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Hydrophobicity parameters determined by reversed-phase liquid chromatography

I. Relationship between capacity factors and octanol–water partition coefficients for monosubstituted pyrazines and the related pyridines

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ABSTRACT

The capacity factors (k') of monosubstituted pyrazines and 2-substituted pyridines were measured on a Capcellpack C_{18} column using methanol–buffer (pH 9.2) mobile phases of different compositions, and the relationship between log P (n-octanol–water partition coefficient) and log k' was analysed. In all instances the amphiprotic substituents were eluted faster than expected on the assumption of log P–log k' linearity, reflecting that these groups act as hydrogen donors. For other substituents, a good linear relationship between log P and log k' was obtained with eluents containing 50–70% (v/v) of methanol. However, as the methanol content decreased, linearity no longer held and correction terms for the electronic effects and specific effects attributed to ester and amide groups were required. The relationship between log k'(pyrazine) and log k'(pyridine) was studied by applying a modified bidirectional Hammett-type correction for the component in π-value attributable to electronic effects of substituents and aza functions on the hydrogen-bonding solvation effect. The retention behaviour changed systematically with changes in mobile phase composition.

INTRODUCTION

The n-octanol–water partition coefficient (log P) is extensively used as a hydrophobicity parameter in quantitative structure–activity relationships (QSAR)[1,2]. Although the measurement of log P values by the shake-flask method is conventional and standard, it is sometimes time consuming and laborious. For some systems the log P values can be predicted by taking advantage of the additive property of substituent hydrophobic constants. However, this method has its limitations for compounds where electronic and steric effects are involved[3].

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Reversed-phase high-performance liquid chromatography (RP-HPLC) has increasingly been used as an alternative method for rapidly measuring the hydrophobicity of bioactive compounds in terms of the logarithm of the capacity factor, k', defined by the equation[4,5]

$$k' = (t_r - t_0)/t_0 \tag{1}$$

where t_r is the retention time of the compound and t_0 is that of an unretained compound. Most HPLC methods for predicting log P have used a combination of a commercially available alkyl-bonded stationary phase and methanol–water eluents[4–6]. Although the partition mechanism between the stationary phase and the mobile phase is complex, the extensive studies so far reported show that, with methanol–water as the eluent, the hydrogen-bonding behaviour of the HPLC system is similar to that of the octanol–water system[5] and log k' values are correlated with log P values by a Collander-type equation[7]:

$$\log k' = a \log P + b \tag{2}$$

It should be noted that an HPLC system using an alkyl-bonded stationary phase often discriminates among the analytes according to the hydrogen-bonding properties of the molecule; non-hydrogen bonders, hydrogen acceptors and hydrogen donors (amphiprotic groups)[4,8–10]. The correlation is then improved by introducing correction terms for differential hydrogen bonding, HB_A ($HB_A = 1$ for hydrogen acceptors and $HB_A = 0$ for others) and HB_D ($HB_D = 1$ for hydrogen donors and $HB_D = 0$ for others), as shown by the equation

$$\log k' = a \log P + bHB_A + cHB_D + d \tag{3}$$

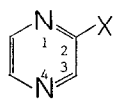
Much effort to minimize this type of hydrogen-bond discrimination has been made[5,8].

Another problem in predicting log P values using eqns. 2 and 3 is the possibility of peak inversion The elution order of solutes is sometimes observed to be reversed, depending on the methanol concentration in the eluent[11–13]. For such a system, the accuracy of the predicted log P values depends on the mobile phase composition used to measure the capacity factors for the series of compounds. To eliminate this uncertainty, recent studies have proposed using a normalized parameter, log k_W, which can be obtained by extrapolation from the linear portion of the plot of log k' against the percentage by volume of methanol in the eluents to 100% water[14]. Many successful examples associated with this log k_w approach have been reported[5,8,10,15–17]. For this purpose, however, retention data at many mobile phase compositions should be measured over a wide range of retention times under the same stationary phase conditions. The linear relationship between log k' and methanol concentration does not always hold. In fact, the plot over a sufficiently wide range of methanol concentrations tends to show a curvature that may fit a quadratic equation better than a linear relationship[18,19]. In addition, the log k_W value estimated using these approximations has no distinct physical meaning, since the log k_W value thus obtained is not the same as the log k' value measured with pure water as the mobile phase[20]. It was claimed that the log k_W approach had the merit of reducing the effect of hydrogen

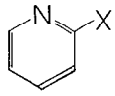
bonds[10]. Under such circumstances, it still seems doubtful whether the use of log k_W can be regarded as a standard method in every case. To gain more insight into this problem, systematic studies on the relationship between log k' and log P at different mobile phase compositions for a variety types of compounds are required

