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Hydrophobicity Parameters Determined by Reversed-Phase Liquid Chromatography. XII.[1] Comparison of Capacity Factors and Octane/Methanol–Water Partition Coefficients for Monosubstituted Pyrazines, and Effect of Octanol Added to both Partitioning Systems

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Partition coefficients (*P*) of pyrazine and its COOMe derivative (strong hydrogen bond acceptor), were measured for the octane/aqueous methanol partitioning system ($P_{O/M\text{-}W}$) at different methanol (MeOH) concentrations and the dependence of $\log P_{O/M\text{-}W}$ on the methanol concentration was compared to the corresponding change in $\log k'$ (k': capacity factor) obtained by reversed-phase liquid chromatography with aqueous methanol. Next, in order to make the chromatographic system approximate more closely to the octanol/water partitioning system, a small quantity of octanol was added to the eluents and also to the octane/aqueous methanol partitioning system and further comparisons were made. It was found that the octanol effect was minimum at 50% MeOH, suggesting that the chromatographic system with eluents containing about 50% MeOH has properties more similar to the octanol/water partitioning system than with eluents of other compositions. This confirms our previous result that the $\log k'$ parameter obtained at 50% MeOH yields a better correlation with $\log P_{oct}$ (P_{oct}: octanol/water partition coefficient) than those obtained at other mobile phase compositions in the range from 5 to 70% MeOH.

Key words capacity factor; partition coefficient; octanol effect; hydrophobicity; hydrogen-bond effect

Hydrophobicity is a very important factor governing biological activity. In quantitative structure–activity relationship (QSAR) studies, the logarithm of the 1-octanol/water partition coefficient, $\log P_{oct}$, measured by the shake-flask method has been widely accepted as a hydrophobicity index.[2] The ability to predict $\log P_{oct}$ values prior to syntheses is important for the purpose of computer-aided rational drug design. In particular, development of a method for predicting $\log P_{oct}$ values of heterocyclic compounds is required, because the usual additivity of π values (π refers to the increment in $\log P$ produced by replacing a hydrogen atom of a parent compound with a certain substituent) tends to be perturbed by hydrogen bond effects between solutes and solvents in most heterocyclic systems.

$\log P_{oct}$ values are also often estimated from the logarithm of the capacity factor, $\log k'$, which is obtained by reversed-phase liquid chromatography (RPLC) on alkyl-modified silicas as the stationary phase with aqueous methanol solutions as the mobile phase. If the retention mechanism is assumed to be governed by partition of solutes between the stationary and mobile phases, retention data are expected to reflect the hydrophobicity of solutes. Thus, extensive studies have been made to develop procedures for predicting $\log P_{oct}$ values from the corresponding $\log k'$ values.[3-7] Many papers have described the use of polycratic methods to derive a standard parameter, $\log k_W$ ($\log k'$ value obtained by extrapolation to 0% organic modifier), which is correlated with $\log P_{oct}$.[3,4] It is claimed that the $\log k_W$ treatment eliminates or minimizes problems such as peak inversion and the hydrogen-bond effects mentioned above, which are often encountered when $\log P$ values are estimated with the use of isocratic retention data.[3,4] Braumann demonstrated that the $\log k_W$ value is identical with $\log P_{oct}$.[3]

With the intention of establishing optimal chromatographic conditions to predict the $\log P$ value, we have been studying systematically the relationship between $\log P$ and $\log k'$ for heteroaromatic compounds. In previous studies,[7-9] we have measured $\log k'$ values of monosubstituted pyrazines under RPLC conditions with a Capcell Pak C_8 or C_{18} column as the stationary phase and various compositions of methanol–water mixtures as the mobile phase, and compared the values with $\log P_{oct}$. With substituents such as alkyls and halogens (nonhydrogen bonders), and OR, SR, CN, Ac, COOR and $CONMe_2$ (hydrogen acceptors), the best correlation between $\log P$ and $\log k'$ was obtained at around 50% MeOH concentration. As the MeOH concentration was decreased, strong hydrogen-accepting groups such as COOR and $CONMe_2$ exhibited upward deviations from a linear relationship in the plot of $\log k'$ against $\log P_{oct}$, and correction terms for hydrogen bond effects were required as shown by Eq. 1.

