized from the ethyl ester of α-picolinic acid.

Whatman No. 4 paper strips were impregnated with solutions of hydrochloric acid of the following concentrations: 0.02 M, 0.05 M, 0.1 M, 0.2 M, 0.5 M, 1.0 M and 2.0 M.'Moist paper' technique was employed, the strips being dried after impregnation

to a humidity of 0.5 g of aqueous solution per 1.0 g of dry paper, as in an earlier paper on paper chromatography of lipophylic acridine and quinoline bases in analogous solvent systems[4].

To determine the actual concentration of HCl in the stationary phase during the development, a calibration line was obtained by plotting the initial concentration of HCl in the impregnating solution (C_0) (multiplied by a factor accounting for partial evaporation of water from the strip) against the final concentration of HCl (C) determined by titrating the acid from the partially dried strip with NaOH. For low and moderate concentrations of HCl, the plot was practically linear, the slope being *ca.* 1.0, which indicated that under these conditions only water evaporated from the strip during the partial drying so that the actual concentration of HCl during development, C, could be calculated from the equation:

$$C = \frac{W_t - W_d}{0.5\ W_d} \cdot C_0$$

where W_d is the weight of the dry strip and W_t is the weight just after impregnation and blotting. ($W_t = 2.0$–$2.2\ W_d$; $0.5\ W_d$ is the weight of the aqueous phase during development.)

For concentrations of HCl higher than 15% $(H_0 < -1.5)$ some loss of HCl during the partial drying was observed, and the calibration line deviated from linearity.

From the actual concentration of HCl, C, the values of H_0 of the aqueous phase were estimated[7].

To avoid elution of the aqueous phase from the chromatogram, more polar solvents were saturated with solutions of HCl of concentrations expected in the strip after partial drying (*ca.* $2\ C_0$). Final equilibration of the phases was expected to occur in the distance between the solvent level and the start line (*ca.* 6 cm).

All-glass tanks ($5 \times 9 \times 24$ cm) were used for descending development. The temperature was $22 \pm 1°$.

Experimental R_F values, average from three or more runs, were plotted directly on R_M vs. H_0 diagrams (Figs. 1–10), using an R_F scale subordinate to proportional R_M scale. As in the preceding papers from this series, the R_M axis is directed downward so that the R_M and R_F both increase ($R_M = \log (1 - R_F)/R_F$).

RESULTS AND DISCUSSION

Effect of acidity of the aqueous phase

In most cases linear R_M vs. H_0 relationships are observed, in accordance with theoretical anticipations (see KEMULA AND BUCHOWSKI[8]); for higher values of H_0, the lines tend to deviate to a horizontal asymptote, like R_M vs. pH plots[9]. However, for some cases, this deviation may be due to gradient effects observed at higher values of R_F (*e.g.*, $R_M < 0.5$, *i.e.*, $R_F > 0.7$). The respective R_F vs. H_0 plots are thus sigmoidal in shape[2], like R_F vs. pH plots (see Fig. 2).

Structural effects of the solutes

It can be seen from the figures that, for both homologous series, the R_M vs. H_0 lines are regularly spaced and parallel, which indicates that for all solvent systems the R_M is linearly dependent on the number of carbon atoms in the alkyl chain. The R_M

values of the 4-pyridyl alkyl ketones vary in the range of optimal accuracy for stronger solvents (e.g., chloroform); for benzene and toluene the points of the first two homologues are beyond the range of accurate R_M values $(-1 < R_M < +1)$. Nonpolar solvents such as carbon tetrachloride and cyclohexane (class N) are not suitable for 4-pyridyl alkyl ketones; also the solvent power of aliphatic ethers (class B) is too low.

The ΔR_M (CH$_2$) value for the solvents used, both weakly polar and nonpolar, is approx. -0.52 to -0.56 R_M units, as in the preceding paper[2]. The selectivity of the partition systems for the solvents investigated is almost constant since it depends mostly on the squeezing effect of the aqueous phase, although their extraction power is differentiated over quite a wide range. In most systems, the value of ΔR_M (Et→Me) is slightly different from ΔR_M (CH$_2$) for the higher homologues $(-0.40$ to $-0.46)$.