While interest in heterocyclic hydrophobicity has been increasing, little has been studied comprehensively except for the investigation of Lewis *et al.*[21]. We have previously measured and analysed log P values of monosubstituted diazines (pyrazines, pyrimidines and pyridazines) in terms of substituent effects[22]. It was shown that the π value (increment in log P attributable to a substitution) for diazine substituents ($\pi_{diazine}$) had considerably different features to the corresponding π value for substituents in monosubstituted benzenes (π_{PhX}), indicating that the electron-withdrawing property of the diazine ring caused a change in the hydrogen-bonding behaviour on the substituent as well as the ring nitrogen atoms. We were interested in analysing the HPLC retention parameters of these azines for several reasons: (1) to examine relationships between log P and log k' measured under various mobile and stationary phase conditions; (2) to compare the retention parameters for the heterocyclic system with those for other systems such as the benzenoid system to examine the influence of the hetero (ring-N) atom(s); (3) to ascertain whether the log k_W value is a reliable measure as a counter part of the *n*-octanol–water partition coefficient, as the retention time for these compounds could be measured experimentally even in mobile phases with high water concentrations; and (4) to find optimum HPLC conditions for the prediction of log P.

It has been difficult to obtain accurate retention times for basic compounds in their non-ionized form because the conventional chemically bonded silica gels cannot be used under alkaline conditions. Recently, silicone-coated silica gels modified with octadecyl or octyl groups have been developed as packing materials (Capcellpack) to ensure long-term stability under alkaline conditions[23]. In this study, as the first step of a systematic study of the HPLC retention behaviour of heterocyclic compounds, we have measured retention data for monosubstituted-pyrazines (2PR) on Capcellpack C_{18} with methanol–water eluents of different compositions. Corresponding data for 2-substituted pyridines (2PY) were also measured. The relationships between log k' and log P and between log k'(2PR) and log k'(2PY) were analysed in terms of substituent effects at each of the various eluent compositions. The changes in the retention behaviour as a function of the eluent composition are discussed.



2PR



2PY

EXPERIMENTAL

Materials

The compounds tested are listed in Table I; their preparation has been described elsewhere[22].

Partition coefficients

n-Octanol–water log *P* values necessary for the discussion were taken from our previous work[22]. Measurements were done at pH values where the solute existed as the neutral form. All the log *P* values of pyrazines (2PR) were measured using an unbuffered aqueous phase. In fact, the log *P* values of even amino derivatives (16–18; pK_a = 3.1–3.5[24]), which are the most basic in the 2PR series, remained unchanged when measured using basic buffer (pH 9.2) solutions. For some pyridines (2PY) having strongly electron-donating substituents, such as alkyl ($pK_a \approx 6.0$[25]) and amino groups ($pK_a \approx 6.9$[25]), basic buffered solutions (pH 9.2) were used.

HPLC procedure

A Shimadzu LC5A liquid chromatograph equipped with a Model 7125 valve loop injector (Rheodyne) and SPD-2A UV (Shimadzu) and Shodex SE-31 refractive index (Shoden) detectors was used. Retention times were measured using a C-R4A Chromatopac (Shimadzu). A commercial Capcellpack C_{18} (15 cm × 4.6 mm I.D.) column (Shiseido)[23] was used without further treatment. This column was demonstrated to be more effective in suppressing the peak tailing caused by hydrogen bonding ion exchange and chelate formation than trimethylsilylation of ODS phases[26]. Commercial HPLC-grade methanol was used without further purification. As an aqueous buffer, 0.01 *M* sodium borate (pH 9.2) was used. Eluents containing 15–70% (v/v) of methanol were prepared. Appropriate amounts of samples were dissolved in methanol and 1–2 μl were injected at 25°C. The concentration of the eluents was adjusted to give an appropriate peak intensity. The flow-rate was 0.2–1.0 ml/min and the peak of methanol was used to estimate the t_0 value.

For 2PR, preliminary examinations showed that methanol–buffer (pH 7.4) and methanol–buffer (pH 9.2) eluents gave identical log *k'* values. However, to compare the results with those for 2PY, all the retention data in this work were taken with methanol–buffer (pH 9.2) eluents.

RESULTS

The capacity factors were measured for 2PR in the range 15–70% methanol and for 2PY in the range 15–50% methanol. The results are summarized in Table I. To check the possibility of the silanol effects, if any, the effects of amines added as masking agents and the effects of buffer concentration were examined for 2PR with the M15 eluent. Neither addition of triethylamine (0.1%) to the aqueous phase nor the use of increased buffer concentrations (0.02 instead of 0.01 *M*) had any significant influence on the retention behaviour, suggesting that the silanol effects are effectively suppressed on this stationary phase.

The parameters used for the correlations are given in Table II. The substituents in Table I can be classified into three groups: non-hydrogen bonders (1–6), hydrogen acceptors (7–16) and amphiprotic substituents (17–20)[9].