$$\log k' = a \log P_{oct} + \rho\sigma_I + h\,HB_{CO} + \text{const}. \qquad (1)$$

In Eq. 1, σ_I represents electronic substituent constants[10] and HB_{CO} is an indicator variable which takes the value of unity for COOR and $CONMe_2$ and 0 for the others. The finding that the retention at 50% MeOH is a better parameter than $\log k_W$ to predict $\log P_{oct}$ values was also valid for other heteroaromatic systems such as furans and thiophenes.[11,12]

To rationalize the above-mentioned features, it would be necessary to know how the eluent composition affects the retention data. Although the partition between alkyl-bonded stationary phases (hydrocarbon layer) and aqueous mobile phases is supposed to be a model of octanol–water partition, differences in properties between stationary phases and octanol, and also between aqueous methanols and pure water restrict the usefulness of

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chromatographic data as an alternative to log P. In addition, there are also factors characteristic of the chromatographic system; the structure of stationary phases is complex and not the same as that of bulk solvents. In fact, Carr and coworkers have used bulk hexadecane as a model for the bonded phase and found that the model works only for non-polar solutes, but fails for polar solutes.[13] With these facts in mind, we attempted to measure partition coefficients of some pyrazines in a partitioning system consisting of octane as an organic phase and methanol–water mixtures as an aqueous phase (log $P_{\mathrm{O/M\text{-}W}}$), which is expected to be more similar to chromatographic systems than the octanol/water partitioning system. By comparing the obtained results with log k' as a function of the mobile-phase composition, we expected to get information about the properties of the stationary-phase, especially its solvation ability. In addition, a small amount of octanol was added to both partitioning systems and its effect on log $P_{\mathrm{O/M\text{-}W}}$ as well as log k' was also investigated.

Experimental

Materials The compounds studied were the monosubstituted pyrazines used in a previous study,[7] but without amphiprotic substituents (see Table 1).

Partition Coefficients Partition coefficients for the octane/MeOH–water partitioning system, log $P_{\mathrm{O/M\text{-}W}}$, were measured by the shake-flask method with methanol contents in the aqueous phase (v/v) of 0, 15, 30, 50, 70, 90 and 100%. Measurements for different ratios of volumes between octane and aqueous methanol gave practically equivalent results. In order to examine the effect of added octanol on log $P_{\mathrm{O/M\text{-}W}}$ values, octane containing 5 or 10% octanol by volume was used as the organic phase instead of pure octane.

RPLC Procedures The apparatus and procedures to obtain log k' values were the same as previously described.[7] A Capcell Pak C_{18} column (4.6 mm × 15 cm, Shiseido) was used as the stationary phase. Aqueous methanols containing 5, 15, 30, 50 and 70% MeOH (hereafter designated as M5, M15, M30, M50 and M70, respectively) prepared by volume were used as the eluent, although methanol–buffer (pH 9.2) mixtures were used in the previous work.[7] Preliminary examination showed that the use of buffered solutions was not necessary for the compounds used in this study.[9] In order to examine the effect of added octanol on log k' values, the eluent was prepared with the use of methanol containing 0.25 or 0.50% octanol by volume. The capacity factor, k', was calculated from the retention time of each compound, t_R, and that of methanol, t_0, by using the equation, $k'=(t_R-t_0)/t_0$. All the measurements were performed at 25 °C. The log k' value was reproducible to within ± 0.005.

