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Hydrophobicity Parameters Determined by Reversed-Phase Liquid Chromatography. IX.[1] Relationship between Capacity Factor and Water–Octanol Partition Coefficient of Monosubstituted Pyrimidines

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The capacity factors, k', of 2- and 5-substituted pyrimidines were determined by reversed-phase high performance liquid chromatography (RPLC). The log k′ values were correlated with log P by using correction terms for the hydrogen-bond effects of the aza functions of the diazine ring and the substituent. By analogy with the case of the pyrazine series previously studied, a correlation equation with indicator variables categorizing the type and strength of the substituent hydrogen-bonding, and the electronic constant of substituent as additional parameters was obtained to describe the correlation between log k' and log P. Suitable mobile-phase conditions to predict reliable log P values are proposed.

Keywords hydrophobicity; capacity factor; partition coefficient; octanol; pyrimidine; hydrogen bond

The hydrophobicity of bioactive compounds is in many cases a major factor governing biological potency.[2] We are interested in methods to estimate the log P value (P, 1-octanol–water partition coefficient), a conventionally used hydrophobicity parameter, of heterocyclic compounds, since many bioactive molecules contain heterocyclic rings. In addition to the standard shake-flask method, procedures using reversed-phase high-performance liquid chromatography (RPLC) have recently been developed to predict log P values.[3–6] The RPLC method assumes a linear relationship between the logarithm of capacity factor (log k'), regarded as a chromatographic hydrophobicity parameter, and log P. This approach seems applicable provided that the compound contains no strongly hydrogen (H)-bonding functional groups. However, in heterocyclic systems, the H-bond effects of ring hetero atom(s) and also of substituent(s) (if any) should always be taken into consideration. Moreover, under such conditions, the electronic interactions between the ring hetero atom(s) and polar substituents usually affect the H-bonding ability of each functional moiety, resulting in complicated relationships between log P and log k'. In previous studies,[7] we have determined log k' values for monosubstituted pyrazines using a Capcell pack C_{18} column[8] and eluents containing different concentrations of methanol as an organic modifier, and analyzed the correlation between log k' and log P[9] in terms of physico-chemical parameters of substituents, obtaining Eq. 1 as a general correlation.

$$\log k' = a \log P + h_{CO} HB_{CO} + h_A HB_A + h_{AM} HB_{AM} + \rho\sigma_I + \text{const.} \quad (1)$$

In this equation, the three HB terms are indicator variables to express H-bonding effects of substituents, and are defined as follows: $HB_{CO}=1$ for esters and amides (–COOR, –CON<), $HB_A=1$ for H-acceptors other than –COOR and –CON< such as OR, SMe, NMe_2 and CN, $HB_{AM}=1$ for amphiprotic (H-donor) substituents; otherwise, each HB parameter is 0. The σ_I value is Charton's electronic constant.[10] The coefficients of these correction terms are thought to reflect the change in the H-bonding effects of each functional group in going from the octanol–water partitioning system to the RPLC system. The h_{CO}, h_A and h_{AM} values represent the H-bonding effect of the substituent and the ρ value represents the H-bonding effect of the ring-N atom(s). Although the HB_A, HB_{CO} and σ_I terms become significant in water-rich eluents, they are almost negligible at medium-range methanol concentrations, and good linear relationships are obtained by treating H-donors or amphiprotic substituents separately.

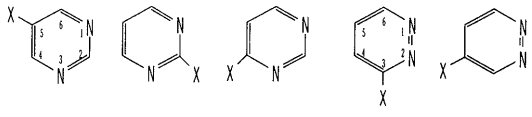
We were interested in whether such a relationship (Eq. 1) is also applicable to other heterocyclic systems. In this work, we determined the log k' values of monosubstituted pyrimidines and the relationship between log P and log k' was examined by applying Eq. 1.