Relationship between log k' and log P

Plots of log *k'* against log *P* for 2PR at different methanol concentrations are shown in Fig. 1. For comparison, the corresponding retention data for mono-substituted benzenes were measured for 30% methanol (data not shown), and the plot

TABLE I

LOG k' VALUES FOR PYRAZINES (2PR) AND PYRIDINES (2PY)

No.	*Substituent*[a]	*2PR*				*2PY*		
		M15[b]	*M30*[b]	*M50*[b]	*M70*[b]	*M15*[b]	*M30*[b]	*M50*[b]
1	H	0.027	−0.301	−0.651	−0.957	0.801	0.442	−0.084
2	F	0.256	−0.016	−0.354	−0.688	0.667	0.349	−0.130
3	Cl	0.603	0.311	−0.106	−0.477	1.004	0.620	0.067
4	Br	–	–	–	–	1.115	0.711	0.147
5	Me	0.398	0.012	−0.482	−0.843	1.196	0.745	0.098
6	Et	0.816	0.367	−0.175	−0.609	1.565	1.066	0.349
7	OMe	0.767	0.392	−0.063	−0.475	1.195	0.796	0.241
8	OEt	1.247	0.847	0.272	−0.233	1.628	1.181	0.516
9	OPr	1.772	1.281	0.629	0.015	–	–	0.881
10	SMe	1.065	0.662	0.144	−0.313	1.396	0.967	0.334
11	CN	0.120	−0.192	−0.549	−0.932	0.471	0.086	−0.386
12	Ac	0.453	0.081	−0.366	−0.744	0.861	0.429	−0.095
13	CO_2Me	0.369	−0.120	−0.602	−1.035	0.781	0.301	−0.277
14	CO_2Et	0.845	0.306	−0.257	−0.749	1.256	0.709	0.031
15	$CONMe_2$	0.063	−0.439	−0.951	–	0.376	−0.175	−0.715
16	NMe_2	1.089	0.542	−0.078	−0.512	1.630	1.113	0.406
17	NH_2	−0.090	−0.463	−0.968	−1.277	0.633	0.294	−0.306
18	NHMe	0.457	−0.027	−0.536	−0.912	–	–	
19	NHAc	0.214	−0.234	−0.797	−1.279	0.628	0.162	−0.406
20	$CONH_2$	−0.195	−0.623	−1.153	–	0.324	−0.111	−0.638

[a] Me = methyl; Et = ethyl; Pr = propyl; Ac = acetyl.

[b] Methanol–buffer (pH 9.2, 0.01 *M* sodium borate); the figures represent the percentage by volume of methanol.

is shown in Fig. 2. It is clear that log k' for 2PR cannot be correlated with log P by a single relationship; in other words, the points for amphiprotic substituents (depicted by triangles) generally deviate downwards. This trend is in sharp contrast with that observed with benzene derivatives, which showed good linearity between log P and log k', reflecting how the electron-withdrawing property of the azine ring affects the hydrogen-bonding capability of these substituents. For simplicity, we first tried to analyse the relationship between log k' and log P, excluding those compounds having amphiprotic substituents. Good linear correlations were obtained when the methanol contents in eluents were 50% (M50) and 70% (M70), as shown by the equations

$$\log k' = 0.581 \log P - 0.511 \quad (4)$$
$$n = 15,\ r = 0.990,\ s = 0.059,\ F = 635.4$$

and

$$\log k' = 0.489 \log P - 0.884 \quad (5)$$
$$n = 14,\ r = 0.986,\ s = 0.052,\ F = 414.2$$

TABLE II

n-OCTANOL–WATER PARTITION COEFFICIENTS (LOG *P*) AND OTHER PARAMETERS USED FOR CORRELATION

No.	*Substituent*[a]	*Log P*		σ_I[c]	HB_{CO}	HB_{NH}	σ_m^0[b]	ρ[b]
		2PR[b]	*2PY*[b]					
1	H	−0.26	0.65	0.00	0.0	0.0	0.00	0.00
2	F	0.29	0.84	0.54	0.0	0.0	0.34	0.00
3	Cl	0.70	1.27	0.47	0.0	0.0	0.37	0.00
4	Br	0.93	1.38	0.47	0.0	0.0	0.37	0.00
5	Me	0.21	1.11	−0.01	0.0	0.0	−0.06	0.00
6	Et	0.69	1.60	−0.01	0.0	0.0	−0.08	0.00
7	OMe	0.73	1.34	0.30	0.0	0.0	0.10	0.27
8	OEt	1.28	1.81	0.28	0.0	0.0	0.10	0.27
9	OPr	1.84	2.38	0.28	0.0	0.0	0.10	0.27
10	SMe	1.17	1.71	0.30	0.0	0.0	0.14	0.20
11	CN	−0.01	0.40	0.57	0.0	0.0	0.62	0.00
12	Ac	0.20	0.83	0.30	0.0	0.0	0.36	0.16
13	CO_2Me	−0.23	0.36	0.32	1.0	0.0	0.35	0.13
14	CO_2Et	0.28	0.87	0.30	1.0	0.0	0.35	0.13
15	$CONMe_2$	−0.80[d]	−0.45[d]	0.28	1.0	0.0	—	—
16	NMe_2	0.93	1.65	0.17	0.0	0.0	−0.10	0.46
17	NH_2	−0.05	0.48	0.17	0.0	1.0	−0.09	0.74
18	NHMe	0.56[d]	1.12[d]	0.13	0.0	1.0	−0.10	—
19	NHAc	−0.03	0.54	0.28	1.0	1.0	0.14	0.91
20	$CONH_2$	−0.50	0.29	0.28	1.0	1.0	0.28	0.45

