Hydrophobicity Parameters Determined by Reversed-Phase Liquid Chromatography. III.¹⁾ Influence of Stationary and Mobile Phases on the Relationship between the Capacity Factor and the Octanol–Water Partition Coefficient for Pyrazine Derivatives

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Our previous study on the relationship between the logarithm of 1-octanol/water partition coefficient ($\log P$) by the shake-flask method and the logarithm of the capacity factor ($\log k'$) by reversed-phase liquid chromatography for monosubstituted pyrazines (2PR) has been extended to the 2-chloro-6-substituted pyrazine series (6PR) and also to other reversed-phase high-performance liquid chromatographic conditions. Analyses have shown that 6PR behave similarly to 2PR. The influences of stationary and mobile phases were studied using several commercially available reversed-phase columns, and various organic modifiers. The results, from any combination of the stationary phases and the modifiers examined, have presented analogous trends to those previously observed: (1) The $\log P$ – $\log k'$ relationship becomes more complicated as the concentration of organic modifier in the mobile phase decreases due to the intervention of the electronic interactions and the retardation effect ascribed to ester and amide substituents. (2) Amphiprotic substituents usually exhibit an acceleration effect and should be treated separately. Practical conditions to be used for predicting the $\log P$ values are proposed.

Keywords reversed-phase HPLC (RPLC); capacity factor; partition coefficient; hydrophobicity; amphiprotic substituent; mobile phase; hydrogen-bond; pyrazine

The hydrophobic property of bioactive compounds, expressed by the logarithm of 1-octanol/water partition coefficient ($\log P$) is one of the most important factors to be considered in quantitative structure–activity relationship (QSAR) studies.^{2,3)} The logarithm of capacity factor ($\log k'$), measured by reversed-phase high-performance liquid chromatography (RPLC) has increasingly been used for predicting the $\log P$ value as a counterpart of the standard and conventional shake-flask method.^{4–8)} The RPLC techniques are considered especially useful for estimation of $\log P$ values of highly lipophilic compounds.

Generally, $\log k'$ values for a series of congeners eluted by methanol (MeOH)–water eluents on an alkyl-bonded stationary phase are considered to correlate linearly with $\log P$. However, the linearity is often perturbed by other factors such as hydrogen-bond effects and selective solute–solvent interactions. At the present stage, most successful examples are found in benzene derivatives,^{6–9)} and little has been studied so far on the relationship between $\log P$ and $\log k'$ for heterocyclic compounds where the important hydrogen-bond effects are expected to be involved. One of the reasons for such paucity is the fact that accumulation of fundamental heterocyclic $\log P$ data has been too meager, except for the pyridines,^{10,11)} to enable systematic studies in this field. Considering that a number of bioactive compounds have various types of heterocyclic rings as the parent skeleton, we started to measure and evaluate the hydrophobicity parameters in various heteroaromatic systems.

In previous works,^{12,13)} we measured the $\log k'$ value for a series of monosubstituted pyrazines (2PR), using the Capcell pak C₁₈ column and different compositions of MeOH–buffer (pH 9.2) eluents, and analyzed the relationship with our experimentally determined $\log P$ values. It was demonstrated that the $\log k'$ value was formulated by the following general equation in the range from 15 to 70 percent of MeOH (% MeOH).

$$\log k' = a \log P + bHB_{CO} + cHB_{AM} + \rho\sigma_1 + \text{const.} \quad (1)$$

where HB_{CO} = 1 for ester and amide substituents and 0 for the others, HB_{AM} = 1 for amphiprotic substituents and 0 for the others, and σ_1 is Charton's electronic constant.¹⁴⁾ The HB_{CO} and σ_1 terms were insignificant or of small significance around 50% MeOH; the plot of $\log k'$ against $\log P$ presented parallel lines belonging to amphiprotics and non-amphiprotics. As the MeOH concentration in the eluents was lowered, the b and ρ values became more positive and more negative, respectively, indicating that the relationship became more complicated. Equation 1 turned out to be insufficient for describing $\log k'$ at 5% MeOH.¹⁾ Analyses with the conventionally used $\log k'_w$ parameter (the $\log k'$ value in 100% water) were also attempted. However, neither the experimentally determined nor the extrapolated value mimicked $\log P$.^{13,15)}

The complicated behavior of the pyrazine derivatives was ascribed to the electron-withdrawing property of the pyrazine ring which affected the over-all hydrogen bonding effect of the molecule.