Experimental

Materials The compounds used in this study are 5-substituted pyrimidines (5PM) and 2-substituted pyrimidines (2PM). Some 4-substituted pyrimidines (4PM), 3-substituted pyridazines (3PD) and 4-substituted pyridazines (4PD) with alkyl and alkoxy groups (X=alkyl, OR) were also studied. The preparation of these compounds was previously described.[9]

Capacity Factors A Shimadzu LC9A liquid chromatograph equipped with a Model 7125 valve loop injector (Rheodyne), a SPD-6A UV detector (Shimadzu) and a Shodex SE-31 refractive index detector was used. A commercial Capcell pack C_{18} column (4.6 mm × 25 cm, Shiseido) was used without further treatment. Eluents consisting of HPLC-grade MeOH and 0.01 M phosphate buffer (pH 7.4) were prepared by volume. Analytes dissolved in methanol were chromatographed at 25 °C and the retention time was measured by using a C-R4A Chromatopac (Shimadzu). Capacity factors, k', were determined from the retention time of analytes, t_R, by using the relation $k'=(t_r-t_0)/t_0$, where t_0 is the retention time of methanol.

Partition Coefficients Partition coefficients necessary for analyses were taken from our previous work.[9]



Results and Discussion

The log k' values of all compounds were determined at 15, 30, 50 and 70% methanol concentrations (M15, M30, M50 and M70) (Table I). The relationships between log P and log k' for 5PM and 2PM are depicted in Figs. 1 and 2. Both figures clearly show that single correlations hardly hold when the methanol concentration becomes very low. The log k_w values (extrapolated log k' values at 0% MeOH) are generally thought to correlate better with log P than isocratic log k'.[3,11] However, the log k_w approach was not effective in the present case. In fact, the plot of log k' against the volume fraction of MeOH, from which log k_w values are calculated, was not linear, indicating that the retention mechanism changes with the change in eluent composition. Moreover, the log k' values seem to exhibit different eluent dependency according to the substituent. To compare the change in the retention behavior for each substituent with that for the pyrazine series, the substituent contribution to the capacity factor, $\kappa[=\log k'(\mathrm{X})-\log k'(\mathrm{H})]$, was derived at each mobile phase composition as was done in our previous study.[7b] The κ values are plotted against the methanol content in mobile phases in Figs. 3 and 4. The general trends are very similar to those observed for the pyrazine series.[7b] The plots for highly electron-withdrawing substituents such as halogens, CN and NO_2 exhibited curves with a maximum, while the κ values for the others increased continuously with decrease in the methanol concentration. The plots for alkyl substituents, which are non-hydrogen bondable and exert little electronic effect were almost linear. It should be noted that the plots for certain substituents intersect the plots for others. This means that the relationship between log P and log k' varies depending on the mobile phase composition.