[a] See Table I.
[b] Taken from ref. 22.
[c] Taken from ref. 27.
[d] Newly measured.

respectively, where n is the number of compounds used for calculations, r is the correlation coefficient, s is the standard deviation and F is the value of the F-ratio between the variances of the observed and calculated values. When the methanol content decreases (M30 and M15), esters and amides containing functional groups such as $-CO_2R$ and $-CON=$ were observed to be retained longer than other hydrogen acceptors. To describe this differentiation effect, an indicator variable, HB_{CO}, was introduced, where $HB_{CO} = 1$ only for ester and amide groups and $HB_{CO} = 0$ for others. Addition of this parameter improved the correlations for M15 and M30 to give

$$\log k' = 0.824 \log P + 0.447 HB_{CO} + 0.184 \quad (6)$$
$$n = 15,\ r = 0.982,\ s = 0.100,\ F = 163.3$$

and

$$\log k' = 0.746 \log P + 0.253 HB_{CO} - 0.151 \quad (7)$$
$$n = 15,\ r = 0.994,\ s = 0.054,\ F = 507.5$$

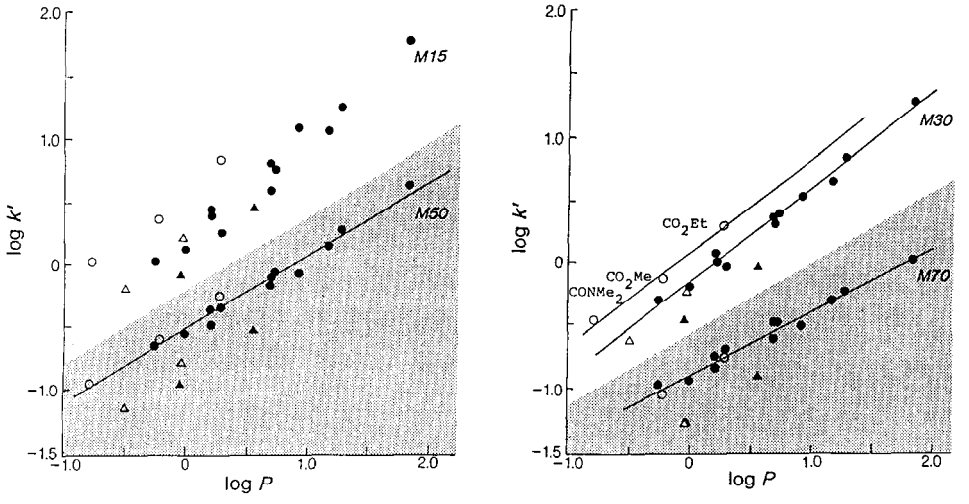


Fig. 1. Relationship between log P and log k' for monosubstituted pyrazines. Non-hydrogen bonders and hydrogen acceptors (substituents 1–16) are represented by the circles (●, $HB_{CO} = 0$; ○, $HB_{CO} = 1$) and amphiprotic substituents (17–20) are represented by the triangles (▲, $HB_{CO} = 0$; △, $HB_{CO} = 1$). The straight lines are the regression lines for non-hydrogen bonders and hydrogen acceptors coresponding to eqns. 12, 14 and 16.

respectively. Although eqn. 6 is statistically acceptable, examination of the residuals from the calculated values showed large deviations for electron-withdrawing substituents such as halogens and acetyl groups, suggesting that the electronic properties of the substituent participate in the retention mechanism. Among the electronic parameters examined, Charton's σ_I constant[27] was found most useful. Addition of σ_I helped significantly in minimizing the residuals and for M15 yielded

$$\log k' = 0.830 \log P + 0.465 HB_{CO} - 0.347\sigma_I + 0.273 \quad (8)$$
$$n = 15,\ r = 0.990,\ s = 0.078,\ F = 181.8$$

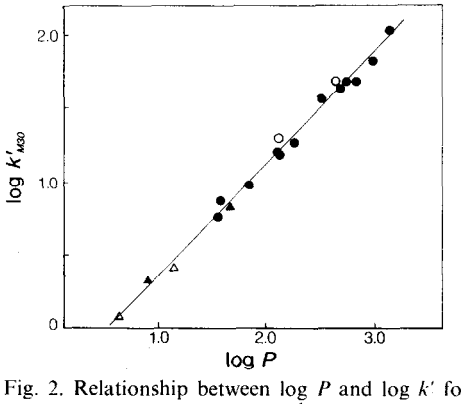


Fig. 2. Relationship between log P and log k' for monosubstituted benzenes [methanol–water (30:70); Capcellpack C_{18}; data taken from our unpublished results]. For symbols, see Fig. 1.

