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Hydrophobicity parameters determined by reversed-phase liquid chromatography

VIII. Hydrogen-bond effects of ester and amide groups in heteroaromatic compounds on the relationship between the capacity factor and the octanol–water partition coefficient

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ABSTRACT

The logarithms of the capacity factors, log k', for several heteroaromatic systems (furan, pyrrole, benzofuran, indole, benzene and their alkyl, ester and amide derivatives) were determined on a Capcell Pak C_{18} column using methanol–buffer (pH 7.4) mobile phases of different compositions. These results and the log k_w values, derived by a linear extrapolation of the plot of log k' against the volume fraction of methanol to 0% methanol, were correlated with experimental log P values by taking into account the hydrogen-acceptor and hydrogen-donor effects. Whereas the hydrogen-donor effect was found to be minimized by using the log k_w parameter, the hydrogen-acceptor effect was found to become insignificant in an eluent containing 50% methanol. Isocratic data determined at this eluent composition gave the simplest and best correlation.

INTRODUCTION

The logarithm of the 1-octanol–water partition coefficient, log P, is a widely used hydrophobicity parameter in quantitative structure–activity relationship (QSAR) studies [1,2]. For compounds without hydrogen-bonding functional groups, their log P values can often be calculated by taking account of the additive property of substituent hydrophobicity constants, π. However, if the compounds include polar groups, such calculations tend to yield erroneous log P values [3–5], and the use of experimental values is required. Recently, reversed-phase liquid chromatographic (RPLC) techniques have found utility in predicting log P values in place of the standard shake-flask method. Extensive examples where the log P value is estimated from the logarithm of the capacity factor, log k', obtained under various chromatographic conditions, have been reported [6–9]. Although the RPLC method is convenient and simple to treat, a universal procedure, including the RPLC conditions, for simulating log P does not seem to have been established.

In our continuing fundamental work on physico-chemical parameters used in QSAR, we have systematically investigated log P values in some heteroaromatic series, such as pyridines and diazines, and have found that the partition

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behaviour is greatly affected by the hydrogen-bond abilities of the ring heteroatom(s) and the substituent on the hetero-ring [5,10]. In order to examine whether the RPLC method can be utilized for determining log P under such circumstances, we have also studied the relationship between log P and log k' obtained with a Capcell Pak C_{18} column and methanol–buffer (pH 7.4) eluents [11–14]. It was demonstrated that the log k_w value (log k' extrapolated to 0% organic modifier) gives accurate estimates of log P for compounds free from strong hydrogen bonders [14], whereas it tends to overestimate the log P value of hydrogen acceptors (H-acceptors) and underestimate that of hydrogen donors (H-donors) [12–14]. In particular, our results obtained so far for several series of compounds ArX (X = variable substituent) have shown that ester groups (CO_2R), being strong hydrogen acceptors, are usually deviants from log P–log k_w relationships, in contrast to other weak hydrogen-accepting substituents such as OR, SR and NMe_2, which lie close to the calibration line [11]. The amide group ($CONH_2$, CONHR) is another functional group of interest. As they are expected to show amphiprotic characters, their log k_w values would involve an overestimating factor as an H-acceptor as well as an underestimating factor as an H-donor.

Keeping the above in mind, we attempted to extend our investigations to a larger number of compounds with such substituents. In this work, we prepared several systematic series of ester and amide derivatives of typical heteroaromatic compounds as shown below, and measured their capacity factors. The relationships between log P and log k_w (log k') were compared with those for the parents and their alkylated compounds to survey the hydrogen-bond effects of ester and amide groups in both chromatographic and octanol–water partitioning systems.

EXPERIMENTAL

Compounds

The compounds used are given in Table I. Some of them have been used previously [13]. The others, if not commercially available, were prepared as described previously [13].

Partition coefficients

Some of the 1-octanol–water log P values were taken from our previous papers [5,13]. Those for the others were measured in this study at 25°C by the conventional shake-flask method. For the measurement of volatile compounds the concentration was determined in both phases by RPLC according to the previous procedure [16].