To establish a RPLC system which could predict reliable $\log P$ values of polar solutes like ours, it would be useful to study the influence of stationary and mobile phases on the relationship between $\log P$ and $\log k'$. In this work we measured the capacity factors for 2PR using alkyl- and phenyl-modified columns as the stationary phase and three different types of solvents as the organic modifier in the mobile phase. The results were analyzed by an analogous treatment. Studies were also extended to the analysis of $\log k'$ values for the 2-chloro-6-substituted pyrazine (6PR) series with the intention of exploring whether Eq. 1 would apply to a disubstituted pyrazines system.

Experimental

Compounds The compounds tested in this work are 2PR and 6PR shown in Table I. The preparation of the compounds has been described elsewhere.^{12,16)}

Partition Coefficients 1-Octanol/water $\log P$ values, measured by the shake-flask method, were taken from our previous work.^{12,16)}

RPLC Procedure The apparatus used was the same as previously described.¹³⁾ Commercial capcell pak¹⁷⁾ C₁₈, C₈ and phenyl columns

TABLE I. $\log k'$ Values for 2PR and 6PR

2PR X	C8 ^{a)}			Ph(CP) ^{b,c)}				Ph(CS) ^{d,e)}			C18 ^{b)}						
	M15	M30	M50	M15	M30	M50	M70	M30	M50	M70	A5	A15	A30	D5	D10	P5	P15
H	0.007	-0.322	-0.648	-0.033	-0.293	-0.602	-0.791	-0.157	-0.478	-0.759	0.006	-0.466	-0.761	-0.303	-0.517	-0.059	-0.457
F	0.158	-0.119	-0.432	0.074	-0.124	-0.404	-0.670	-0.078	-0.355	-0.703	0.359	0.060	-0.172	0.159	0.033	0.012	-0.311
Cl	0.474	0.158	-0.201	0.368	0.116	-0.222	-0.547	0.164	-0.178	-0.554	0.739	0.368	0.056	0.564	0.402	0.413	0.049
Me	0.342	-0.071	-0.496	0.299	-0.034	-0.431	-0.724	0.046	-0.331	-0.686	0.451	-0.133	-0.520	0.156	-0.093	0.369	-0.130
Et	0.732	0.269	-0.224	0.655	0.275	-0.193	-0.595	0.323	-0.127	-0.553	0.934	0.323	-0.130	0.660	0.402	0.733	0.167
OMe	0.662	0.290	-0.140	0.580	0.291	-0.138	-0.490	0.277	-0.108	-0.506	0.860	0.368	-0.020	0.656	0.453	0.579	0.129
OEt	1.138	0.700	0.181	1.007	0.645	0.127	-0.326	0.596	0.127	-0.350	1.377	0.821	0.340	1.165	0.933	1.035	0.529
OPr	1.641	1.149	0.519	1.478	1.038	0.399	-0.147	0.944	0.374	-0.188	1.948	1.330	0.734	1.727	1.466	1.554	0.992
SMe	0.958	0.538	0.041	0.875	0.524	0.038	-0.396	0.532	0.078	-0.363	1.229	0.699	0.241	1.043	0.816	0.905	0.409
CN	0.023	-0.276	-0.595	0.053	-0.162	-0.466	-0.732	-0.050	-0.349	-0.715	0.378	0.065	-0.169	0.120	-0.021	-0.253	-0.640
Ac	0.350	-0.035	-0.430	0.324	0.030	-0.331	-0.643	0.146	-0.240	-0.613	0.652	0.118	-0.247	0.368	0.143	0.103	-0.392
CO ₂ Me	0.256	-0.193	-0.634	0.264	-0.078	-0.482	-0.753	0.061	-0.337	-0.703	0.572	-0.094	-0.526	0.216	-0.090	-0.032	-0.603
CO ₂ Et	0.720	0.210	-0.312	0.652	0.257	-0.223	-0.607	0.358	-0.116	-0.552	1.072	0.344	-0.168	0.713	0.391	0.433	-0.248
CONH ₂	-0.235	-0.670	-1.126	-0.166	-0.488	-0.880	—	-0.306	-0.707	-1.128	-0.041	-0.725	-1.383	-0.348	-0.649	-0.650	-1.460
CONMe ₂	—	-0.470	-0.923	—	—	—	—	—	—	—	—	—	—	-0.189	-0.559	—	—
NH ₂	-0.110	-0.477	-0.902	-0.085	-0.385	-0.757	-0.988	-0.262	-0.649	-1.068	-0.084	-0.674	-1.201	-0.338	-0.582	—	—
NHMe	0.391	-0.067	-0.551	0.353	-0.029	-0.460	-0.801	0.085	-0.378	-0.806	0.513	-0.143	-0.639	0.264	-0.014	—	—
NMe ₂	0.947	0.422	-0.145	0.906	0.456	-0.087	-0.481	0.511	-0.010	-0.467	1.165	0.464	-0.082	0.894	0.584	—	0.280
NHAc	0.121	-0.326	-0.786	0.130	-0.213	-0.635	-0.903	-0.097	-0.516	-0.928	0.348	-0.379	-0.855	0.031	-0.279	-0.167	-0.874