TABLE III

RELATIONSHIP BETWEEN LOG P AND LOG k'[a]

$\text{Log}k' = a\text{log}P + h_1HB_{CO} + h_2HB_{AM} + \rho\sigma_I + c$

System	*Mobile phase*[b]	*Log P*	HB_{CO}	HB_{NH}	σ_I	*c*	*n*	*r*	*s*	*F*	*Eqn. no.*
2PR	M15	0.817	0.385	−0.292		0.200	19	0.984	0.099	152.7	10
		0.824	0.414	−0.319	−0.371	0.292	19	0.991	0.078	186.7	11
	M30	0.744	0.237	−0.305		−0.147	19	0.996	0.050	565.7	12
		0.747	0.248	−0.315	−0.137[c]	−0.113	19	0.997	0.045	513.6	13
	M50	0.582		−0.349		−0.511	19	0.992	0.060	503.2	14
		0.620	0.100	−0.361		−0.549	19	0.995	0.047	543.2	15
	M70	0.495		−0.348		−0.887	17	0.991	0.051	376.7	16
2PY[d]	M15	0.760	0.403	−0.446		0.203	17	0.964	0.126	56.7	17
		0.719	0.374	−0.451	−0.543	0.407	17	0.988	0.076	123.6	18
	M30	0.706	0.244	−0.362		−0.150	17	0.980	0.091	107.5	19
		0.674	0.222	−0.365	−0.414	0.006	17	0.995	0.048	299.5	20
	M50	0.540		−0.235	−0.219	−0.450	18	0.991	0.060	260.8	21
		0.591	0.117	−0.293		−0.591	18	0.991	0.061	251.6	22
		0.579	0.109[e]	−0.296	−0.205	−0.518	18	0.994	0.050	284.4	23

[a] Unless noted otherwise, all of the terms except for the intercept values are justified above the 99.5% level
[b] See the footnotes in Table I.
[c] Justified at the 94% level.
[d] Substituent NH_2 was not included for correlations 17–23.
[e] Justified at the 98% level.

Next, analyses including amphiprotic substituents were done by using an additional indicator variable, HB_{AM}, which takes the value 1 only for amphiprotic substituents (17–20) and 0 for others, as shown in Table II. Stepwise regression analyses were made in terms of the following general equation:

$$\log k' = a \log P + h_1HB_{CO} + h_2HB_{AM} + \rho\sigma_I + c \tag{9}$$

The significance of each term was judged on the basis of statistical considerations. The most plausible correlations at each methanol concentration and statistically equivalent equations are summarized in Table III together with other equations necessary for the discussion.

For pyridines (2PY), plots of log k' against log P were generally similar to those for the pyrazine series, although the points became more scattered with decrease in the methanol content in the mobile phase than in the case of 2PR (Fig. 3). Excellent correlation equations were also obtained by application of eqn. 9 as the general formula, except for NH_2, which showed large deviations in all the mobile phases studied, for which rationalization is difficult at present[a]. The results are included in Table III.

[a] A possible explanation might be that aminopyridine is partially ionized under the experimental conditions. The log P values for octanol–water, octanol–buffer (pH 9.2) and octanol–0.1 M sodium hydroxide systems are 0.4–0.44, 0.48 and 0.51, respectively. It is difficult to judge whether the difference between the last two values should be regarded as significant to support this reasoning or not.

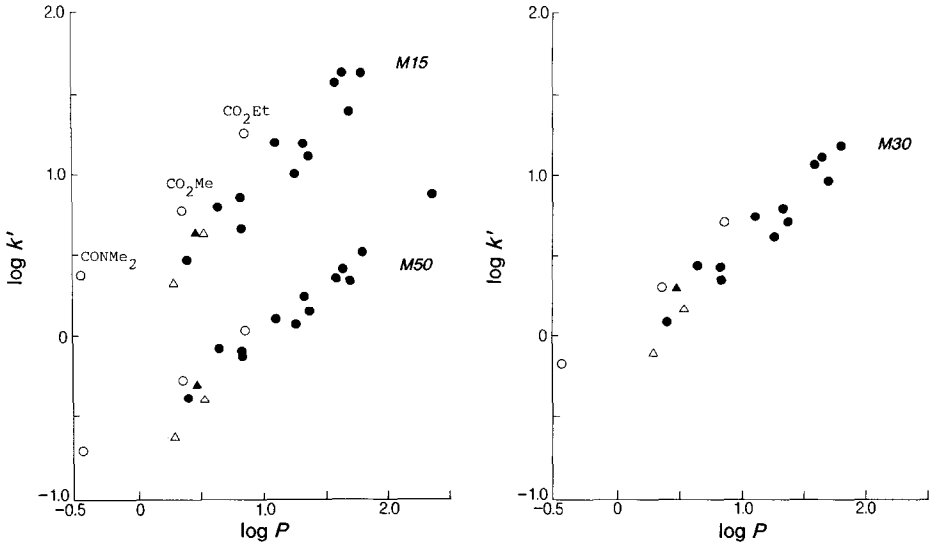


Fig. 3. Relationship between log P and log k' for 2-substituted pyridines. For symbols, see Fig. 1.