Results and Discussion

Relationship between log P_{oct} and log k' The log k' values were measured with unbuffered M5-M70 eluents under the same chromatographic conditions as those in the previous work,[7] and are listed in Table 1. The values were reanalyzed by using Eq. 1 so that the correlations (Table 2) could be directly compared with the results of this work, as shown later. Comparison of the present results with the previous ones[7] showed that subtle changes in stationary and mobile phase conditions in separate runs may slightly alter the ρ and constant values, whereas the coefficients of the other terms, "a" and h, are almost unchanged. The contributions of hydrogen bond effects expressed by the $\rho\sigma$ and h HB_{CO} terms are clearly increased as the methanol concentration in the mobile phase is decreased. In order to examine how the hydrogen bond effects involved in the log k' values change as a function of mobile phase composition, we were interested in comparing the chromatographic data with the log P value derived from the corresponding partitioning system consisting of bulk solvents. We therefore first examined the relationship between log P_{oct} and log P for the octane/water system (log P_{octane}) to elucidate the solvation effect of octanol. Next log P values for the octane/aqueous methanol system (log $P_{\mathrm{O/M\text{-}W}}$) were investigated. Octane was used as a model of the stationary phase. Ideally, a hydrocarbon with a chain length in the neighborhood of octadecane should be utilized since the chain length is reported to alter the structure of the stationary phase.[14] However, our choice seems to be reasonable since log k' values obtained on the C_{18} bonded column have been shown to correlate linearly with those on a Capcell Pak C_8 column.[9] The use of methanol-water mixtures as the aqueous phase was expected to reveal the hydrogen bond effects exerted by the mobile phase.

Relationship between log P_{oct} and log P_{octane} In RPLC, solutes are partitioned between the stationary phase

Table 1. log k' Values of Monosubstituted Pyrazines Determined by Using Aqueous Methanols with and without Octanol as Eluents

Substituent	M5[a]	M15	M30	M50	M70	Octanol (0.25%)[b]				Octanol (0.5%)[c]			log P_{oct}[d]
						M5	M15	M30	M50	M5	M15	M30	
1 H	0.567	0.188	−0.270	−0.518	−0.738	0.259	−0.113	−0.383	−0.577	0.189	−0.164	−0.460	−0.26
2 F	0.578	0.287	0.030	−0.314	−0.629	0.260	−0.010	−0.190	−0.401	0.163	−0.071	−0.252	0.29
3 Cl	0.947	0.635	0.300	−0.079	−0.425	0.596	0.320	0.119	−0.155	0.476	0.261	0.029	0.70
4 Me	1.040	0.559	−0.008	−0.335	−0.646	0.639	0.177	−0.132	−0.411	0.518	0.095	−0.276	0.21
5 Et	1.482	0.953	0.360	−0.079	−0.477	0.981	0.512	0.180	−0.177	0.854	0.408	0.029	0.69
6 OMe	1.207	0.822	0.424	−0.022	−0.402	0.801	0.413	0.178	−0.112	0.669	0.333	0.103	0.73
7 OEt	1.696	1.290	0.851	0.295	−0.185	1.192	0.838	0.561	0.185	1.037	0.749	0.461	1.28
8 OPr	[e]	1.770	1.316	0.628	0.045	1.676	1.289	0.976	0.503	1.498	1.195	0.865	1.84
9 SMe	1.571	1.133	0.666	0.179	−0.258	1.100	0.748	0.455	0.081	0.967	0.652	0.365	1.17
10 CN	0.462	0.127	−0.166	−0.513	−0.837	−0.027	−0.305	−0.450	−0.603	−0.163	−0.387	−0.533	−0.01
11 Ac	0.900	0.477	0.073	−0.329	−0.654	0.306	−0.038	−0.235	−0.427	0.137	−0.161	−0.299	0.20
12 CO_2Me	0.902	0.396	−0.116	−0.527	−0.845	0.202	−0.191	−0.450	−0.642	−0.023	−0.384	−0.592	−0.23
13 CO_2Et	1.418	0.861	0.307	−0.214	−0.636	0.656	0.188	−0.093	−0.349	0.421	0.006	−0.238	0.28
14 $CONMe_2$	0.678	0.138	−0.385	−0.819	−1.110	−0.029	−0.518	−0.807	−0.928	−0.245	−0.727	−0.993	−0.80

a) Eluent; the figure represents the volume% of MeOH. *b*) Methanol component contains 0.25 volume% of octanol. *c*) Methanol component contains 0.50 volume% of octanol. *d*) Taken from reference 7. *e*) The retention time was too long to be measured.

