Measurement and Prediction of Hydrophobicity Parameters for Highly Lipophilic Compounds: Application of the HPLC Column-Switching Technique to Measurement of log *P* of Diarylpyrazines

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Received April 12, 1999. Accepted for publication September 20, 1999.

Abstract □ In the preparatory stage of structure–activity relationship (QSAR) studies of anti-platelet aggregant pyrazine derivatives, log *P* values (*P*: 1-octanol/water partition coefficient) of diarylpyrazines were measured by a newly developed HPLC column-switching technique. The system consists of two processes: (1) adsorption of the sample at the top end of a short precolumn, and then (2) quantifying the enriched analyte by a conventional analytical column. By using the log *P* values thus obtained, the correction factor for the steric hindrance caused by the vicinal diphenyl groups was estimated. The log *k* values (*k*; retention factor) were also measured with methanol-buffer (pH 7.4) eluents and related to log *P*. The eluent of 50% methanol content (M50) gave a good linear relationship over a wide range of log *P* ($-0.3 < \log P < 5.2$), indicating that log k_{M50} parameter is useful for predicting the log *P* value.

Introduction

The logarithm of 1-octanol/water partition coefficient, log *P*, has been used as a measure of hydrophobicity of organic compounds and has played an important role in structure–activity relationship studies.[1,2] A conventional procedure for measurement of log *P* is the shake-flask method which consists of determining the equilibrium concentration of a substance in the aqueous or in both the aqueous and the octanol phases. This method, however, is not usually applicable to highly lipophilic compounds (log $P > 3$) because of extremely low concentrations of test compounds in the aqueous phase. To solve this problem, some procedures, such as re-extraction of the aqueous phase before the measurement of concentrations and the use of radiolabeled solutes, have been used to get a satisfactory response with the detector used.[3]

In addition to direct measurements, the log *P* value is often estimated by calculations or by retention parameters derived from reversed-phase HPLC, hereafter referred to as the HPLC method. The most widely used methodology for calculating log *P* was first proposed by Fujita et al., being based on an additive-constitutive, free energy related property of log *P*.[4] Thus, by using π defined as the difference in log *P* between a derivative with a given X substituent (RX) and the parent compound (RH),

$$\pi_X = \log P_{(RX)} - \log P_{(RH)} \quad (1)$$

log *P* values for multisubstituted compounds might be expressed as:

$$\log P_{(YRX)} = \log P_{(HRH)} + \pi_X + \pi_Y \quad (2)$$

Although many other empirical ways for log *P* calculations have been proposed subsequently,[5–8] most of them essentially assume such additivity. In accord with expectation, eq 2 fails in solute systems which involve electronic and/or steric interactions between the substituents, and various correction methods are proposed so that the predicted log *P* values may approach the experimental results.[6,7]

In HPLC approaches, the logarithm of the retention factor, *k*, is used as a hydrophobicity parameter. Usually C-18 bonded silica is used as a stationary phase with methanol–water mixtures as the mobile phase, and a Collander type relationship as shown by eq 3 is assumed.[9]

$$\log P = a \log k + b \quad (3)$$

Since the log *k* value depends on the mobile phase composition, extensive studies have been made to find optimal mobile phase conditions producing a good linear relationship.[10–15] Many investigators have used the log k_W parameter, (log *k* extrapolated to 0% methanol) to eliminate organic solvent effects.[10,11,16,17] On the other hand, other reports use isocratic log *k* values at an appropriate methanol concentration.[13–15,18,19] It is very often observed that hydrogen bonding effects of solutes with the surrounding molecules break a linearity, making the HPLC approach less effective for the estimate of log *P*. In an attempt to establish a standard HPLC procedure for predicting reliable log *P* values, we have studied the relationship between log *P* and log *k* for various series of fundamental heteroaromatic compounds, such as monosubstituted pyridines, diazines, and thiophenes, under various HPLC conditions.[13–15,20] Our results have demonstrated that hydrogen donating solutes (amphiprotics) usually behave differently from nonamphiprotics and should be treated separately.[13–15] Moreover, the log k_W method tends to overestimate log *P* for strong hydrogen acceptors. For this reason, most of pyridines and diazines which have *N*-atom(s), a strong hydrogen acceptor, in the parent rings have been found to yield values for log k_W much higher than that corresponding to the true value for log *P*. Instead, we have found that isocratic log *k* obtained with an eluent containing around 50% methanol usually results in a good linear relationship with log *P*, permitting us to reliably predict values for log *P* over the range, $-1 < \log P < 3$.[13–15,20]