2PR X	C8 ^{d)}		Ph(CP) ^{b)}								log P ^{g)}	
	TMG5	TMG10	A5	A15	A30	D5	D10	D15	P5	P15	TMG5	TMG10
H	0.180	0.015	-0.178	-0.509	-0.800	-0.323	-0.484	-0.598	-0.210	-0.499	0.067	-0.040
F	0.181	0.070	-0.071	-0.260	-0.437	-0.167	-0.276	-0.358	-0.232	-0.431	0.043	-0.052
Cl	0.478	0.303	0.233	-0.030	-0.284	0.148	0.011	-0.089	0.103	-0.124	0.324	0.224
Me	0.500	0.237	0.158	-0.248	-0.611	0.029	-0.165	-0.310	0.131	-0.222	0.427	0.218
Et	0.762	0.497	0.492	0.046	-0.358	0.389	0.150	-0.010	0.415	0.036	0.691	0.519
OMe	0.640	0.457	0.374	0.013	-0.329	0.300	0.131	-0.002	0.277	-0.019	0.510	0.401
OEt	1.010	0.869	0.774	0.339	-0.072	0.703	0.501	0.342	0.655	0.326	0.864	0.740
OPr	1.434	1.307	1.244	0.718	0.212	1.164	0.910	0.723	1.090	0.728	1.264	1.124
SMe	0.920	0.778	0.686	0.271	-0.122	0.623	0.425	0.271	0.575	0.239	0.797	0.662
CN	-0.076	-0.157	-0.028	-0.205	-0.399	-0.152	-0.256	-0.339	-0.326	-0.571	-0.054	-0.159
Ac	0.112	0.013	0.188	-0.169	-0.483	0.063	-0.118	-0.248	-0.052	-0.399	0.160	0.026
CO ₂ Me	0.001	-0.140	0.150	-0.262	-0.653	-0.006	-0.222	-0.384	-0.095	-0.534	0.147	-0.007
CO ₂ Et	0.378	0.222	0.514	0.042	-0.389	0.359	0.115	-0.064	0.241	-0.216	0.467	0.294
CONH ₂	-0.457	-0.572	-0.273	-0.709	-1.057	-0.387	-0.587	-0.753	-0.501	-0.973	-0.225	-0.384
CONMe ₂	-0.355	-0.500	—	—	—	—	—	—	—	—	—	—
NH ₂	-0.054	-0.216	-0.191	-0.567	-0.949	-0.313	-0.483	-0.620	-0.235	-0.547	-0.041	-0.175
NHMe	0.505	0.301	0.241	-0.199	-0.636	0.156	-0.067	-0.238	0.218	-0.165	0.441	0.265
NMe ₂	0.968	0.675	0.730	0.205	-0.272	0.630	0.375	0.174	0.660	0.190	0.909	0.684
NHAc	-0.054	-0.298	0.019	-0.434	-0.881	-0.120	-0.340	-0.508	-0.176	-0.618	0.048	-0.117

6PR X	C18 ^{d)}				Ph(CS) ^{d)}			log P ^{h)}	log k _w ⁱ⁾
	M15	M30	M50	M70	M30	M50	M70		
H	0.603	0.311	-0.106	-0.477	0.164	-0.178	-0.554	0.70	0.87 (-0.999)
F	0.924	0.636	0.265	-0.182	0.335	0.001	-0.415	1.16	1.24 (-0.999)
Cl	1.236	0.927	0.495	0.012	0.533	0.153	-0.293	1.53	1.58 (-0.999)
Me	0.978	0.579	0.120	-0.302	0.377	-0.036	-0.443	1.04	1.30 (-0.999)
OMe	1.479	1.100	0.582	0.049	0.729	0.259	-0.229	1.65	1.88 (-1.000)
OEt	1.974	1.535	0.919	0.270	1.052	0.487	-0.089	2.22	2.45 (-1.000)
OPr	—	2.045	1.278	0.515	1.421	0.730	0.062	2.71	3.19 ^{j)} (-1.000)
CN	0.770	0.448	0.025	-0.455	0.343	-0.025	-0.448	0.79	1.11 (-1.000)
CO ₂ Me	0.859	0.383	-0.093	-0.525	0.391	-0.039	-0.453	0.47	1.16 ^{k)} (-0.994)
CONH ₂	0.478	0.052	-0.414	-0.885	0.089	-0.308	-0.715	0.28	0.82 (-0.998)
NH ₂	0.648	0.264	-0.224	-0.710	0.159	-0.268	-0.729	0.95	1.01 (-1.000)
NMe ₂	1.857	1.290	0.626	0.005	0.975	0.381	-0.174	1.95	2.33 (-0.999)
NHAc	0.933	0.484	-0.029	-0.520	0.385	-0.086	-0.534	1.10	1.30 (-0.999)