It should be noted that eqn. 9 shows that hydrogen-bonding acceptors other than ester and amide groups, such as alkoxy and cyano, behave similarly to non-hydrogen bonders. To examine the hydrogen-bond effect, if any, attributable to these ordinary hydrogen acceptors, we tried to introduce an additional indicator variable, HB_A, which takes the value 1 for the hydrogen-bond acceptors other than ester and amide groups (substituents 7–12 and 16). Regression analyses using log P, σ_I and three kinds of HB parameters (HB_{CO}, HB_{AM} and HB_A) indicated that the HB_A parameter was statistically significant (at the 93% level) only for M15 in 2PY. Even in this instance, the correlation was not much improved by the introduction of the parameter HB_A, indicating that eqn. 9 is sufficient as a general empirical equation to relate two kinds of hydrophobicity parameters from octanol–water and HPLC systems.

Relationship between log k'(2PR) and log k'(2PY)

In our previous work[22], we tried to analyse the π value of 2PR (π_{2PR}) in terms of physico-chemical parameters of substituent, such as π_{PhX}, and electronic and steric parameters. Contrary to our initial expectation in correlating π_{2PR} with π_{PhX}, no simple correlation could be derived because of strong electronic interactions between the substituent and ring nitrogen atoms. Therefore, as a preliminary step, we tried to correlate π_{2PR} with the π value of structurally related 2-substituted pyridines (π_{2PY}). Applying the bidirectional Hammett-type treatment proposed for the analysis of π values for disubstituted benzenes, YC_6H_4X (X and Y are variable and fixed substituents respectively)[3], to the pyrazine series where the ring N-4 atom is considered as the substituent Y, the π_{2PR} value was demonstrated to fit excellently the equation

$$\pi_{2PR} = a\pi_{2PY} + \rho_Y\sigma_X^0(m) + \sigma_Y^0(m)\rho_X + c \quad (24)$$

TABLE IV

RELATIONSHIP BETWEEN LOG k'(2PR) AND LOG k'(2PY)

$k^*_{2PR} = ak^*_{2PY} + \rho_Y\sigma^0_X(m) + \sigma^0_Y(m)\rho_X + c$

Mobile phase[a]	*Correlation*[b]	n[c]	r	s	F	*Eqn.no*
M15	$k^*_{2PR} = 1.257k^*_{2PY} + 0.940\sigma^0_X(m) + 0.323\rho_X - 0.022$	16	0.990	0.068	205.1	26
M30	$k^*_{2PR} = 1.312k^*_{2PY} + 1.034\sigma^0_X(m) + 0.286\rho_X - 0.020$	16	0.991	0.064	209.4	27
M50	$k^*_{2PR} = 1.302k^*_{2PY} + 0.847\sigma^0_X(m) + 0.106\rho_X$[d] $- 0.002$	16	0.993	0.059	308.0	28

[a] See the footnotes in Table I.

[b] Unless noted otherwise, all of the terms except for the intercept values are justified above the 99.5% level

[c] The substituents NHMe and $CONMe_2$ were not included in calculations because their ρ_X values were unknown.

[d] Justified at the 90% level.

where $\sigma^0_X(m)$ is the electronic substituent constant σ^0_m of the substituent X and ρ_X is the susceptibility constant of X to the solubility-modifying effects of the Y (N-4) atom. While the interaction between X and N-1 is assumed to be cancelled by using π_{2PY} as the reference, the interaction between X and N-4 is expressed by the $\sigma^0_X(m)$ and ρ_X terms: the former expresses the effects of X (or strictly N-1 + X) on the change in the hydrogen-bonding ability of N-4, and the latter expresses the reversed effect of N-4 on X (or N-1 + X). The intercept, c, should theoretically be zero. By introducing the values of π_{2PY}, σ^0_m and ρ for X substituents the regression coefficients, a, ρ_Y and $\sigma^0_Y(m)$ are calculated by the least-squares method. If the analysis is limited to the series of 2PR, the use of σ_I instead of σ^0_m gave a statistically equivalent correlation to that formulated by eqn. 24. However, a comprehensive analysis of the π value of diazines, including not only pyrazines but also pyrimidines and pyridazines, showed that σ^0_m works better than σ_I in this type of analysis.

To compare the chromatographic retention behaviour with that observed from the octanol–water partitioning system, analogous analyses for log k' were tried at M15, M30 and M50, for which the retention data for 2PY are available. The correlation equation corresponding to eqn. 24 can be formulated by

$$k^*_{2PR} = ak^*_{2PY} + \rho_Y\sigma^0_X(m) + \sigma^0_Y(m)\rho_X + c \qquad (25)$$

where k^* is the increment in log k' attributable to the substitution. The analysis using eqn. 25 yielded excellent correlations at each mobile phase composition, as shown in Table IV.

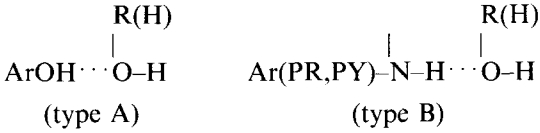
DISCUSSION

Examination of Table III shows that the quality of correlation equations varies with the mobile phase composition, suggesting that the retention mechanism becomes more complicated with the decrease in methanol concentration. It is clearly demonstrated that the linearity of the log P–log k' relationship becomes perturbed more significantly by other factors as the methanol concentration decreases. For instance, the HB_{CO} term is statistically significant at M50, but neglecting this term from

ithe correlation would have little effect on the estimated log P value. However, for M15 and M30, the HB_{CO} term is essential.