Even when such empirical predictive methods are known to be reliable, accurate experimental values of log *P* for several compounds are required.

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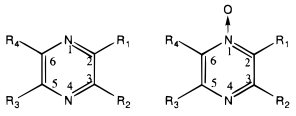
10.1021/js990112s CCC: $18.00
Published on Web 10/22/1999

In the course of our QSAR studies of bioactive compounds, we were interested in analyzing the structure–activity relationship of a series of diaryl pyrazines which has recently been found, by one of the authors, to have potent anti-platelet aggregation activity.[21] Knowing reliable log *P* values of these compounds was a prerequisite for this purpose. We, therefore, undertook measurement and evaluation of log *P* values for these compounds. The conventional shake-flask method failed not only because of their high lipophilicity but also of very low solubility of some compounds even in octanol. Although we were well experienced in predicting the log *P* value of pyrazines with log $P < 3$ by the HPLC method, it seemed dangerous to apply our relationship to a wider lipophilicity range (log $P > 4$). Accordingly, we attempted to develop a method to determine experimentally log *P* values for highly lipophilic compounds by using the HPLC column-switching system. The column-switching technique is widely used for on-line sample enrichment and cleanup, and has been increasingly applied to environmental analyses and the isolation of clinical samples.[22,23] We expected that this method would be applicable to our measurements of log *P* by loading large volumes of the sample solutions onto a precolumn and succeeded in obtaining log *P* values ranging from 3.5 to 5.2.



This report describes a novel procedure to measure log *P* values above 3 by using HPLC with a column-switching system. The log *P* values thus obtained were evaluated in terms of the additivity of π values and also related to log *k* values to examine how the log *P* values might be predicted by the HPLC approach. For comparison, some pyrazine *N*-oxides (PR→O) were also studied.

Materials and Methods

Materials—Compounds used in this study are given in Tables 1 and 2. Most of the diaryl pyrazines in Table 1 (**1**, **3**, **4**, **8**, **10**, **12**, **13**, **15**–**17**, **20**-**22**, and **24**) and monosubstituted pyrazines (**28**–**35**) in Table 2 were prepared as previously described.[21,24] Compounds **5**, **6**, **18**, and **19** were prepared by treatment of the 5-Cl derivatives with an appropriate sodium alkoxide according to the method for preparing the corresponding monosubstituted pyrazines.[24] Alkylpyrazines (**36**–**45**) were purchased from Tokyo Kasei Organic Chemicals (Tokyo, Japan). The references for preparation of the other compounds are given in the footnotes to Tables 1 and 2. HPLC-grade methanol and water, 1-octanol of the grade for log *P* measurements, and a phosphate buffer solution (pH 7.4) were purchased from Nacalai Tesque.

Measurement of log *P*: Conventional Shake-Flask Method—The log *P* for compounds with medium or low hydrophobicity (log $P < 3$) was measured by a conventional shake-flask method. The concentration of samples in both of the water and octanol phases was determined by HPLC.

Column-Switching Method—Samples (0.5–30 mg) were dissolved in 1 mL of octanol and then shaken with 100 mL of distilled water. The phases were allowed to separate overnight at 25 °C. Persistent emulsions were not observed to occur with our compounds. After pipetting off the octanol phase, the aqueous phase was collected into centrifuge tubes, preventing contamination by octanol, and then centrifuged twice for 5–10 min. As the solute concentration of lipophilic compounds in the aqueous phase was too low to detect by HPLC, sample enrichment on a precolumn was performed before conventional quantitative analysis by using a column-switching technique as follows.