Table 2. Effect of Octanol Added to Eluents on Correlation with Eq. 1

Eluent[a]	a	ρ	h	const.	n[b]	r[c]	s[d]	Eq. No.
M5	0.784	−0.790	0.558	0.874	13	0.981	0.091	2
M5 (oct-0.25%)[e]	0.731	−0.787	0.229	0.466	13	0.995	0.046	3
M5 (oct-0.5%)[f]	0.725	−0.813	0.122	0.354	13	0.995	0.056	4
M15	0.781	−0.532	0.407	0.413	14	0.994	0.063	5
M15 (oct-0.25%)	0.738	−0.504	0.132	0.030	14	0.998	0.038	6
M15 (oct-0.50%)	0.727	−0.488		−0.048	14	0.996	0.049	7
M30	0.740		0.255	−0.135	14	0.992	0.063	8
M30 (oct-0.25%)	0.672			−0.312	14	0.992	0.063	9
M30 (oct-0.50%)	0.688			−0.427	14	0.994	0.054	10
M50	0.544			−0.426	14	0.994	0.045	11
M50 (oct-0.25%)	0.536			−0.520	14	0.995	0.040	12
M70	0.427			−0.743	14	0.989	0.046	13

a) Eluents are the same as given in Table 1. For symbols, see the footnote of Table 1. *b*) Number of compounds. *c*) Correlation coefficient. *d*) Standard deviation from regression. *e*) Methanol component contains 0.25 volume% of octanol. *f*) Methanol component contains 0.50 volume% of octanol.

(hydrocarbon-like) and the eluent (aqueous methanol). Since the bonded hydrocarbon moiety has no hydrogen-bonding ability, using the chromatographic method to predict the $\log P_{\mathrm{oct}}$ value will require correction primarily for hydrogen bondings between solutes and octanol. Hydrogen-bonding effects exerted by octanol could be estimated by comparing $\log P_{\mathrm{oct}}$ values with $\log P_{\mathrm{octane}}$. As shown in Fig. 1, where $\log P_{\mathrm{oct}}$ values are plotted against $\log P_{\mathrm{octane}}$, an almost linear relationship was found except for COOR and $\mathrm{CONMe_2}$ (represented by open circles). The $\log P_{\mathrm{oct}}$ value for all the compounds tested was greater than that of $\log P_{\mathrm{octane}}$, and both were observed to approach each other as the hydrophobicity of the molecule was increased. This means that molecules with hydrophilic substituents are more stabilized in octanol than in octane by hydrogen bonding, to a greater extent than those with hydrophobic substituents. In particular, substituents such as COOR and $\mathrm{CONMe_2}$, which cause upward deviations form the average straight line drawn through the other points, are considered to show more effective hydrogen bonding with octanol than the others. This feature is in good agreement with the results of correlation analyses by using Eq. 1, where the HB_{CO} value of 1 was required only for these compounds, indicating that solutes of strong hydrogen-bonding ability with octanol are deviants in terms of the $\log k' - \log P_{\mathrm{oct}}$ relationship.

Interestingly, the correlation of $\log k'$ with $\log P_{\mathrm{oct}}$ was much better than with $\log P_{\mathrm{octane}}$; for example, while plots of $\log k'_{\mathrm{M50}}$ values against $\log P_{\mathrm{oct}}$ gave a nearly linear relationship, those of $\log k'_{\mathrm{M50}}$ against $\log P_{\mathrm{octane}}$ were more scattered (not shown). This means that the structure of the stationary phase, which is a dynamic solvated layer, is too complex to be described using bulk octane as a model. The difference may be attributed to the fact that the stationary phase is more polar than the bulk solvent due to the enhanced concentration of imbedded organic solvent from the mobile phase.[13,15,16] We therefore thought it useful to study how the partition between octane and methanol–water mixtures, $\log P_{\mathrm{O/M\text{-}W}}$, is affected with the methanol concentration in comparison with the corresponding change in $\log k'$, since many investigations have shown that a change in the mobile phase composition causes a conformational change of the bonded stationary phase.[13,17] The results will be described below.