In both series, 2PR and 2PY, the slope of log P and the intercept, c, increased with decreasing methanol concentration according to the general trend so far observed[4]. At a constant mobile phase composition, the slope of log P for 2PR is slightly but significantly higher than that for 2PY.

The coefficient of the HB_{AM} term was found to be negative in all the cases examined here, in accordance with the general behaviour that hydrogen donors have shorter retention times than expected from the log k' *vs.* log P calibration line[4]. Typical examples are the acceleration effects of phenol derivatives when eluted with methanol–water mixtures[6,8,28]. In this instance, the OH group is thought to undergo hydrogen bonding with the solvent as follows (type A):



As octanol is more basic than methanol and water, this type of solvation participates to a greater extent in an octanol–water system than in a stationary phase–aqueous methanol mobile phase system, and hence the OH derivatives appear to be more hydrophobic in the former system. The present results could be understood in a similar way, as the electron-withdrawing property of the azine rings increases the hydrogen-donating ability of the amphiprotic groups and, hence, hydrogen bonding as in type B would occur.

The coefficient of the HB_{CO} term is positive and becomes larger with a decrease in methanol concentration. The reason why only functional groups such as ester and amide are retained more than the other ordinary hydrogen acceptors is difficult to explain. A similar effect was observed with other modifiers such as dioxane and also with other stationary phases such as phenyl- and cyano-bonded columns, although the slopes of the HB_{CO} term varied[29]. Interestingly, our current work has demonstrated that such an ester effect is also observed when the log P values of 2PR are compared between octanol–water and chloroform–water partitioning systems[29]. It seems from these results that the structural characteristics of solutes are responsible for the origin of the apparent retardation effect associated with ester and amide groups. Further studies of the retention behaviour exerted by such substituents involved in other reference systems would be required to explain this problem.

The fact that the σ_I term is significant, especially in water-rich mobile phases, demonstrates that the selective solute–solvent interactions are important in governing the log k' values. Probably this effect is associated with the pK_a of the solute. Ehrenson *et al.*[30] have shown that the pK_a values of 2-substituted pyridines can be described mainly by σ_I. The observed tendency that the contribution of the σ_I term becomes greater with an increase in the polarity of the mobile phase could be understood by the fact that the electronic effects are generally more important in a polar solution. At a fixed methanol concentration, the coefficient σ_I is larger for 2PY than for 2PR, probably because the electronic substituent effects affect the pK_a value for stronger bases (2PY) to a greater extent than for weaker bases (2PR). With increase in the

contribution from the electronic effects to the overall retention, the linear relationship between log P and log k' becomes poorer.

It should be noted that although the HPLC partitioning mechanism seems very complex, the relationship expressed by eqn. 25 holds very well in the range from M15 to M50. We had also found that the same type of correlation held in correlating the capacity factors of *m*- and *p*-substituted phenylacetanilides (PhA) with those of monosubstituted benzenes in the range from M50 to M70[6]. These results suggest that the partitioning in the HPLC system may be governed by similar factors to those operating in the octanol–water system. Table IV shows that the coefficient of k^*_{2PY} is fairly constant (*ca.* 1.3) and those of σ^0_m and ρ_X seem to reflect the properties of the mobile phase. The coefficient of σ^0_X(m), ρ_Y, increased by a factor of 1.2 on going from M50 to M30 [$\rho_{Y(M30)}/\rho_{Y(M50)} = 1.2$]. The same factor had been obtained with PhA when the equations for M50 and M70 were compared [$\rho_{Y(M50)}/\sigma_{Y(M70)} = 1.2$], indicating that the overall hydrogen-bonding behaviour of methanol–water mixtures varies linearly with the methanol concentration in the M30–M70 region. This linear change no longer seems assured when the methanol content is lower than 30%, as demonstrated by a decrease in the ρ_Y value at M15 relative to that at M30, indicative of a discontinuity in the retention mechanism at very high water contents in the mobile phase. In such a region of methanol concentrations it is possible that the properties of the stationary phase would also be changed non-linearly[31].

From the above discussion, the log k_W value obtained as an extrapolated value would have complex characteristics. To examine the validity of the log k_W approach, precise measurements of capacity factors over a wide range of methanol concentrations are required. Therefore, we shall discuss this problem only briefly here. In Table V, the log k_W values obtained by the linear extrapolation are presented. It is clearly seen that log k_W depends on the range over which the retention data were measured, as has often been observed[8]. The analysis of these log k_W values using eqn. 9 gave the following equations:

2PR, for the range 15–50% methanol:

$$\log k_{W(15-50)} = 0.949 \log P + 0.521HB_{CO} - 0.267HB_{AM} - 0.499\sigma_I + 0.606 \quad (29)$$
$$n = 16,\ r = 0.985,\ s = 0.105$$