HPLC System—The column-switching system (Figure 1) consisted of two isocratic HPLC pumps (LC 9A, Shimadzu, Kyoto), an autoinjector (IS-25, Kyoto Chromato, Kyoto,

Table 1—Log *P* Values of (Di)arylpyrazines

	R_1	R_2	R_3	R_4	log *P*[a]	log P_{add}[b]
1	Ph	H	H	H	2.07	
2[c]	4-OMe-Ph[d]	H	H	H	2.24	
3	Ph	Ph	H	H	3.19	4.4
4	Ph	Ph	Me	H	3.52	4.9
5	Ph	Ph	OMe	H	4.21	5.4
6	Ph	Ph	OEt	H	4.73	5.9
7[e]	Ph	Ph	Cl	H	4.05	5.4
8	Ph	Ph	*i*-Pr	H	4.71	
9[e]	4-Me-Ph	4-Me-Ph	H	H	4.10	
10	4-F–Ph	4-F–Ph	Me	H	3.75	
11[e]	4-CN–Ph	4-CN–Ph	Me	H	2.52	
12	Ph	Ph	2-OMe-Bn[f]	H	5.18	
13	Ph	Ph	3-OMe-Bn[f]	H	5.20	
14[e]	4-OMe-Ph	4-OMe-Ph	H	H	3.42	4.7
15	4-OMe-Ph	4-OMe-Ph	Me	H	3.66	5.2
16	4-OMe-Ph	4-OMe-Ph	Et	H	4.22	5.7
17	4-OMe-Ph	4-OMe-Ph	Cl	H	4.30	5.7
18	4-OMe-Ph	4-OMe-Ph	OMe	H	4.47	5.7
19	4-OMe-Ph	4-OMe-Ph	OEt	H	5.00	6.3
20	4-OMe-Ph	4-OMe-Ph	CN	H	3.70	5.0
21	4-OMe-Ph	4-OMe-Ph	COOMe	H	3.41	4.8
22	4-OMe-Ph	4-OMe-Ph	Me	Me	4.10	5.2
23[g]	Ph	Me	Ph	H	4.28	4.9
24	Ph	Me	H	Ph	4.29	4.9
25[h]	Ph	Et	Ph	Et	4.94	6.3
26[i]	Ph	Et	H	Ph	4.66	5.4
27[j]	Ph	Et	Et	Ph	4.83	6.3

[a] Measured by the column-switching method except for **1** and **2**. [b] Sum of log *P* of pyrazine (–0.26) and $\sum$ pyrazine-π (R_1, R_2, R_3, R_4). [c] Superscript of the compound number identifies the reference for the preparation: Ohta, A. et al. *J. Heterocycl. Chem*. **1982**, *19*, 1061–1067. [d] 4-X-Ph refers to 4-X-substituted phenyl. [e] Ohta, A. et al. *J. Heterocycl. Chem*. **1982**, *19*, 465–473. [f] 2-OMe-Bn and 3-OMe-Bn refer to 2-OMe-benzyl and 3-OMe-benzyl, respectively. [g] Ohta, A. et al. *Heterocycles* **1984**, *22*, 2317–2321. [h] Ohta, A. et al. *Heterocycles* **1986**, *24*, 785–792. [i] Ohta, A. et al. *Heterocycles* **1987**, *26*, 2449–2454. [j] Ohta, A. et al. *J. Heterocycl. Chem*. **1983**, *20*, 311–320.