a) Aqueous phase: buffer (pH 9.2). b) Aqueous phase: buffer (pH 7.4). c) Capcell Pak Phenyl. d) Aqueous phase: water. e) Cosmosil Phenyl. f) Organic modifier, M: methanol, A: acetonitrile D: dioxane, P: 1-propanol, TMG: trimethylene glycol, the figure represents the volume % of organic modifier. g) Taken from ref. 12. h) Taken from ref. 16. i) Derived from the linear extrapolation using the data on C18 column for M15—M70. Figures in parentheses are correlation coefficients. j) Derived from the linear extrapolation using the data for M30—M70. k) Not exactly linear in the range of M15 to M70. The log k_w value derived from the linear extrapolation using the data for M30—M70 is 1.01 (-1.000).

(4.6 mm $\times$ 15 cm, Shiseido) [C18, C8 and Ph(CP), respectively] and a Cosmosil 5Ph packed column (4.6 mm $\times$ 15 cm, Nakalai tesque) [Ph(CS)] were used without further treatment. Commercial high performance liquid chromatographic (HPLC) grade MeOH, acetonitrile, dioxane and 1-propanol were used without further purification. As an aqueous buffer, 0.01 M sodium borate (pH 9.2) or phosphate buffer (pH 7.4) was used. Eluents were prepared by volume. Appropriate quantities of samples were dissolved in MeOH and 1–2 μ l was injected at 25°C. The flow-rate was 0.2–1.5 ml/min. The capacity factor ($\log k'$) was obtained as previously.¹³⁾

In the beginning of this series of studies, the buffer solutions of pH 9.2 were used because of the necessity of measuring stronger bases (pyridines) simultaneously.¹³⁾ Since preliminary examination showed that the eluents at pH 7.4 and 9.2 gave practically equivalent retention data for the pyrazine series, measurements were done afterward at pH 7.4. Measurements for 6PR were done using a MeOH–water mobile phase system because it was shown that the use of buffered solutions was not needed for the compounds used.

Results and Discussion

To examine the effects of stationary phases on the retention data, the series of 2PR was chromatographed on three different stationary phases, C8, Ph(CP) and Ph(CS), with eluents containing 15 to 70% MeOH. The $\log k'$ values obtained are listed in Table I with all the other $\log k'$ values obtained in this study. As demonstrated in our previous work,¹³⁾ the possibilities of partial ionization of the solutes and the silanol–solute interactions could be excluded under the conditions we used.

Preliminary analyses of the retention data using Eq. 1 had suggested that the H-acceptors other than ester and amide groups also exerted small but significant hydrogen-bond effects in phenyl-columns. Such an effect usually became involved at very high water contents even on the C18 column. Therefore, the general formula to be investigated was as follows.

$$\log k' = a \log P + bHB_{CO} + cHB_{AM} + \rho\sigma_1 + dHB_A + \text{const.} \quad (2)$$

where the parameters except for HB_A are the same as in Eq. 1, therefore,

$$HB_{CO} = 1: \text{CO}_2\text{R, CONH}_2, \text{CONMe}_2, \text{NHAc}; \quad 0 \text{ for the others}$$

$$HB_{AM} = 1: \text{NH}_2, \text{NHMe, NHAc, CONH}_2; \quad 0 \text{ for the others}$$

$$HB_A = 1: \text{OR, SMe, CN, Ac, NMe}_2, \text{NH}_2, \text{NHMe}; \quad 0 \text{ for the others}$$

The significance of each term was tested statistically. Among the correlations obtained for 2PR with different columns, those considered to be most reasonable are summarized in Table II along with our previous results with the C18 column.