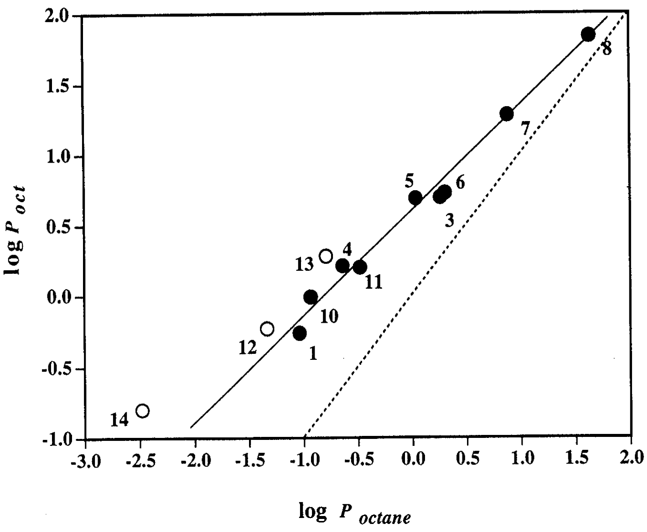

Fig. 1. Relationship between Octane–Water and Octanol–Water Partition Coefficients of Monosubstituted Pyrazines

○, Strong hydrogen-accepting substituents ($HB_{\mathrm{CO}}=1$); ●, others ($HB_{\mathrm{CO}}=0$). The dotted line represents a straight line for $\log P_{\mathrm{oct}} = \log P_{\mathrm{octane}}$. $\log P_{\mathrm{octane}}$ values were taken from reference 18.

Effect of Methanol Concentration on $\log P_{\mathrm{O/M\text{-}W}}$ The $\log P_{\mathrm{O/M\text{-}W}}$ values were measured for pyrazines with substituents H and COOMe, nonhydrogen-bonding and strongly hydrogen-accepting groups, respectively, at various methanol contents in the aqueous phase. The results are shown in Fig. 2. The $\log P_{\mathrm{O/M\text{-}W}}$ value is always negative and decreases almost linearly as the methanol concentration is increased up to about 70%. This trend indicates that the solutes are more stabilized in the aqueous phases than in octane due to the strong hydrogen-bonding ability of the hydrophilic solvents and also more stabilized in aqueous solutions of higher methanol content. With a methanol content above 70%, the $\log P_{\mathrm{O/M\text{-}W}}$ value increased with increase in methanol concentration. Preliminary measurements of the aqueous phase content in the octane phase and the octane content in the aqueous phase after the establishment of partition equilibrium showed that the penetration into the other phase is almost negligible at methanol concentrations less than 70%.

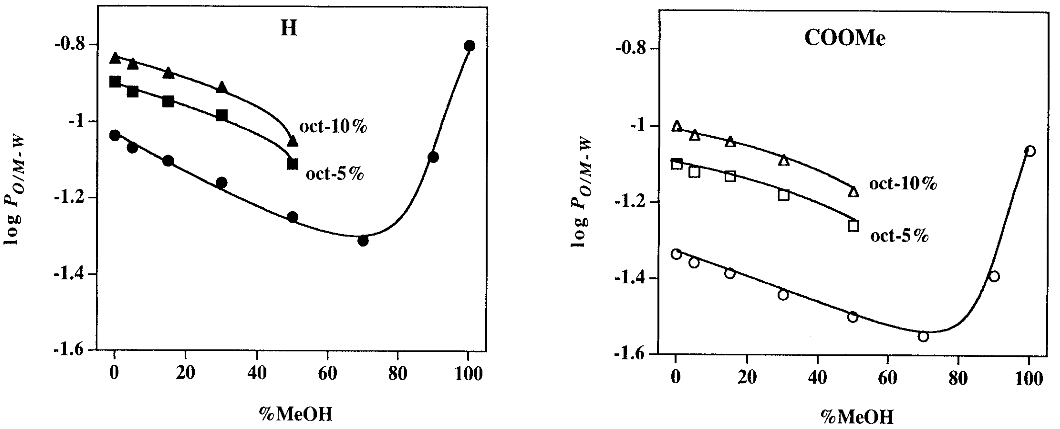

Fig. 2. Dependence of $\log P_{O/M\text{-}W}$ on the Aqueous Phase Composition for Pyrazine and Its Ester Derivative, and the Effect of Octanol Added to the Partitioning Systems

Circles, without octanol; squares, partitioned with octane containing 5.0 volume% of octanol; triangles, partitioned with octane containing 10.0 volume% of octanol.