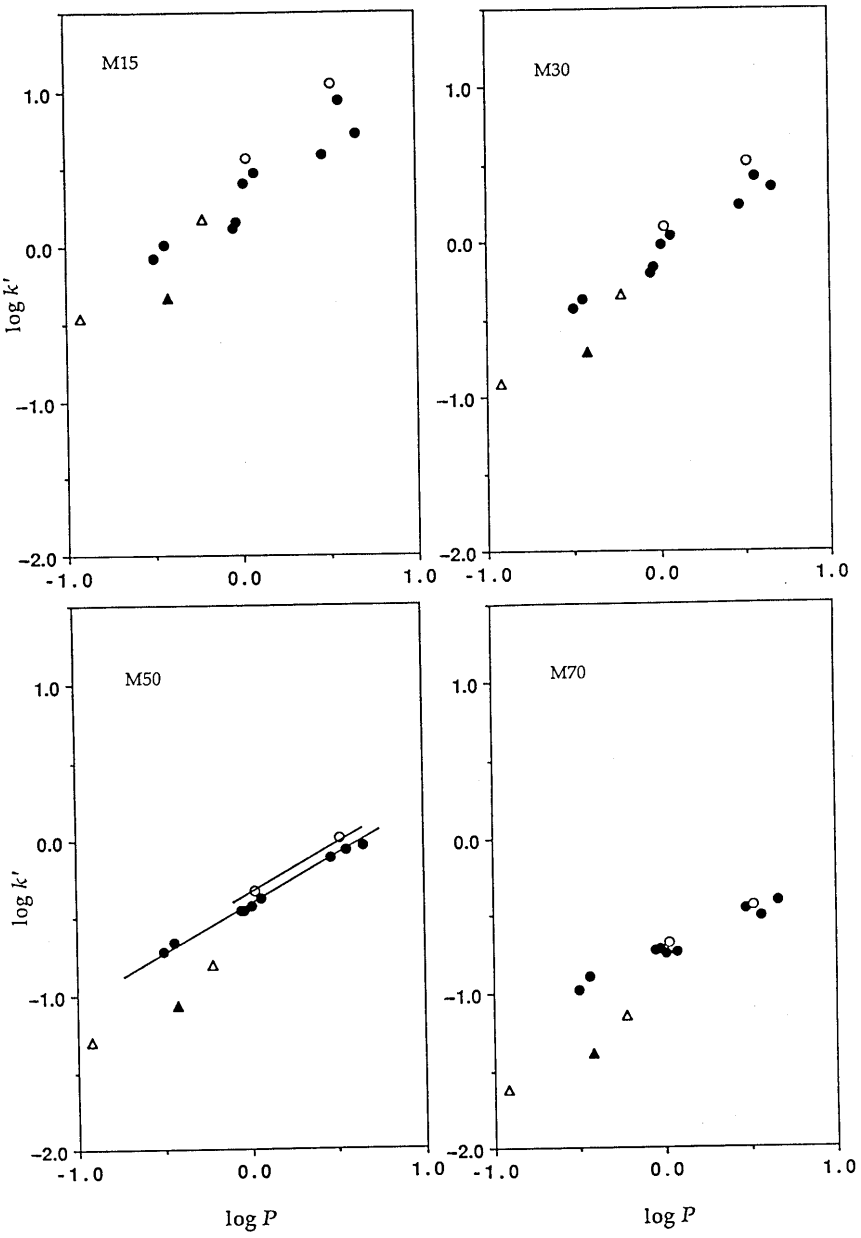


Fig. 1. Relationship between log P and log k' for 5-Substituted Pyrimidines (5PM)

Circles, nonhydrogen-bonders and H-acceptors; triangles, amphiprotics. The open symbols represent the esters and amides ($HB_{CO}=1$).

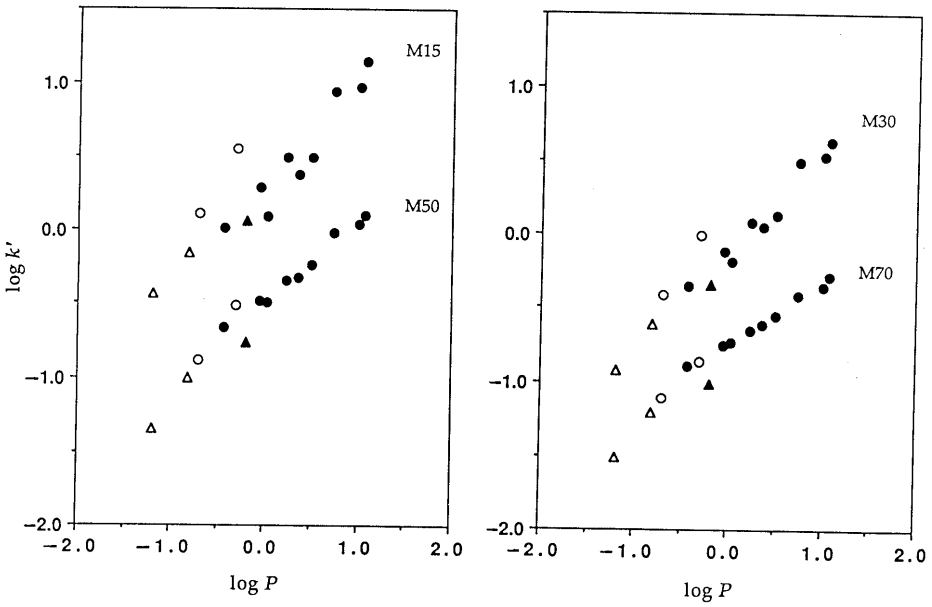


Fig. 2. Relationship between log P and log k' for 2-Substituted Pyrimidines (2PM)
For symbols, see the caption of Fig. 1.