We tried to extend our procedure to the 6PR series. The retention data was taken with C18 and Ph(CS) columns (Table I), and was analyzed similarly. Although the number of samples was not enough, good correlations that were similar to those for 2PR were yielded. The results are given in Table III. Since the corresponding coefficients of each term in the correlations for 2PR and 6PR coincided within the 95% interval for 30–70% MeOH, the analyses were done with the combined data set to give very good fits as shown in the lower part in Table III. Again an almost linear relationship was observed at 50% MeOH, so far as amphiprotics were treated separately (Fig. 1).

The $\log k_w$ values for 6PR, derived from the extrapolation using the data on the C18 column (Table I), are included in Table I. Most of them were higher than the $\log P$ values, as was the case with 2PR.¹³⁾

Next, we investigated the influence of mobile phases. The $\log k'$ values for 2PR were measured on C18 and Ph(CP) columns, using acetonitrile, dioxane and 1-propanol as the organic modifiers. Again, Eq. 2 worked well, though the data was limited to narrow ranges of the mobile phase compositions because the retention times at higher organic modifier concentrations were too short to measure. The results are presented in Table IV.

The fact that Eq. 2 applied to $\log k'$ values for various combinations of stationary- and mobile-phases indicates that our treatment is reasonable in spite of using the discrete-type parameters as a first approximation. Examples where the hydrogen-bond effects are successfully described by indicator variables, have often been found not only in the $\log P$ – $\log k'$ relationships^{4,18)} but also in structure–activity relationships.¹⁹⁾

Inspection of the obtained correlations presents the

TABLE II. Correlations for 2PR on Various Columns by Eq. 2

Column	Mobile ^{a)} phase	<i>a</i>	<i>b</i>	<i>c</i>	ρ	<i>d</i>	Const	<i>n</i>	<i>r</i>	<i>s</i>	<i>F</i>
C18 ^{b)}	M15	0.824	0.414	−0.319	−0.371		0.292	19	0.991	0.078	186.7
	M30	0.744	0.237	−0.305			−0.147	19	0.996	0.050	565.7
		0.747	0.248	−0.315	−0.137		−0.113	19	0.997	0.045	513.6
	M50	0.582		−0.349			−0.511	19	0.992	0.060	503.2
		0.620	0.100	−0.361			−0.549	19	0.995	0.047	543.2
	M70	0.495		−0.348			−0.887	17	0.991	0.051	376.7
C8	M15	0.794	0.340	−0.248	−0.404		0.242	18	0.993	0.066	218.1
	M30	0.697	0.228	−0.286	−0.203		−0.165	19	0.995	0.054	317.1
	M50	0.564	0.088	−0.305			−0.577	19	0.995	0.045	485.5
Ph(CP) ^{c)}	M15	0.669	0.438	−0.287	−0.506	0.169	0.155	18	0.996	0.047	292.8
	M30	0.587	0.307	−0.278	−0.256	0.123	−0.153	18	0.998	0.031	550.0
	M50	0.471	0.120	−0.245			−0.496	18	0.995	0.035	468.5
	M70	0.309	0.064	−0.222			−0.746	17	0.991	0.032	239.7
Ph(CS) ^{d)}	M30	0.479	0.322	−0.271	−0.294	0.137	−0.039	18	0.995	0.040	226.7
	M50	0.402	0.140	−0.240			−0.391	18	0.991	0.040	268.9
	M70	0.293	0.082	−0.296			−0.726	18	0.991	0.036	253.4

a) The figure represents the % MeOH. b) The data taken from ref. 13. c) Capcell pak phenyl. d) Cosmosil phenyl.

TABLE III. Correlations for 6PR by Eq. 2

System	Column	Mobile ^{a)} phase	<i>a</i>	<i>b</i>	<i>c</i>	ρ	<i>d</i>	Const	<i>n</i>	<i>r</i>	<i>s</i>	<i>F</i>
6PR	C18	M15	0.780	0.446	-0.349	-0.212	0.177	0.124	12	0.994	0.072	97.1
		M30	0.811	0.241	-0.268			-0.256	13	0.994	0.076	230.1
		M50	0.607		-0.243			-0.451	13	0.990	0.075	249.8
			0.638	0.136	-0.301			-0.507	13	0.994	0.060	265.9
		M70	0.460		-0.300			-0.762	13	0.989	0.066	220.4
	Ph(CS) ^{b)}	M30	0.517	0.372	-0.271	0.163	0.091	-0.222	13	0.993	0.059	135.6
		M50	0.382	0.238	-0.266			-0.440	13	0.996	0.032	272.3
		M70	0.286	0.154	-0.260			-0.724	13	0.995	0.027	297.8
	2PR + 6PR ^{c)}	C18	M30	0.754	0.229	-0.302	-0.260	-0.156	31	0.996	0.061	1020.4
			M50	0.632		-0.283		-0.515	31	0.990	0.082	712.8
				0.664	0.144	-0.324		-0.566	31	0.995	0.058	701.0
			M70	0.518		-0.294		-0.879	29	0.987	0.074	495.4
		Ph(CS) ^{b)}	M30	0.473	0.334	-0.288		-0.060	30	0.994	0.047	416.5
			M50	0.383	0.200	-0.262		-0.426	30	0.997	0.029	931.3
			M70	0.294	0.126	-0.265		-0.732	29	0.992	0.035	487.5