RPLC procedure

The apparatus and the procedure used were the same as described previously [16]. Commercial Capcell Pak C_{18} columns [17,18] (5 or 25 cm × 4.6 mm I.D.) (Shiseido) were used without further treatment (silanol effects were demonstrated to be negligible in previous and preliminary work [11]). Commercial HPLC-grade methanol and water were used. As an aqueous phase, 0.01 M phosphate buffer (pH 7.4) was used. The methanol–buffer eluents were prepared by volume. Samples were dissolved in methanol (about 0.5%) and 1-2 μl was injected at 25°C. The flow-rate was 0.5–2.0 ml/min. The capacity factor, k', was determined from the retention time of the sample, t_R, by the equation $k' = (t_R - t_0)/t_0$, where the t_0 value is the retention time of methanol. Solutes were chromatographed on the 5-cm column for an eluent containing 15% methanol and on the 25-cm column for eluents containing 30, 50 and 70% methanol.

RESULTS OF REGRESSION ANALYSES

Relationship between log k_w and log P

The compounds examined were furan (Fr), benzofuran (BF), pyrrole (Pyr), 1-methylpyrrole (1-Me-Pyr), indole (In), 1-methyl-indole (1-Me-In), benzene (Bz) and their alkyl, ester and amide derivatives. The 1-Me-Pyr and 1-Me-In series were studied to examine the hydrogen-donor effect of the acidic hydrogen attached to the ring-N atom (N*H*). Although some of them were studied in previous work [13,14], measurements were made for all compounds in this work under the same conditions. As one of the most widely used chromatographic parameters for predicting log P, log k_w values were derived by linear extrapolations using log k' values determined at methanol concentrations ranging from 30 to 70%:

$$\log k' = \log k_w - SX \tag{1}$$

where X represents the volume fraction of methanol in the mobile phase. In previous work it was shown that the log k_w value obtained in this way correlates better with log P than log k_w values obtained otherwise [12]. Although retention data in an eluent containing 15% methanol were also measured, they tended to deviate from the linear line. Therefore, the data in this eluent were excluded from the analyses. The log k_w values are given in Table I together with the log P values.

It is known empirically that the log k_w value derived for the methanol–water mobile phase system with a C_{18}-modified stationary phase can be a direct measure of log P for certain compounds (log k_w method) [6,11]. Although the correlation between log k_w and log P for compounds given in Table I is fairly good ($r = 0.98$, Fig. 1), close examination of the differences between log k_w and log P show the following tendencies in accordance with those observed in our previous work [14]: (i) the log k_w values for Fr, BF, 1-Me-Pyr, 1-Me-In, Bz and their alkyl derivatives, which are non-hydrogen bonders or very weak H-acceptors, agreed well with log P; (ii) for Pyr, In and their alkyl derivatives, classified as H-donors, the log k_w method tended to underestimate the log P values, *i.e.*, log $k_w <$ log P; (iii) for esters with the parent nucleus noted in (i), classified as strong H-acceptors, the log k_w method overestimated the log P values, *i.e.*, log $k_w >$ log P.

For the compounds not included in (i)–(iii), the relationship between log P and log k_w was more complicated because those compounds contain various combinations of H-donating, H-accepting and amphiprotic substituents. To separate these hydrogen-bond effects, the compounds were divided into subgroups depending on the hydrogen-bond types as follows: system N, parent compounds: Fr, BF, 1-Me-Pyr, 1-Me-In and their alkyl derivatives, and substituents (subgroups): CO_2R (E), CONHR and $CONH_2$ (AM); and system H, parent compounds: Pyr, In and their alkyl derivatives, and substituents (subgroups) CO_2R (E), CONHR and $CONH_2$ (AM), where in system H the aromatic rings have H-donors and in system N the aromatic rings are non-hydrogen-bonders or very weak H-acceptors. Alkyl derivatives were included in the parent compounds because they exhibit no hydrogen-bond effect. Classification of the furan ring into this group is rationalized also by a theoretical approach: preliminary semi-empirical molecular orbital calculations by the PM3 method suggested that the interaction energy between furan and water molecules is relatively insignificant. Analyses were made by using the hydrogen-bond indicator variables as follows: $HB_H = 1$ for compounds of system H, $HB_H = 0$ for those of system N; $HB_E = 1$ for esters, $HB_E = 0$ for the others; and $HB_{AM} = 1$ for amides, $HB_{AM} = 0$ for the others. The parameter used are listed in Table II