TABLE I. log k' and log P Values of Monosubstituted Pyrimidines and Pyridazines

Series	Substituent	log P[a]	log k'			
			M15[b]	M30	M50	M70
5PM	H	−0.440	0.004	−0.363	−0.663	−0.889
	F	−0.030	0.153	−0.158	−0.456	−0.714
	Cl	0.470	0.593	0.234	−0.124	−0.459
	Br	0.660	0.729	0.348	−0.047	−0.410
	Me	0.010	0.400	−0.014	−0.426	−0.746
	OMe	0.070	0.470	0.038	−0.380	−0.741
	OEt	0.560	0.941	0.425	−0.068	−0.503
	CN	−0.500[c]	−0.079	−0.426	−0.714	−0.979
	NO_2	−0.050[c]	0.113	−0.197	−0.462	−0.728
	CO_2Me	0.030	0.574	0.096	−0.329	−0.678
	CO_2Et	0.500	1.049	0.519	0.006	−0.439
	NH_2	−0.420	−0.329	−0.706	−1.068	(−1.384)[d]
	NHAc	−0.220	0.174	−0.339	−0.800	(−1.143)
	$CONH_2$	−0.920	−0.455	−0.909	(−1.293)	(−1.617)
2PM	F	0.020	0.080	−0.191	−0.500	−0.731
	Cl	0.360	0.370	0.046	−0.328	−0.617
	Br	0.500	0.485	0.124	−0.239	−0.552
	Me	−0.050	0.282	−0.118	−0.480	−0.751
	OMe	0.230	0.485	0.077	−0.352	−0.651
	OEt	0.740	0.942	0.483	−0.026	−0.421
	SMe[e]	1.010	0.971	0.530	0.035	−0.355
	NMe_2[e]	1.070	1.146	0.629	0.094	−0.291
	CO_2Me[e]	−0.710	0.106	−0.415	−0.860	(−1.102)
	CO_2Et[e]	−0.310	0.542	−0.017	−0.516	−0.860
	NH_2[e]	−0.200	0.053	−0.350	−0.764	−1.008
	NHAc[e]	−0.810	−0.162	−0.615	−1.000	(−1.201)
	$CONH_2$[e]	−1.200	−0.435	−0.918	(−1.341)	(−1.512)
4PM	Me	−0.050	0.346	−0.103	−0.500	−0.761
	OEt	0.970	1.193	0.701	0.189	−0.225
4PD	H	−0.730	−0.256	−0.641	−0.983	(−1.134)
	Me	−0.320	0.195	−0.265	−0.721	−0.974
	OMe	−0.310	0.240	−0.224	−0.682	−0.971
	OEt	0.200	0.751	0.209	−0.330	−0.727
3PD	OMe	0.080	0.459	0.023	−0.417	−0.733
	OEt	0.630	0.945	0.448	−0.083	−0.492

a) Taken from ref. 9 unless otherwise noted. *b*) Mobile phase composition; the figure represents the volume % of MeOH. *c*) This work. *d*) The reliability of the data in parentheses is low because the retention times are too short. *e*) Not included in the analyses.

TABLE II. Parameters Used in Eq. 1

Substituent	σ_I	HB_{CO}	HB_A	HB_{AM}
H	0.00	0	0	0
F	0.54	0	0	0
Cl	0.47	0	0	0
Br	0.47	0	0	0
Me	−0.01	0	0	0
OMe	0.30	0	1	0
OEt	0.28	0	1	0
SMe	0.30	0	1	0
CN	0.57	0	1	0
NMe_2	0.17	0	1	0
NO_2	0.67	0	1	0
CO_2Me	0.32	1	0	0
CO_2Et	0.30	1	0	0
NH_2	0.17	0	0	1
NHAc	0.28	1	0	1
$CONH_2$	0.28	1	0	1

Finding similar features to those observed in pyrazines led us to analyze the log k' values by use of Eq. 1. Since amphiprotic substituents, which exhibit not only an H-acceptor effect but also an H-donor effect, behave differently from nonamphiprotics, the analyses were first done, excluding the amphiprotic substituents (NH_2, NHAc and $CONH_2$). The results obtained for 5PM by using the parameters given in Table II are summarized in Table III (Eqs. 2—5). It is apparent that Eq. 1 can describe very well the data obtained in all mobile phase compositions. The number of correction terms was increased as the methanol concentration was decreased, indicating that the H-bonding effects are enhanced in highly water-rich eluents: the HB_A and σ_I terms, which were insignificant in the M50 and M70 eluents, were found to make important contributions to the log k' values at 15 and 30% MeOH. Addition of the amphiprotic substituents to the above data set produced correlations whose coefficients were similar