Table 2—Log *P* Values of Variously Substituted Pyrazines (PR) and Pyrazine *N*-Oxides (PR→O)

	substituents	log *P*[a]		substituents	log *P*[a]
	PR		**40**	2-Me,3-Bu	2.10
28	H	–0.26	**41**	2-Me,3-*i*Bu	1.96
29	Me	0.21	**42**	2,3-diEt	1.51
30	Et	0.69	**43**	triMe	0.95
31	OMe	0.73	**44**	2,3-diEt,5-Me	1.95
32	OEt	1.28	**45**	tetraMe	1.28
33	OPr	1.84	**46**[b]	2,5-diMe-3,6-diCl	2.40
34	COOMe	–0.23		**PR→O**	
35	COOEt	0.28	**47**[c]	2,5-diMe-3Cl	0.44
36	2,3-diMe	0.54	**48**[d]	2,5-diMe-6Cl	0.19
37	2,6-diMe	0.54	**49**[e]	2,5-diMe-3,6-diCl	1.08
38	2-Me,3-Et	1.07	**50**[f]	2,3-bis(4-Cl–Ph),5-Cl	3.66
39	2-Me,3-Pr	1.57	**51**[f]	2,3-bis(4-Br–Ph)	3.41

[a] The data for compounds **28**–**35** and **36**–**45** were taken from refs 24 and 29, respectively. Those for the others were measured in this work. [b] Superscript of the compound number identifies the reference for the preparation: Baxter, R. A. et al. *J. Chem. Soc*. **1948**, 1859–1862. [c] Ohta, A. et al. *Chem. Pharm. Bull*. **1979**, *27*, 2027–2041. [d] Ohta, A. et al. *J. Heterocycl. Chem*. **1981**, *18*, 555–558. [e] Ohta, A. et al. *J. Heterocycl. Chem*. **1982**, *19*, 781–784. [f] Ohta, A. et al. *J. Heterocycl. Chem*. **1982**, *19*, 465–473.