However, at 90% MeOH, about 35% octane was detected in the aqueous phase, whereas no detectable amount of methanol or water was observed in the octane phase. Between pure methanol and octane phases, about 40% octane was observed to pass into the methanol phase. As the content of octane is increased in the aqueous phase, the extent of hydrogen-bonding associations between the solute and surrounding solvents would be decreased, which would raise $\log P_{O/M\text{-}W}$. This explains why the $\log P_{O/M\text{-}W}$ value increased with increasing methanol content past 70%. These results demonstrate that the $\log P_{O/M\text{-}W}$ parameter derived with aqueous phases containing over 70% MeOH can no longer be regarded as a model of the chromatographic partition ($\log k'$). Therefore, the following discussion will be limited to the range from 0 to 70% methanol concentration.

As shown in Table 1, the $\log k'$ value of all the pyrazines examined was decreased as the methanol concentration increased from 5 to 70%, but not linearly (not shown), while an approximately linear relationship was observed between $\log P_{O/M\text{-}W}$ and %MeOH. These results suggest that the $\log k'$ parameter is governed mainly by the same factor as that controlling the partition between the corresponding bulk solvents, but is also affected by additional interactions involved in the retention process in RPLC. The difference between the two parameters can be seen in Fig. 3 where $\log k'$ values at various methanol concentrations are plotted against the corresponding $P_{O/M\text{-}W}$ values. It should be noted that the compounds, H and COOMe, present two different curves, indicating that their relative hydrophobicity changes depending on the mobile phase composition. The COOMe derivative is always more hydrophilic than H on the $P_{O/M\text{-}W}$ scale. However, the same compound is regarded as being more hydrophobic than H on the basis of the $\log k'$ scale with M5, M15 and M30 eluents, provided $\log k'$ is linearly correlated with $\log P_{O/M\text{-}W}$. By extrapolating such a tendency to 0% MeOH, it can be expected that the $\log k_W$ treatment will fail even to predict $\log P_{O/M\text{-}W}$ values correctly. From this reasoning, the fact that the $\log k_W$ treatment resulted in a poor correlation with $\log P_{oct}$ can also be understood. All these results suggest that the choice of eluent composition is very important for estimation of the $\log P$ value by RPLC.

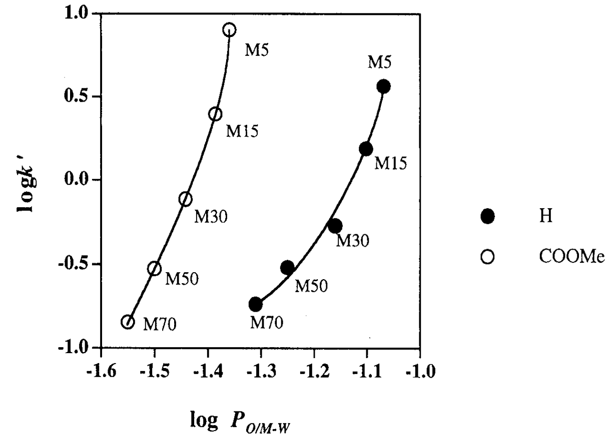

Fig 3. Relationship between $\log k'$ at Various Methanol Concentrations and the Corresponding $\log P_{O/M\text{-}W}$ Values

Figures after M represent the volume% of MeOH in aqueous phases or in eluents. $\log k'$ values were taken from Table 1. $\log P_{O/M\text{-}W}$ values are the same as those in Fig. 2.

Addition of Octanol to Partitioning Systems Minick and coworkers have studied in detail the RPLC conditions suitable to optimize the correlation between $\log P_{oct}$ and $\log k'$ and found that the hydrogen bond effect involved in the correlation is minimized by adding traces of *n*-decylamine (a masking agent) and 1-octanol to the eluent.[4] Since inclusion of octanol in the eluent is expected to make the RPLC system more similar to the octanol/water partitioning system, we were interested in examining the effect of octanol on the correlations formulated by Eq. 1. $\log k'$ values obtained with eluents containing 0.25 and 0.50% octanol in methanol (Table 1) were analyzed by Eq. 1, yielding excellent correlation equations as given in Table 2. No masking agent was used, because our earlier work showed that addition of triethylamine to the eluent did not have any significant influence on the retention behavior of the compounds used in this work.[7] It can be clearly seen that addition of octanol makes all the solutes less retentive, that is, $\log k'$ values become smaller. This acceleration effect was more pronounced with decreasing methanol concentration, as shown in Fig. 4, where the $\log k'$ value with eluents containing 0.25% octanol is