First, pre-analyses in terms of eqn. 2 were made step by step on the assumption that the hydrogen-bond effects attributable to each functional group are additive:

$$\log k_w = a \log P + \sum_i b_i HB_i + c \tag{2}$$

where HB_i represents one of the HB parameters given above. The coefficients and constant values can be obtained by regression analyses. By this treatment, the b_i value should reflect the hydrogen-bond effect contributed from each hydrogen-bonding group.

Effect of the ring NH (H-donor effect). To see the H-donor effect attributable to the ring N*H* atom, we tried the analysis for the parent compounds of both N and H systems, yielding an excellent correlation, with an improvement of eqn. 4 compared with the single correlation (eqn. 3):

$$\log k_w = 1.023 \log P - 0.190$$

$$n = 15, r = 0.990, s = 0.101, F = 627 \tag{3}$$

$$\log k_w = 1.012 \log P - 0.152 HB_H - 0.097$$

$$n = 15, r = 0.997, s = 0.062, F = 843 \tag{4}$$

In these equations and throughout this work, n is the number of compounds used for calculations, r is the correlation coefficient, s is the standard

TABLE I

RETENTION PARAMETERS AND 1-OCTANOL–WATER LOG P VALUES

Compound	System	Log P^a	Log k_w ^{b}	S^b	r^c
Furan (Fr)	*N*	1.34	1.17	1.88	0.998
-2-Me		1.85	1.79	2.41	1.000
-2-Et		2.40	2.39	2.92	1.000
-2-CO_2Me		1.00	1.32	2.50	1.000
-2-CO_2Et		1.50	1.84	2.96	1.000
-2-CONHMe		0.23	0.45	2.07	0.999
-2-CONHEt		0.61	0.82	2.32	0.999
-2-CONHPr		1.10^d	1.28	2.66	0.999
-2-$CONH_2$		−0.11	0.19	2.05	0.999
-3-CO_2Me		1.28	1.52	2.59	1.000
-3-CO_2Et		1.78	2.07	3.07	1.000
-3-CO_2Pr		2.36^d	2.66	3.58	1.000
-3-CONHMe		0.34	0.43	1.95	1.000
-3-CONHEt		0.72	0.75	2.15	1.000
-3-CONHPr		1.20^d	1.19	2.49	1.000
-3-$CONH_2$		0.09	0.22	1.94	1.000
Benzofuran (BF)	*N*	2.67	2.72	3.43	1.000
-2-CO_2Me		2.53	2.83	3.79	0.999
-2-CO_2Et		3.05^d	3.37	4.27	0.999
-2-CONHMe		1.85^d	2.00	3.34	0.999
-2-CONHEt		2.22^d	2.34	3.62	0.999
-2-CONHPr		2.65^d	2.80	4.04	0.998
-2-$CONH_2$		1.54^d	1.79	3.24	0.999
Pyrrole (Pyr)		0.75	0.52	1.62	0.999
-2-Et		1.59	1.42	2.36	1.000
-2,5-di-Me		1.47	1.30	2.25	1.000
-2-CO_2Me		1.27^d	1.40	2.67	1.000
-2-CO_2Et		1.73^d	1.90	3.09	1.000
-2-CONHMe		0.42^d	0.44	2.03	0.999
-2-CONHEt		0.80^d	0.79	2.28	0.999
-2-CONHPr		1.25^d	1.25	2.62	0.999
-2-$CONH_2$		0.09^d	0.22	2.00	0.999
1-Me-pyrrole (1Me-Pyr)	*N*	1.15	1.06	1.72	0.999
-2-CO_2Me		1.80^d	2.06	3.04	1.000
-2-CONHMe		0.71^d	0.88	2.32	0.999
-2-CONHEt		1.09^d	1.21	2.55	0.999
-2-CONHPr		1.63^d	1.65	2.91	0.999
-2-$CONH_2$		0.45^d	0.72	2.32	0.999
Indole (In)	*H*	2.14	1.90	2.88	1.000
-2-Me		2.53	2.31	3.22	1.000
-3-Me		2.80	2.50	3.34	0.999
-5-Me		2.68	2.45	3.32	1.000
-2-CO_2Me		2.78^d	2.84	3.94	1.000
-2-CO_2Et		3.22^d	3.35	4.39	1.000
-2-CONHMe		1.90^d	1.85	3.24	0.999
-2-CONHEt		2.32^d	2.17	3.49	0.999
-2-CONHPr		2.80^d	2.59	3.87	0.999
-2-$CONH_2$		1.61^d	1.69	3.17	1.000
-3-CO_2Me		2.57^d	2.54	3.83	0.999
-3-CO_2Et		3.04	3.07	4.32	0.999