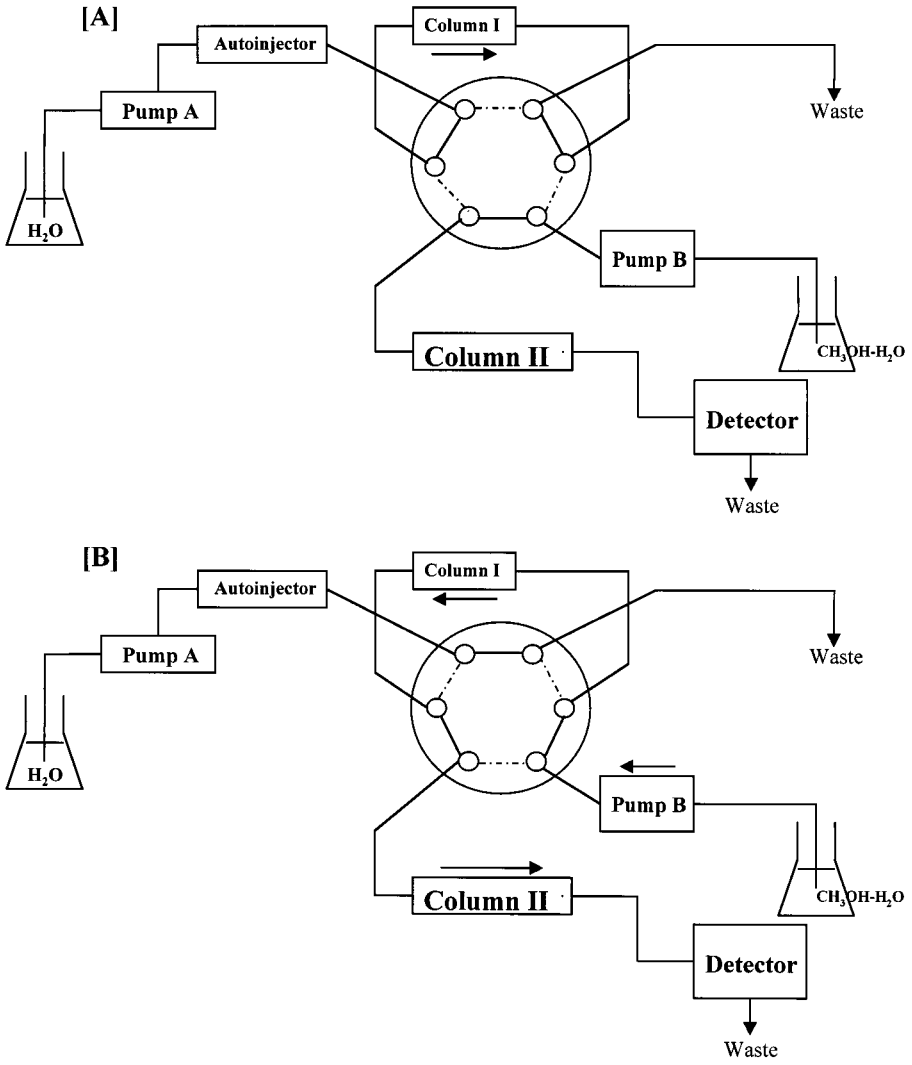


Figure 1—Schematic representation of the HPLC column-switching system. (A) Sample enrichment process. (B) Analytical process.

Japan) fitted with a 10 mL sample loop, a 10 mL syringe and a Rheodyne 7125 injection valve, and a universal valve-switching module ACP 1010 with a Rheodyne 7000 six-port, two-position valve (Anachem, Luton, England). The following columns were connected to the six-port valve. Column I (precolumn for concentration): Capcell Pak C18, UG 80 (Shiseido, Tokyo, Japan), 35 × 4.6 mm i.d. Column II (analytical column): Cosmosil 5C18-AR−II (Nacalai Tesque, Kyoto, Japan). 150 × 4.6 mm i.d.

Column-Switching HPLC Analysis—A 5−9 mL aliquot of the centrifuged aqueous phase, giving an appropriate peak area on the final chromatogram, was automatically injected onto column I by pump A using pure water as the mobile phase at a flow rate of 5.0 mL/min (at position A, Figure 1). This process allowed the solute to be adsorbed at the top end of the column I. After 4 min, the valve was switched to the position B (Figure 1), and the preconcentrated (adsorbed) analyte was eluted to the analytical column II (back flush mode) with an aqueous solution containing 70−90% methanol delivered by pump B. The signals were analyzed by a photodiode array detector SPD-MV10A (Shimadzu, Kyoto, Japan). Eluent compositions were chosen so that the analytical time would be less than 10 min for each sample. The average peak area of a given sample for the aqueous phase, A_W, was obtained from repeated 5−7 cycles. To analyze the corresponding octanol phase under comparable conditions to those for the aqueous phase, the following two-step dilution was performed. First, a 200 μL aliquot of the octanol phase was diluted with methanol to 1.00 mL. The methanolic solution thus obtained was then diluted with pure water by an appropriate factor as described below. The resulting homogeneous solution was analyzed quantitatively in the same manner as in the analysis of the aqueous phase. Injection volume was the same as that for the aqueous phase. The second dilution with water was done at two dilution factors so that the resulting peak areas, A_o (1) and A_o (2), obtained as described above would be $A_o(1) < A_W < A_o(2)$ and also the ratio of $A_o(2)/A_o(1)$ would be 2−5. With these data, the P value was calculated as $P = fA_o/A_W$, where f represents the overall dilution factor. Agreement of log P from two A_o values, A_o (1) and A_o (2), was usually obtained to within 0.05 log unit. The mean value was taken as the P value. For each compound, measurements were carried out at least in duplicate, and the P values thereby obtained were reproducible to within ±0.1 log unit for most cases.

Measurement of log *k*—The log k value was determined as previously described with a Capcell Pak C18 column (50 × 4.6 mm i.d., Shiseido, Tokyo, Japan) at 25 °C.[13] Methanol−0.01 M phosphate buffer (pH 7.4) mixtures containing 50, 60, and 70% MeOH (hereafter designated as M50, M60, and M70, respectively) prepared by volume were used as the eluent. Log k parameters were determined by $k = (t_R - t_0)/t_0$, where, t_0 and t_R are retention times of methanol and a given analyte, respectively.