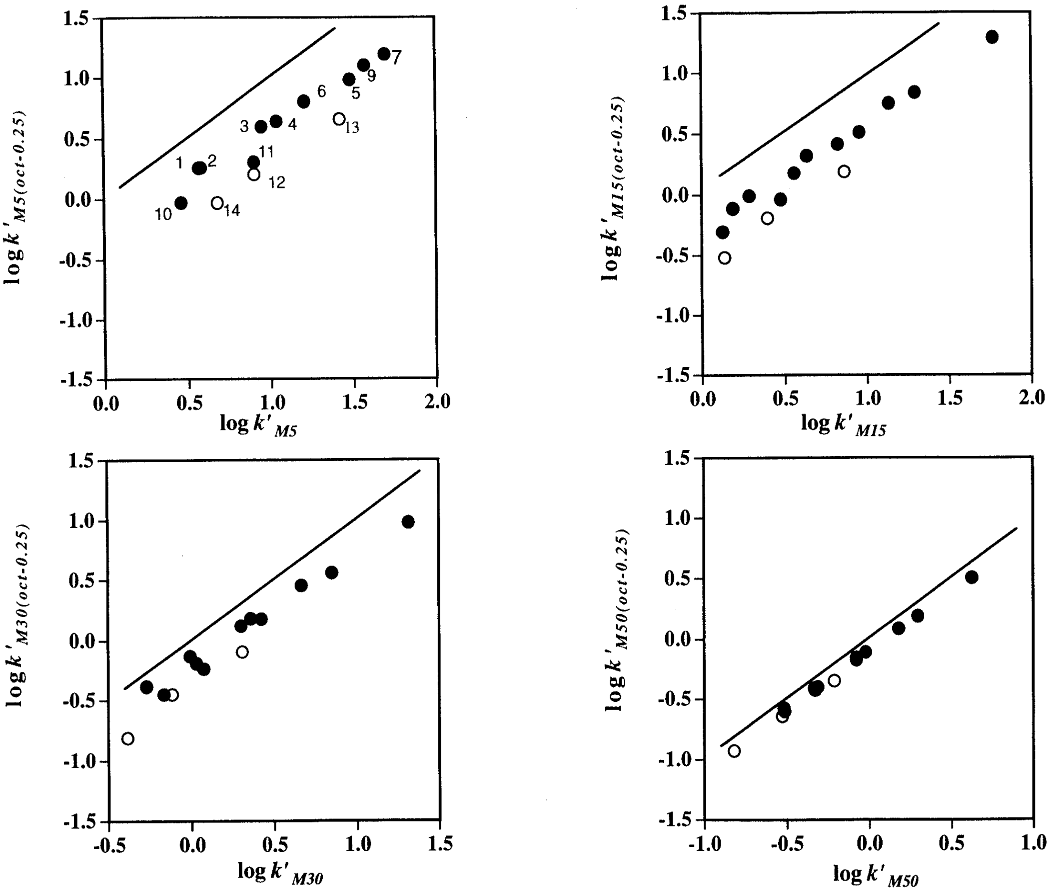

Fig. 4. log k' Values with Eluents Containing 0.25% Octanol in MeOH and log k' Values without Octanol

○, Strong hydrogen-accepting substituents ($HB_{\mathrm{CO}}=1$); ●, others ($HB_{\mathrm{CO}}=0$). Solid lines represent the straight line $y=x$.

plotted against that without octanol. With eluents containing 0.50% octanol, the acceleration effect was more enhanced (not shown). It should be noted that the strong hydrogen acceptors **12**—**14** underwent greater acceleration effect than the others. This is expressed in Table 2 by the reduced contribution of the HB_{CO} term on addition of octanol; the h value decreased or became null in going from the eluent without octanol to that containing 0.5% octanol, when the methanol content is less than 50%. On the other hand, the "a" value was decreased only slightly with the same change in octanol content and the ρ value was almost unchanged. On the other hand, inclusion of octanol in the M50 eluent had little influence on the correlation. The trends are clearly shown in Fig. 4: the points for both strong hydrogen-acceptors (open circles) and others (closed circles) form a straight line approaching the line $y=x$ in the case of M50.