a) The figure represents the % MeOH. b) Cosmosil Phenyl. c) Combination of mono(2PR)- and di(6PR)-substituted pyrazines.

Fig. 1. Relationship between $\log P$ and $\log k'$ with C18 Column for 2PR and 6PR

(A): Relationship between $\log P$ and $\log k'_{M50}$. (B): Relationship between $\log P$ and $\log k'_{M15}$. Non-hydrogen bonders and H-acceptors are represented by the circles (●, $HB_{CO}=0$; ○, $HB_{CO}=1$) and amphiprotics by the triangles (▲, $HB_{CO}=0$; △, $HB_{CO}=1$).

TABLE IV. Correlations for 2PR from Various Mobile Phase Systems by Eq. 2

Column	Mobile ^{a)} phase	<i>a</i>	<i>b</i>	<i>c</i>	ρ	<i>d</i>	Const	<i>n</i>	<i>r</i>	<i>s</i>	<i>F</i>
C18	A5	0.813	0.583	-0.418	-0.222	0.172	0.277	18	0.993	0.077	162.8
	A15	0.770	0.240	-0.416			-0.255	18	0.993	0.069	243.0
	A30	0.610		-0.536			-0.612	18	0.989	0.088	203.2
	D5	0.855	0.484	-0.373	0.138	0.115	-0.032	19	0.996	0.057	423.1
	D10	0.837	0.359	-0.386			-0.227	19	0.996	0.058	420.0
	P5	0.850	0.238	-0.383			0.156	15	0.999	0.030	1244.8
	P15	0.768		-0.517	-0.453		-0.320	16	0.995	0.064	433.0
Ph(CP) ^{b)}	A5	0.612	0.422	-0.260	-0.447	0.163	0.016	18	0.992	0.062	140.4
	A15	0.545	0.171	-0.228			-0.332	18	0.987	0.062	178.6
	A30	0.408		-0.274			-0.677	18	0.992	0.045	292.3
	D5	0.649	0.392	-0.234	-0.404	0.156	-0.116	18	0.995	0.052	214.8
	D10	0.601	0.299	-0.229			-0.305	18	0.997	0.039	340.7
	D15	0.572	0.210	-0.219			0.093	18	0.996	0.037	437.3
	P5	0.642	0.274	-0.185	-0.718	0.115	-0.026	18	0.995	0.051	221.4
	P15	0.622		-0.143			-0.344	18	0.995	0.048	416.9

a) Organic modifier A: acetonitrile, D: dioxane, P: 1-propanol; the figure represents the volume % of the organic modifiers. b) Capcell Pak Phenyl.

following trends.

(a) The sign of the coefficient of HB_{AM} was always negative in all the cases. This feature is shown in

Fig. 1 through the downward deviation of the points for amphiprotic substituents. This can be explained, as was discussed in our previous paper.¹³⁾ The electron-

withdrawing property of the pyrazine ring enhances the H-donating ability of amphiprotic groups like NHR, CONH₂ and NHAc so that such H-donors would behave in a similar manner as phenols which are well known to present an acceleration effect relative to neutral benzenoid compounds^{6,20}; the acidic hydrogen in the amphiprotic substituents would undergo hydrogen-bonding more effectively with octanol than any stationary phase examined because of the higher basicity of octanol. Hence, the amphiprotic compounds appear to be more hydrophobic in the octanol/water system than in the HPLC system.

(b) The compounds in the 2PR and 6PR series were well incorporated in the same correlations in the range of 30 to 70% MeOH as shown in Table III, which means that no significant modification of hydrogen-bond effects arises from the introduction of the non-hydrogen bonding second substituent (C1) into the 2PR. This conforms to our previous finding¹⁶) that the electronic interactions between two substituents in 6PR were small enough to enable the prediction of their log *P* values by the summation of the π values of the corresponding 2PR as far as the substituents examined were concerned. The situation would, however, not be the same in such a case where one or both of the substituents are amphiprotic(s) of pronounced hydrogen-bond ability.