TABLE I (*continued*)

Compound	System	Log P^a	Log k_w ^{b}	S^b	r^c
-3-CONHMe		1.25[d]	1.35	2.96	0.999
-3-CONHEt		1.62[d]	1.71	3.24	0.999
1-Me-indole (1Me-In)	*N*	2.64	2.60	3.27	1.000
Benzene (Bz)		2.13	1.99	2.48	0.999
-Me		2.69	2.59	2.99	1.000
-CO_2Me		2.12	2.34	3.23	1.000
-CO_2Et		2.67	2.90	3.73	0.999
-CONHMe		0.90[d]	1.00	2.42	0.999
-CONHEt		1.28[d]	1.29	2.61	0.999
-CONHPr		1.72[d]	1.70	2.95	0.999
-$CONH_2$		0.64	0.86	2.42	0.999

[a] Taken from refs. 13 and 14, unless indicated otherwise.
[b] See eqn. 1.
[c] Correlation coefficient.
[d] This work.

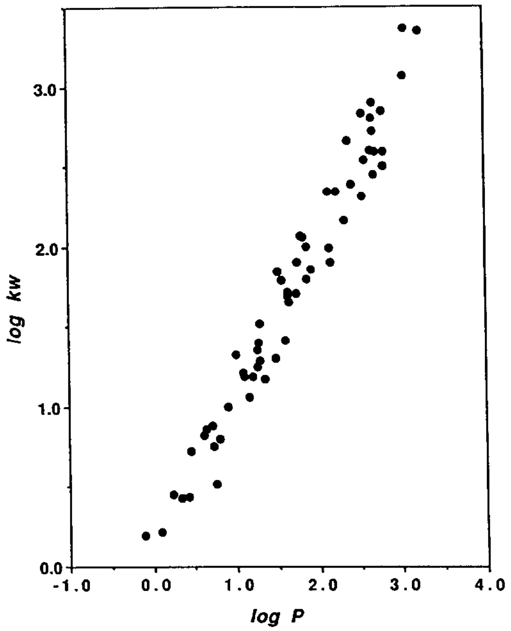

Fig. 1. Relationship between log k_w and log P for all compounds given in Table I.

deviation and F is the value of the F-ratio between regression and residual variances.

Effect of the ester group (H-acceptor effect). To estimate the contribution of the H-acceptor effect attributable to the ester group, analyses for the parent compounds and the esters of system N were carried out and eqns. 5 and 6 were obtained:

$$\log k_w = 0.938 \log P + 0.223$$

$$n = 16, r = 0.948, s = 0.191, F = 124 \qquad (5)$$

$$\log k_w = 1.019 \log P + 0.351 HB_E - 0.097$$

$$n = 16, r = 0.995, s = 0.061, F = 674 \qquad (6)$$

Addition of the parent and ester compounds of system H to the above data set gave the following correlations:

$$\log k_w = 0.998 \log P + 0.042$$

$$n = 31, r = 0.956, s = 0.210, F = 309 \qquad (7)$$

$$\log k_w = 0.990 \log P + 0.334 HB_E - 0.175 HB_H - 0.041$$

$$n = 31, r = 0.997, s = 0.059, F = 1437 \qquad (8)$$

In eqn. 8, the coefficients of the HB terms are very close to those in eqns. 4 and 6.