Table 3—Pyrazine-π (π_{PR}) and Benzene-π(π_{PhX})

X	π_{PR}[a]	π_{PhX}[b]
F	0.55	0.14
Cl	0.96	0.71
Br	1.19	0.86
Me	0.47	0.56
Et	0.95	1.02
OMe	0.99	–0.02
OEt	1.54	0.38
CN	0.25	–0.57
COOMe	0.03	–0.01
COOEt	0.54	0.51
Ph	2.33[c]	1.96
4-MeO-Ph	2.50[c]	–

[a] Taken from ref 24. [b] Taken from ref 2. [c] This work.

Results and Discussion

log *P* of Diarylpyrazines—Before measuring log *P* values for our compounds, we attempted to determine the log *P* value of curcumin with our column-switching system. Curcumin has attracted our interest in the previous structure−activity relationship study,[25] and its experimental log *P* value is reported to be 4.26.[26] We obtained a value of 4.28, very close to 4.26, indicating that this newly developed procedure works satisfactorily. Therefore, we applied this method to determining log *P* values of diaryl pyrazines (Table 1). Inspection of Table 1 shows that introduction of two hydrophobic aryl substituents increases extensively the hydrophobicity of molecules. We have previously shown[24] that the π value in the pyrazine system (Table 3), derived from the log *P* values of monosubstituted pyrazines and pyrazine itself, often differs from the widely used benzene-π values and, therefore, that pyrazine-π values should be used in the analysis of log *P* values of pyrazines. By using pyrazine-π values, where available, estimated log *P* values, log P_{add}, were calculated on assumption of the additivity, that is, the sum of log *P* of pyrazine (−0.26) and pyrazine-π values of the substituents (Table 1).

It is noteworthy that experimental log *P* values of all compounds in Table 1 were much lower than log P_{add} values. In particular, in 2,3-diarylpyrazines, **3**−**7** and **14**−**22**, the difference was over one log unit. Steric hindrance between the two benzene rings may contribute to reducing the hydrophobicity of molecules (*ortho*-diaryl effect). This argument would also be supported by comparing the log *P* values of the three isomeric diphenyl-methylpyrazines (**4**, **23**, and **24**). Among them, only the isomer **4** having two adjacent phenyl groups gave a log *P* value (3.52) much smaller than those for **23** (4.28) and **24** (4.29), which have two phenyl substituents at 2,5- and 2,6-positions, respectively. An X-ray analysis of 2,3-diphenylpyrazine **3** has demonstrated that the dihedral angles between the two phenyl substituents and the pyrazine ring are approximately 50° and 40°.[27] Our semiempirical MO calculations of 2,3-diphenylpyrazine by the AM1 method[28] (the MOPAC 93 program package incorporated in an ANCHOR II modeling system, Fujitsu, Japan) gave a similar result. Such nonplanar conformation would cause resonance inhibition, decreasing the degree of conjugation and consequently reducing hydrophobicity below expectations for a molecule with full delocalization of π-electrons. The use of the additivity rule overestimated log *P* for **3** by 1.2 and that for **14** by 1.3, suggesting that the steric hindrance between the two vicinal benzene rings makes a negative contribution of about −1.2 to log *P*. This finding conforms to a well-known phenomenon that an extension of the resonating system requires a positive correction factor.[2]

Table 4—Substituent Effects of R_3

compound	R_3	R_1,R_2	log P_{obsd}	log P_{pred}[a]	compound	R_3	R_1,R_2	log P_{obsd}	log P_{pred}[b]
4	Me	Ph	3.52	3.66	**15**	Me	4-OMe-Ph	3.66	3.89
5	OMe	Ph	4.21	4.18	**16**	Et	4-OMe-Ph	4.22	4.37
6	OEt	Ph	4.73	4.73	**17**	Cl	4-OMe-Ph	4.30	4.38
7	Cl	Ph	4.05	4.15	**18**	OMe	4-OMe-Ph	4.47	4.41
					19	OEt	4-OMe-Ph	5.00	4.96
					20	CN	4-OMe-Ph	3.70	3.67
					21	COOMe	4-OMe-Ph	3.41	3.45

[a] Sum of log *P*(**3**) and pyrazine-π(R_3). [b] Sum of log *P*(**14**) and pyrazine-π(R_3).