The R_F values obtained for the other homologous series of 2-pyridyl alkyl ketones are considerably higher and for almost all solvents used are in the range of optimal accuracy for H_0 values in the range of -1, $+1$. The R_M vs. H_0 plots are also in this case linear, parallel and almost equidistant; the ΔR_M (CH$_2$) values are approx. -0.50 to -0.52 R_M units. A slightly higher selectivity is observed for cyclohexane, benzene and di-n-butyl ether; however, the solvents are weak extractants and the ΔR_M (CH$_2$) values, especially for lower homologues, are less accurate.

Comparing the chromatographic results for 2- and 4-pyridyl alkyl ketones, the ΔR_M values due to 4–2 isomerism could be estimated. The ΔR_M $(\gamma \to \alpha)$ is usually in the range of 1.1 to 1.3 R_M units. The ΔR_M values for the corresponding 2- and 4-isomers, determined from the R_M vs. H_0 plots by linear interpolation (or extrapolation) to $H_0 = 0$, are presented in Table I.

The observed differences of R_M values of the corresponding 4- and 2-isomers are presumably due to an *ortho* effect involving the shielding of the aromatic nitrogen by the vicinal R–CO–group. The inductive effects in the 2- and 4-positions are probably similar[10]; however, some difference in the pK_A values of 2- and 4-isomers can also contribute to the value of ΔR_M $(4 \to 2)$.

Extraction power of the solvents

For both homologous series, the solvents can be arranged in the following sequence of decreasing solvent power:

TABLE I

ΔR_M VALUES OF ISOMERIC 2- AND 4-PYRIDYL ALKYL KETONES
The numbers in parentheses are less accurate.

Fig. No.	Solvent (mobile phase)	Homologue				
		Me	Et	Pr	Bu	Am
1	Cyclohexane					
2	Benzene			1.30	1.30	1.30
3	Toluene		(1.34)	1.38	1.38	1.34
4	Carbon tetrachloride		(1.30)	1.30	1.30	1.30
5	Chloroform	1.20	1.20	1.12	1.06	(0.94)
6	1,2-Dichloroethane	1.24	1.24	1.24	1.12	0.98
7	Tetrachloroethane	1.12	1.10	1.10	0.90	0.82
8	Anisole		1.10	1.14	1.04	1.04
9	Di-n-butyl ether			1.30	1.40	1.40
10	Diethyl ether		(1.10)	1.30	1.38	1.40

chloroform, tetrachloroethane > 1,2-dichloroethane > anisole > benzene, toluene > ethyl ether, carbon tetrachloride > di-*n*-butyl ether > cyclohexane. The sequence is in agreement with the chromatographic spectra obtained for the partition of quinoline bases[11]. The extraction power of the solvents is largely determined by the electron-donor properties of the solutes which are thus better extracted by solvents of class A and weakly extracted by solvents of classes N and B, even more polar ones.

When *n*-hexanol (class AB) was used as the mobile phase, the R_F coefficients were found to be constant at lower H_0 values, which indicated extraction of ion pairs (*cf.* ref. 4).

CONCLUSIONS

The experiments demonstrate that in using solutions of hydrochloric acid as the stationary phase and organic solvents of various polarities as the developing phase suitable ranges of R_M values of the two homologous series investigated were obtainable.

In the range of moderate acidity of the aqueous phase ($-1 < H_0 < +1$), accurate R_M values of the first five 4-pyridyl alkyl ketones could be determined for stronger solvents (class A, *e.g.*, chloroform) while for 2-pyridyl alkyl ketones the range of solvents could be extended for weaker proton-donor solvents (*e.g.*, ethylene chloride) electron-donor and neutral solvents (classes B and N, respectively).

Constancy of ΔR_M (CH_2) values qualifies the compounds and especially 2-pyridyl alkyl ketones, as reference series for the determination of parameters analogous to KOVATS' indices[2] in chromatography of weak lipophilic bases in liquid–liquid partition systems with moderately acidic aqueous phase ($-1 < H_0 < +1$).

The selectivity to molecular size of the system investigated was found to be much higher than in the case of usual systems of lower alcohol–hydrochloric acid–water; thus, ΔR_M (CH_2) was *ca.* 0.5 units while NEUHÄUSER AND WOLF[1] report ΔR_M (CH_2) values in the range of -0.1 to -0.3.

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