In order to examine whether this octanol effect is characteristic of chromatographic partition or not, we measured $\log P_{\mathrm{O/M\text{-}W}}$ values using octane containing 5% or 10% octanol (v/v). The octanol % was chosen so as to afford a significant difference in a batchwise equilibrium system. The results are depicted in Fig. 2. Addition of octanol was seen to raise the $\log P_{\mathrm{O/M\text{-}W}}$ value for both compounds, but the effect was greater for the COOMe derivative. Although octanol itself is partitioned between the organic and aqueous phases, preliminary tests showed that a large portion of octanol exists in the organic (octane) phase when the aqueous phase is in the range from M0 to M50. Therefore an enhanced octanol effect in the case of COOMe is considered to reflect the fact that octanol contained in the octane phase interacts with the ester (COOMe) more strongly than with the unsubstituted (H) derivative. In Fig. 2, the plots for the octane containing 5% octanol (squares) and 10% octanol (triangles) are nearly parallel to those for pure octane (circles), indicating that the octanol effect is observed to a comparable extent regardless of %MeOH. This is in sharp contrast to the octanol effect on log k' where the effect decreases with an increase in %MeOH to reach a minimum at 50% MeOH. This discrepancy suggests the complexity of the retention process in RPLC, which can not be modeled by the corresponding bulk solvent partition. The stationary phase is known to be enriched in the organic solvent component.[13,16)] Therefore the dynamic solvated stationary phase is thought to have an alcohol-like nature whose properties vary with the mobile phase composition. The finding that the octanol effect on log k' was minimum at 50% MeOH, allows us to conclude that an RPLC system with the M50 eluent most closely resembles the octanol-water partitioning system. The present results seem to support our conclusion derived from previous studies that hydrogen bond acceptors are barely discernible from non-hydrogen-bonders at around 50% MeOH, and therefore a good linear relationship between $\log P_{\mathrm{oct}}$ and log k' exists with such eluents.

References

1) Part XI: Yamagami C., Katashiba N., *Chem. Pharm. Bull.*, **44**, 1338—1343 (1996).
2) Hansch C., Fujita T., *J. Am. Chem. Soc.*, **86**, 1616—1626 (1964).
3) Braumann T., *J. Chromatogr.*, **373**, 191—225 (1986).
4) Minick D. J., Frenz J. H., Patrick M. A., Brent D. A., *J. Med. Chem.*, **31**, 1923—1933 (1988).
5) Terada H., *Quant. Struct.-Act. Relat.*, **5**, 81—88 (1986).
6) Lambert W. J., *J. Chromatogr. A.*, **514**, 469—484 (1993) and references cited therein.
7) Yamagami C., Ogura T., Takao N., *J. Chromatogr.*, **514**, 123—136 (1990).
8) Yamagami C., Takao N., *Chem. Express*, **7**, 385—388 (1992).
9) Yamagami C., Takao N., *Chem. Pharm. Bull.*, **39**, 2924—2929 (1991).
10) Charton M., *Prog. Phys. Org. Chem.*, **13**, 119—251 (1981).
11) Yamagami C., Takao N., *Chem. Pharm. Bull.*, **40**, 925—929 (1992).
12) Yamagami C., Masaki Y., *Chem. Pharm. Bull.*, **43**, 2238—2242 (1995).
13) Carr P. W., Li J., Dallas A. J., Eikens D. I., Tan L. C., *J. Chromatogr. A*, **656**, 113—133 (1993).
14) Sentell K. B., *J. Chromatogr.*, **656**, 231—263 (1993).
15) Stahlberg J., Almgren M., *Anal. Chem.*, **57**, 817—821 (1985).
16) Schunk T. C., Burke M. F., *J. Chromatogr. A*, **656**, 289—316 (1993).
17) McNally M. E., Rogers L. B., *J. Chromatogr.*, **331**, 23—32 (1985).
18) Yamagami C., Takao N., Fujita T., *J. Pharm. Sci.*, **82**, 155—161 (1993).