2PR, for the range 30–70% methanol:

$$\log k_{W(50-70)} = 0.961 \log P + 0.403HB_{CO} - 0.153HB_{AM} - 0.264\sigma_I + 0.447 \quad (30)$$
$$n = 16,\ r = 0.991,\ s = 0.084$$

2PY, for the range 15–50% methanol:

$$\log k_{W(15-50)} = 0.844 \log P + 0.459HB_{CO} - 0.386HB_{AM} - 0.656\sigma_I + 0.722 \quad (31)$$
$$n = 16,\ r = 0.986,\ s = 0.089$$

It is worth noting that the correlation becomes more complex with the log k_W parameter, although the coefficient of log P becomes nearly unity. As an alternative extrapolation method, a quadratic model was also tried, but led to poorer correlations. Use of the $E_T(30)$ solvent polarity parameter[32] did not improve the correlation (data not shown). As studies on the log k_W approach have mostly treated benzenoid compounds, we also attempted to examine some monosubstituted benzenes on the

TABLE V

LOG k_W VALUES DERIVED BY LINEAR EXTRAPOLATION

No.	*Substituent*	*2PR*		*2PY:*	*PhX:*	
		$k_{W(30-70)}$[a]	$k_{W(15-50)}$[b]	$k_{W(15-50)}$[b]	k_W[c]	*(log P)*[d]
1	H	0.18(0.9993)[e]	0.30(0.9980)	1.19(0.9997)	2.04	(2.13)
2	F	0.49(1.0000)	0.51(0.9998)	1.02(0.9994)	2.22	(2.27)
3	Cl	0.89(0.9994)	0.89(0.9998)	1.41(0.9998)	2.77	(2.84)
4	Br	—	—	1.54(0.9999)	2.94	(2.99)
5	Me	0.63(0.9960)	0.77(0.9999)	1.68(0.9998)	2.65	(2.69)
6	Et	1.08(0.9980)	1.23(0.9996)	2.10(0.9998)	3.21	(3.15)
7	OMe	1.04(0.9996)	1.11(0.9997)	1.61(0.9999)	2,12	(2.11)
8	OEt	1.65(0.9993)	1.67(0.9998)	2.12(0.9995)	2.60	(2.51)
9	OPr	2.22(0.9999)	2.26(1.0000)	—	—	—
10	SMe	1.38(0.9994)	1.45(0.9999)	1.86(0.9999)	—	—
11	CN	0.37(0.9998)	0.40(0.9990)	0.83(0.9997)	1.65	(1.56)
12	Ac	0.69(0.9988)	0.80(0.9996)	1.26(0.9996)	1.77	(1.58)
13	CO_2Me	0.56(0.9995)	0.76(0.9963)	1.22(0.9996)	2.30	(2.12)
14	CO_2Et	1.09(0.9993)	1.29(0.9980)	1.77(0.9998)	2.85	(2.64)
15	$CONMe_2$	—[f]	—[f]	—	—	—
16	NMe_2	1.30(0.9949)	1.57(0.9990)	2.16(1.0000)	—	—
17	NH_2	—[f]	—[f]	—[f]	—	—
18	NHMe	0.86(0.9963)	0.86(0.9977)	—	—	—
19	NHAc	0.54(0.9990)	0.64(0.9999)	1.06(0.9997)	1.21	(1.16)
20	$CONH_2$	—[f]	—[f]	0.73(0.9997)	0.90	(0.64)

[a] Log k_W derived from the linear extrapolation using the data for 30–70% methanol.
[b] Log k_W derived from the linear extrapolation using the data for 15–50% methanol.
[c] Log k_W derived from the linear extrapolation using our unpublished data dor 30–70% methanol[29].
[d] Taken from ref. 22.
[e] Correlation coefficients.
[f] Not accurately determined.

same column. The log k_W values obtained are included in Table V for comparison. It is seen that the log k_W value agrees well with the log P value, indicative of the usefulness of log k_W as a possible candidate for the hydrophobicity of the benzenoid compounds. The different features observed in our system could therefore be produced by the ring nitrogen atom(s), which must increase the solute–solvent interactions by the hydrogen-bond effect. The difference in relative solvation effects through the hydrogen bonding between the stationary phase–mobile phase and octanol–water systems is expected to be increased with decrease in the methanol concentration in the eluent. In such instances, the eluent composition used to predict log P should be selected very carefully.

In conclusion, this work has shown that the log k' value of 2PR at different methanol concentrations can be expressed by eqn. 9 as a general equation. One of the most troublesome problems hampering the use of the RP-HPLC techniques for the determination of log P arises from the contribution of electronic effects (the $\rho\sigma$ term) which is responsible for perturbing the log P *vs.* log k' linearity. These electronic effects were more important in highly water-rich mobile phases. This finding leads us to conclude that the log k_W obtained as an extrapolated value would fail to describe the

hydrophobic properties of very polar solutes. To gain a closer insight into this subject, the analysis by eqn. 9 should be extended to a wider range of eluent compositions and to different kinds of stationary phases and also to other heterocyclic systems of solutes. Detailed discussion on this subject will be presented in a forthcoming paper. The physical meaning of the HB_{CO} parameter remains to be studied and work is in progress.

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