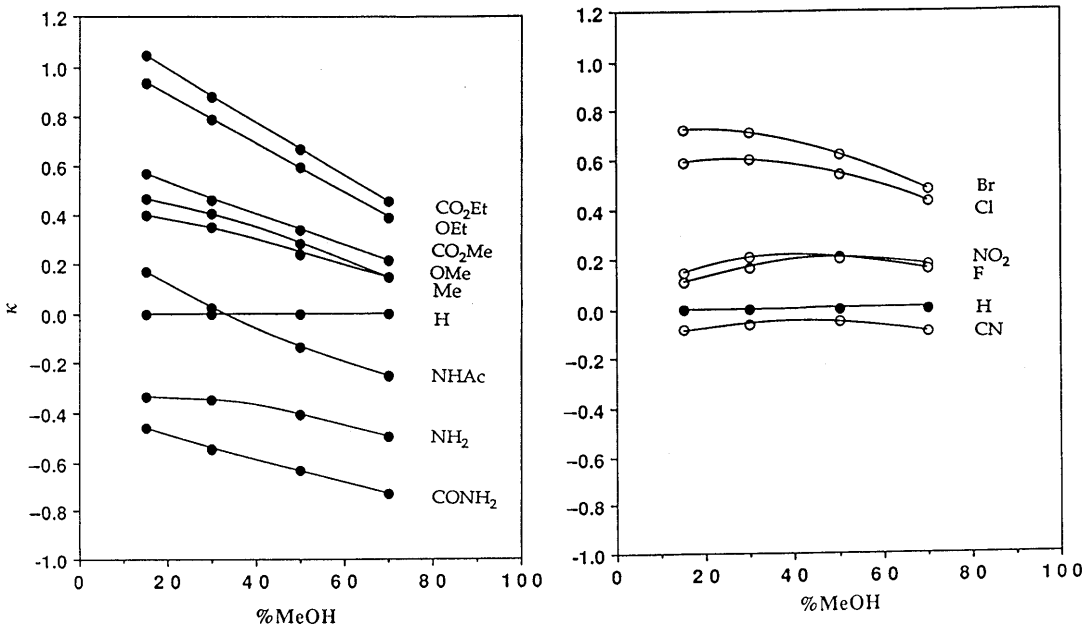


Fig. 3. Effect of Mobile-Phase Composition on κ for 5-Substituted Pyrimidines (5PM)

The % MeOH represents the volume % of MeOH in the mobile phase. Open circles, highly electron-withdrawing substituents ($\sigma_I > 0.4$).

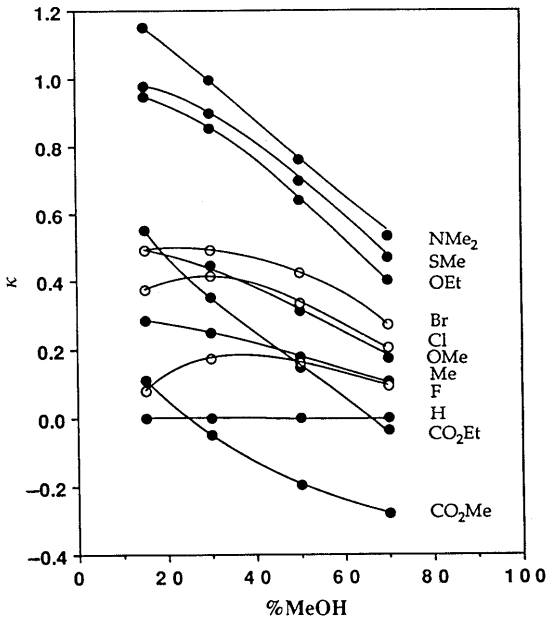


Fig. 4. Effect of Mobile-Phase Composition on κ for 2-Substituted Pyrimidines (2PM)

The % MeOH represents the volume % of MeOH in the mobile phase. For the symbols, see the caption of Fig. 3.

to the corresponding values for nonamphiprotics in Eqs. 2—5. They are also included in Table III (Eqs. 6—9).

In the case of 2PM, no reasonable correlation could fit all the substituents. This is not unexpected if we take into consideration the solute structure, where the substituent is located adjacent to the two ring-N atoms. The α-substituent may interfere with the solvation of the ring N-atoms. There is also a possibility that bulky substituents containing lone-pair electrons are unable to adopt a coplanar conformation. This would result in a change in the H-bonding ability of both the substituent and the ring-N atoms. In analyses of log P values of 2PM,[9] we have treated SMe, CO_2R, NMe_2 and amphiprotic substituents as outliers. Preanalyses by Eq. 1 for 2PM including all substituents also demonstrated that the above outliers exhibited an outlying behavior in water-rich eluents. Therefore, the analyses were done excluding these substituents. The correlations thus obtained are summarized in Table IV (Eqs. 10—13). It is a matter of course that these correlations contain neither an HB_{CO} term nor an HB_{AM} term, because the H-bondable substituents used for the analysis were limited to OR groups.