We also examined the substituent effect of R_3 on log *P* when R_3 is introduced to 2,3-diarylpyrazines, **3** and **14**, in terms of the *ortho*-diaryl effect discussed above and pyrazine-π values. As the R_3 substituent hardly suffers from steric effects, we may expect normal substituent effects in these pyrazines. The sum of log *P* of **3** or **14**, and pyrazine-π of R_3 was calculated (log $P_{pred.}$) and compared with the experimental log *P*. As shown in Table 4, they were in fairly good agreement. It is not unexpected that the deviation from the experimental value is somewhat larger than the expected experimental error in some compounds because some additional minor corrections for the electronic interactions between the substituents and the ring *N*-atoms as well as for the steric effect between R_2 and R_3 should be taken into account for detailed analysis.[24] These results revealed that the pyrazine-π is very helpful in making a rough estimate of log *P* of even highly lipophilic pyrazines, provided severe steric effects are not involved. It should be emphasized that the use of benzene-π instead of pyrazine-π for the substituent on the pyrazine ring would lead to erroneous estimated values.

Comparisons of log *P* for a series of variously substituted alky-diphenylpyrazines (**4** and **23**−**27**) indicates that steric repulsion by the vicinal alkyl and phenyl substituents also requires a correction of −0.6 to −0.7 in log *P*. As the corresponding reduction in log *P* in the vicinal dialkyl substituents is very small (∼−0.15),[29] large correction factor suggests twisting of the benzene ring even in this case. Nonplanar conformations were also indicated by the MO calculations. Measurements and more quantitative analyses of log *P* for a larger size data set including more lipophilic compounds are now in progress.

Log *k*: Prediction of log *P* by the HPLC Approach—Our previous studies on several series of monosubstituted heteroaromatic compounds have revealed that the relationship between log *P* (−1 < log *P* < 3) and log *k* obtained with various compositions of methanol−water eluents usually require some correction terms for hydrogen-bonding effects and can be expressed by the following general equation for nonamphiprotics:[30]

$$\log k = a \log P + \rho\sigma_I + sS_{HA} + \text{const} \qquad (4)$$

In this equation, the σ_I parameter represents Charton's electronic substituent constant,[31] and the S_{HA} parameter is that we have recently proposed as the hydrogen-accepting indicator for the substituent.[30] In general, contributions of the two correction terms are of minor importance when the eluent contains 50% MeOH (M50), so that we could assume the log *k*−log *P* linearity. However, these contributions are found to increase with decreasing methanol content. Strong hydrogen-accepting substituents, such as COOR and $CONMe_2$, deviate substantially from the line formed by non-hydrogen bonders and weak hydrogen acceptors in the plot of log *k* against log *P*. If such a predictive capability of M50 eluent holds for compounds

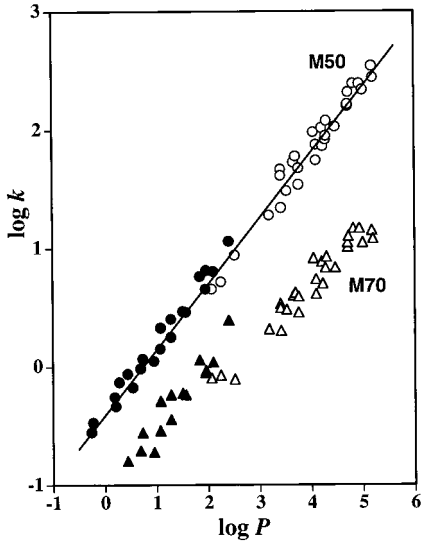


Figure 2—Relationship between log *k* and log *P* for variously substituted pyrazines. Open symbols represent (di)arylpyrazines, and closed symbols represent the other pyrazines.