(c) High linearities between the log *k'* values for C8 and C18 stationary phases were observed to hold, indicating that both the stationary phases behave similarly. Likewise, the two kinds of Ph-columns from different suppliers also showed a resemblance in retention behavior, except that Ph(CP) is somewhat more dependent on log *P* than Ph(CS) because, for a given mobile phase, the slope of the log *P* term for Ph(CP) is higher than that for Ph(CS).

(d) The contributions of electronic effects, represented by ρ values, became more negative as the concentration of organic modifier in the mobile phases was lowered. As mentioned above, the HB_A term also became significant in highly water-rich mobile phases. These results suggest that a RPLC system with an eluent of low organic modifier concentrations discriminates subtle changes in the hydrogen bonding properties of solutes more effectively than eluents with intermediate modifier concentrations. The reason why attempts to incorporate the 2PR and 6PR series into a common correlation failed at 15% MeOH would be understood on this basis. The difference in polarity between the mobile and stationary phases becomes more pronounced as the water content in the mobile phase increases. It should also be mentioned that the change in the stationary phase structure tends to occur as the composition of eluents approaches the pure water.⁵) Such concomitant effects would make the retention mechanism more complicated in highly water-rich eluents.

It is of interest to observe positive ρ values for acetonitrile (A15 and A30 on C18, and A30 on Ph(CP)). This would mean that the ρ value, being negative at 100% water, increases continuously as the organic modifier-content increases. Since the ρ value obtained with the C18-phase varied with % CH₃CN in a similar manner to that with Ph(CP), it seems that the $\rho\sigma_1$ term is mostly ascribed to the solute-solvent interactions. McCormick and Karger²¹) reported that acetonitrile was extracted into the stationary phase (octyl bonded Hypersil) to a much

larger extent than MeOH. It might then be possible that the solute-solvent interaction in the stationary phases also plays a role in retention behavior when the modifier is acetonitrile. More comprehensive study would be required to elucidate the physical meaning of the coefficients.

(e) A retardation effect ascribed to ester and amide groups (ester effect) was commonly observed with water-rich mobile phases. In the previous paper,^{13,22}) we speculated that this ester effect was rather due to the interaction with the mobile phase but not with the stationary phase including silanol effects. The present finding that the reproducible ester effect exists regardless of the stationary phases, seems to supply additional support to our view. Although the origin of this effect remains to be clarified, one possibility is that the ester and amide groups are more sensitive to the change in the solvent system than other ordinary H-acceptors in the sense that ester type functional groups have two H-accepting sites. It would be interesting to extend the measurements to higher contents of organic modifiers to see whether a negative coefficient of the HB_{CO} term could be obtained using an appropriate model system which gives retention times long enough to allow the measurements. To know whether this effect is specific for α -substituted azines or not would also be useful. Studies to elucidate this problem are under way.

(f) The HB_A term was significant, in some cases, only with Ph-columns but not with alkyl-columns, suggesting that the Ph-phases are more discriminating between non-hydrogen-bonders and H-acceptors than the alkyl-phases relative to the octanol/water partitioning system. Therefore, the Ph-columns are supposed to be inferior in simulating log *P* for our solutes. The situation is shown in Fig. 2, where the relationship between log *P* and log *k'* obtained using the Ph(CS) column and 50% aqueous methanol as the eluent is illustrated. Comparison between Figs. 1A and 2 reveals that the C18 stationary phase gives a higher linearity than the Ph(CS) one. Thus and coworkers have reported that the retention for various benzenoid compounds on a phenyl-bonded stationary phase (Phenyl-Bondapack) with aqueous MeOH as the eluent correlates better with the log *P* values than that on a C18-modified phase (Hypersil ODS).⁸) However, the preliminary experiments on monosubstituted benzenes under our RPLC conditions again showed that the Ph-columns were more discriminating between H-acceptors and non-hydrogen bonders than the C18 column (the data

Fig. 2. Relationship between log *P* and log *k'*_{M50} with Ph(CS) Column for 2PR and 6PR

For Symbols see Fig. 1.

not shown). Especially, with MeOH as the modifier, the relative contribution of the $\log P$ term to $\log k'$ was usually higher in C18 than phenyl-ones (Tables II and III). All of these support the preference of C18 over Ph-phases.