Although the number of data is insufficient, especially in 2PM, the fact that the correlation equation derived from the analyses for 2PR could work satisfactorily in the present series suggests that the H-bonding effects which hamper the log P − log k' linearity can be treated as a linear combination of indicator variables which categorize the substituent depending on the type of H-bonding (H-acceptor or H-donor) and H-bond strength. In using Eq. 1, H-acceptable substituents were classified into two groups, one of which, using the HB_{CO} parameter, contains strong H-acceptors with two H-bondable sites, and the other, using the HB_A parameter, contains weak H-acceptors.[12] This classification has also been found useful to analyze the relationship of log P values obtained in the octanol–water and $CHCl_3$–water partitioning systems in the pyrazine series.[13]

The plots of coefficients of the log P term in Eq. 1, "a," against the volume % of MeOH in eluents gave good linear relationships, yielding extrapolated values at 0% MeOH of 0.96 for 5PM and 0.92 for 2PM. The fact that these values are close to 1 indicates that the hydrophobic component was well separated from other factors. In fact, for solutes whose retentions are mostly governed by the partition mechanism, the extrapolated "a" values are expected to approach 1 while the extrapolated constant values approach 0, as observed in benzene derivatives.[3,7a]

TABLE III. Correlations for 5-Substituted Pyrimidines by Using Eq. 1

Mobile phase	Coefficients					Const.	n	r	s	F	Eq. No.
	$\log P$	HB_{CO}	σ_I	HB_A	HB_{AM}						
Compounds without amphiprotic substituents											
M15[a]	0.865	0.329	−0.474	0.160		0.399	11	0.994	0.052	127	2
M30	0.744	0.174	−0.189			−0.006	11	0.991	0.051	123	3
M50	0.601	0.101				−0.421	11	0.997	0.021	713	4
M70	0.472					−0.717	11	0.981	0.038	234	5
All compounds											
M15	0.867	0.343	−0.470	0.164	−0.261	0.394	14	0.997	0.047	241	6
M30	0.750	0.185	−0.183		−0.330	−0.010	14	0.996	0.047	254	7
M50	0.614	0.103			−0.380	−0.423	14	0.999	0.024	1132	8
M70	0.496				−0.404	−0.720	14	0.992	0.049	350	9

a) Mobile phase composition; the figure represents the volume % of MeOH.

TABLE IV. Correlations for 2-Substituted Pyrimidines by Using Eq. 1

Mobile phase	Coefficients			Const.	n	r	s	F	Eq. No.
	$\log P$	σ_I	HB_A						
M15[a]	0.799	−0.513	0.144	0.331	7[b]	0.999	0.022	422	10
M30	0.688	−0.268	0.101	−0.077	7	0.998	0.026	215	11
M50	0.530			−0.480	7	0.981	0.045	126	12
M70	0.366			−0.726	7	0.990	0.023	234	13

a) Mobile phase composition; the figure represents the volume % of MeOH. b) The compounds used for analyses are H, F, Cl, Br, Me, OMe and OEt, see the text.

It should also be noted that the "a" values for 5PM are very close to those for 2PR (0.82, 0.74, 0.62 and 0.50 for M15, M30, M50 and M70, respectively). On the other hand, the plot of the constant values against the volume % of MeOH was not linear and quadratic extrapolations to 0% MeOH yielded the values of 0.86 for 5PM and 0.83 for 2PM, presenting large deviations from zero. This means that the parent compound, pyrimidine, itself is a strong H-bond acceptor.[14]