with much higher log *P* values, the log k_{M50} approach would be of practical value in prediction of log *P*. Accordingly, we measured log *k* values for the present compounds under the same conditions and studied the relationship between log *P* and log *k*. To make the results comparable with those previously studied, we selected from many of the related compounds studied earlier, typical compounds including esters and *N*-oxides which were previously classified as deviants in water rich eluents.[13] Some *N*-oxides of diarylpyrazines were also added to the data set. Compounds used for log *k* measurements in addition to the (di)arylpyrazines in Table 1 are listed in Table 2 with their log *P* values. It should be noted that the compounds studied in this work are non-hydrogen bonding or hydrogen accepting. Log *k* values of all the compounds in Tables 1 and 2 obtained with the M50 and M70 eluents are plotted against log *P* in Figure 2. The M50 eluent again produced a satisfactory linear relationship of the form shown in eq 5:

$$\log k_{M50} = 0.554 \log P - 0.384 \quad (5)$$
$$n = 51,\ r = 0.996,\ s = 0.085$$

The relationship for the M60 eluent was somewhat inferior to eq 5 (the data not shown). As the data set contains molecules having strongly hydrogen-accepting substituents and/or a sterically hindered conjugated system, finding a simple linear equation covering a range of hydrophobicity of 6 log units seems to be rather surprising. It is of interest to notice that our earlier work on a series of monosubstituted pyrazines found the value of 0.58 as the slope of the log *P* term, being very close to the value of 0.55 shown by eq 5, in the corresponding equation. Although measuring log *k* values of monosubstituted pyrazines in more methanol-rich eluents such as M60 and M70 has been technically difficult, it has now become very clear, by combination of all the results obtained so far, that an optimum eluent composition yielding almost linear relationships would be at 50% MeOH or at methanol concentrations near 50%. Since the measurement of log *k* is easier and quicker than log *P*, particularly in very hydrophobic compounds, the log k_{M50} parameter can be expected to be a powerful tool for predicting the log *P* value. Care should be taken, however, in treating amphiprotic compounds by the HPLC method. When alkyl-bonded stationary phases are used, amphiprotics often give separate lines parallel to that for nonamphiprotics in the log *k*−log *P* plot.[13,15] This phenomenon is unavoidable because octanol is more hydrogen accepting than the stationery phase. Finding an effective procedure for treating these is still to be investigated.

Conclusion

In this study, we developed a novel procedure for measuring the log *P* value of highly lipophilic diaryl pyrazines by the HPLC column-switching technique. This method enabled us to measure the extremely low concentration of solutes by direct injection of a large volume (5−9 mL) of aqueous phase onto a precolumn. The log *P* values thus obtained ranged from about 3 to 5.2. By using these data, a large correction factor of about −1.2 was estimated as the *o*-diphenyl effect, which was thought to be attributable to the loss of coplanarity. The effects of sterically unhindered substituents attached to the pyrazine ring on log *P* could be well estimated by using the pyrazine-π value obtained in our earlier work.[24] The log *k* values were also measured with different compositions of methanol−buffer (pH 7.4) eluents, and a good linear relationship between log *P* and log *k* was found to hold at 50% MeOH, in conformity with our previous finding,[13,14] over the log *P* range from −0.3 to 5.2. To our knowledge, there have been no previous studies where log *k* values for a set of compounds determined under exactly the same HPLC conditions have been related to experimentally measured log *P* values over such a wide range. The present result seems to afford a nice example demonstrating that the use of the k_{M50} parameter provides reliable and practical method to predict quickly the log *P* value.

Development of procedures for obtaining reliable log *P* values for lipophilic compounds has become increasingly important not only in QSAR studies of bioactive compounds but also in environmental chemistry and toxicology where the log *P* is used as a parameter for evaluating the fish bioconcentration and toxicity.[32−34] Our present study would be also expected to be helpful for research in such fields.

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Acknowledgments

We thank Professor Toshio Fujita of EMILE project for useful discussion. This work was partly supported by Kobe Pharmaceutical University Collaboration Fund.

JS990112S