This work has revealed that $\log k'$ values for 2PR and 6PR obtained from various combinations of stationary- and mobile-phase systems correlate very well with $\log P$ by the general Eq. 2. In all cases, the retention mechanism was shown to vary systematically: it became more complicated with the decrease in organic modifier content. The electronic interactions between the ring-N atoms and the substituents are thought to play an important role in governing the retention mechanism. The solutes having amphiprotic substituents usually exhibited the acceleration effect compared to non-amphiprotic compounds with the same $\log P$. The results led us to conclude that care should be taken to treat the RPLC capacity factor in connection with the $\log P$ value in heterocyclic systems. The fact that the $\log P$ - $\log k'$ linearity is usually broken by other disturbing factors in highly water rich eluents strongly demonstrates that the conventional $\log k_w$ approach is not effective in predicting the $\log P$ values in the present cases. It is rather more practical to use the RPLC system consisting of the C18 column and the mobile phase containing about 50% MeOH, where the disturbing terms (σ_1 , HB_{CO} and HB_A terms) are negligible or of small significance and hence $\log k'$ depends almost linearly on $\log P$, as far as amphiprotic substituents are treated separately, as shown in Fig. 1A.

To elucidate the effect of amphiprotics as well as ester groups on the retention behavior, analyses with a number of additional data would be required to parametrize the hydrogen-bonding effects in heteroaromatic systems. Our future work is being directed towards this problem.

References

- 1) Part II: C. Yamagami and N. Takao, *Chem. Pharm. Bull.*, **39**, 1217 (1991).
- 2) T. Fujita, J. Iwasa and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).
- 3) A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
- 4) H. Terada, *Quant. Struct.-Act. Relat.*, **5**, 81 (1986); K. Miyake, N. Mizuno and H. Terada, *J. Chromatogr.*, **439**, 227 (1988).
- 5) Th. Braumann, *J. Chromatogr.*, **373**, 191 (1986).
- 6) C. Yamagami, H. Takami, K. Yamamoto, K. Miyoshi and N. Takao, *Chem. Pharm. Bull.*, **32**, 4994 (1984).
- 7) D. J. Minick, J. H. Frenz, M. A. Patrick and D. A. Brent, *J. Med. Chem.*, **31**, 1923 (1988).
- 8) J. L. G. Thus and J. C. Kraak, *J. Chromatogr.*, **320**, 271 (1985).
- 9) W. E. Hammers, G. J. Meurs and C. L. De Ligny, *J. Chromatogr.*, **247**, 1 (1982).
- 10) T. Fujita, *Prog. Phys. Org. Chem.*, **14**, 75 (1983).
- 11) S. J. Lewis, M. S. Mirrlees and P. J. Taylor, *Quant. Struct.-Act. Relat.*, **2**, 100 (1983).
- 12) C. Yamagami, N. Takao and T. Fujita, *Quant. Struct.-Act. Relat.*, **9**, 313 (1990).
- 13) C. Yamagami, T. Ogura and N. Takao, *J. Chromatogr.*, **514**, 123 (1990).
- 14) M. Charton, *Prog. Phys. Org. Chem.*, **13**, 119 (1981).
- 15) C. Yamagami and N. Takao, *Chem. Express*, **6**, 113 (1991).
- 16) C. Yamagami, N. Takao and T. Fujita, *J. Pharm. Sci.*, **80**, 772 (1991).
- 17) Y. Ohtsu, H. Fukui, T. Kanda, K. Nakamura, M. Nakano, O. Nakata and Y. Fujiyama, *Chromatographia*, **24**, 380 (1987); Y. Ohtsu, Y. Shiojima, T. Okumura, J. Koyama, K. Nakamura, O. Nakata, K. Kimata and N. Tanaka, *J. Chromatogr.*, **481**, 147 (1989).
- 18) T. Fujita, T. Nishioka and M. Nakajima, *J. Med. Chem.*, **20**, 1071 (1977).
- 19) C. Hansch, A. Vittoria, C. Slipo and P. Y. C. Jow, *J. Med. Chem.*, **18**, 546 (1975).
- 20) N. Tanaka, H. Goodell and B. Karger, *J. Chromatogr.*, **158**, 233 (1978).
- 21) R. M. McCormick and B. L. Karger, *Anal. Chem.*, **52**, 2249 (1980).
- 22) C. Yamagami, N. Takao and T. Fujita, "Progress in Clinical and Biological Research," Vol. 291, ed. by J. L. Fauchere, Alan R. Liss, Inc., New York, 1989, p. 83.