Inspection of Tables III and IV demonstrates that the contributions of correction terms to H-bonding effects become greater with decrease in the methanol content. This was more the case in 2PM than in 5PM, probably because the intramolecular interactions between the substituent and the ring N-atoms are enhanced in 2PM. The coefficient of the HB_{CO} term was larger than that of the HB_A term for a given eluent, reflecting the relative H-bonding abilities of the substituents belonging to each HB parameter group.[12] The $\rho\sigma_I$ term expresses the electronic effect of the X substituent on the H-accepting ability of the ring N-atoms. This effect was slightly larger in 2PM than in 5PM, probably because transmission of the electronic effect of the X substituent is more effective in 2PM where the X substituent is located closer to the ring N-atoms than in 5PM. The ρ value was more negative with decrease in methanol content, indicating that the selective solute–solvent interaction becomes more significant in a more water-rich eluent. It is clear that eluents of high water content are not appropriate to predict $\log P$. So far as nonamphiprotic substituents are concerned, the M50 eluent seems to give the best correlation in the sense that eluents around this composition discriminate less between nonhydrogen bonders and H-acceptors. Although statistical treatments require the HB_{CO} term for M50, exclusion of this term still leads to a satisfactory correlation with a standard deviation of 0.046, which is better in quality than the correlation at 70% MeOH, where the deviation of each substituent from the regression line was rather high.

The coefficient of the HB_{AM} term was always negative, as is usually observed.[7a,15] In particular, amphiprotic substituents involved in heterocyclic systems exhibit stronger H-donor effects than when they are involved in benzene systems due to the electron-withdrawing property of the ring hetero atom, which makes the acidic hydrogen atom in the amphiprotic group undergo H-bonding more effectively with octanol than with less basic methanol and water: in other words, amphiprotics are more hydrophobic in the octanol–water system than in the RPLC system relative to nonamphiprotics under conditions of equivalent $\log P$.

It would be interesting to compare the correlations for 5PM and 2PM. However, as the substituents incorporated into the correlation as formulated by Eq. 1, are rather limited in 2PM, closer comparisons of the corresponding coefficients at the same level may be difficult. Considering that OR substituents fit Eq. 1 in both cases and that their H-bonding effect is usually small,[5,7,15] we next examined alkyl and alkoxy derivatives of 5PM, 2PM, 3PM, 4PD and 3PD (X=alkyl, OR) listed in Table I. At 30 and 50% MeOH concentrations, excellent single correlations between $\log k'$ and $\log P$ were obtained as shown by Eqs. 14 and 15, regardless of the parent nucleus and the substitution site.

$$\text{M30}: \quad \log k' = 0.764 \log P - 0.036$$
$$n=15, \quad r=0.993, \quad s=0.043, \quad F=971 \qquad (14)$$

$$\text{M50:}\quad \log k' = 0.652 \log P - 0.467 \qquad (15)$$
$$n=15,\quad r=0.992,\quad s=0.041,\quad F=793$$

In these equations, n is the number of compounds used for calculations, r is the correlation coefficient and s is the standard deviation. F is the value of the F ratio between the variances of observed and calculated values. At 15% and 70% MeOH concentrations, each series showed different correlations and the deviations for compounds of similar structure from the average line (the regression line) became irregular, although the apparent overall correlations were good ($r=0.98$ with M15 and M70 eluents). These trends again conform with the tentative conclusion that eluents containing around 50% MeOH are less capable of discriminating between nonhydrogen bonders and H-acceptors: the pyrimidine and pyridazine series gave single straight lines in eluents containing a medium-range methanol content in spite of the fact that both pyrimidine and pyridazine are H-acceptors, but of different H-bonding abilities, while each series produced different correlations in highly water-rich or methanol-rich eluents, which is thought to reflect the fact that pyridazine is a stronger H-acceptor than pyrimidine.[14)]

In conclusion, the relationship between $\log k'$ and $\log P$ could be successfully formulated by a general equation (Eq. 1) which has been derived from analyses of the $\log k'$ values for the pyrazine series. The relationship became more complicated as the methanol content in the mobile phase was decreased, and hence the $\log k_w$ parameter was not thought to be a useful predictor of $\log P$ values. It was found more practical to use mobile phases containing around 50% MeOH, in which the selective solute–solvent interactions expressed by the $\rho\sigma_I$ term as well as the H-acceptor effect of the substituent expressed by the HB parameters become small or almost negligible, resulting usually in good linear $\log k' - \log P$ relationships. We should be careful, however, in treating amphiprotic (H-donating) substituents, because H-donating ability is expected to be enhanced in heterocyclic systems. It can be said that the mobile phase composition giving better correlations for nonhydrogen bonders and H-acceptors may also yield better results for amphiprotics, which have both H-acceptor and H-donor abilities. Studies on amphiprotics (H-donors) are in progress.

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