Effect of the amide group (amphiprotic effect). Similarly, the contribution of amide groups was described by eqn. 10, derived from the parent compounds and the amide derivatives of system N:

TABLE II

OBSERVED AND CALCULATED HYDROPHOBICITY PARAMETERS AND HYDROGEN-BOND PARAMETERS

Compound	Log k_w (Δ^b) calcd.^{a}	Log k'_{M50} obsd.	Log k'_{M50} (Δ^d) calcd.^{c}	Log P (Δ^e) calcd.^{c}	HB_E	HB_{AM}	HB_H	HB_D
Furan (Fr)	1.29 (−0.12)	0.259	0.25 (0.01)	1.37 (−0.03)	0	0	0	0
-2-Me	1.78 (0.01)	0.599	0.54 (0.06)	1.96 (−0.11)	0	0	0	0
-2-Et	2.31 (0.08)	0.926	0.84 (0.09)	2.54 (−0.14)	0	0	0	0
-2-CO_2Me	1.31 (0.01)	0.043	0.06 (−0.02)	0.99 (0.02)	1	0	0	0
-2-CO_2Et	1.79 (0.05)	0.351	0.34 (0.01)	1.53 (−0.03)	1	0	0	0
-2-CONHMe	0.41 (0.04)	−0.609	−0.57 (−0.04)	0.18 (0.05)	0	1	0	1
-2-CONHEt	0.77 (0.05)	−0.359	−0.36 (0.00)	0.62 (−0.01)	0	1	0	1
-2-CONHPr	1.24 (0.04)	−0.072	−0.08 (0.01)	1.13 (−0.03)	0	1	0	1
-2-$CONH_2$	0.08 (0.11)	−0.847	−0.76 (−0.09)	−0.24 (0.13)	0	1	0	1
-3-CO_2Me	1.58 (−0.06)	0.223	0.22 (0.00)	1.30 (−0.02)	1	0	0	0
-3-CO_2Et	2.06 (0.01)	0.528	0.50 (0.03)	1.84 (−0.06)	1	0	0	0
-3-CO_2Pr	2.61 (0.05)	0.858	0.82 (0.04)	2.42 (−0.06)	1	0	0	0
-3-CONHMe	0.51 (−0.08)	−0.561	−0.51 (−0.05)	0.27 (0.07)	0	1	0	1
-3-CONHEt	0.88 (−0.13)	−0.335	−0.30 (−0.04)	0.67 (0.06)	0	1	0	1
-3-CONHPr	1.34 (−0.15)	−0.066	−0.03 (−0.04)	1.14 (0.06)	0	1	0	1
-3-$CONH_2$	0.27 (−0.05)	−0.756	−0.65 (−0.11)	−0.08 (0.17)	0	1	0	1
Benzofuran (BF)	2.57 (0.15)	0.992	1.00 (−0.01)	2.65 (0.02)	0	0	0	0
-2-CO_2Me	2.78 (0.05)	0.912	0.92 (−0.01)	2.51 (0.02)	1	0	0	0
-2-CO_2Et	3.28 (0.09)	1.198	1.21 (−0.01)	3.02 (0.03)	1	0	0	0
-2-CONHMe	1.96 (0.04)	0.293	0.34 (−0.05)	1.77 (0.08)	0	1	0	1
-2-CONHEt	2.32 (0.02)	0.494	0.55 (−0.06)	2.12 (0.10)	0	1	0	1
-2-CONHPr	2.73 (0.07)	0.733	0.79 (−0.06)	2.54 (0.11)	0	1	0	1
-2-$CONH_2$	1.66 (0.13)	0.135	0.16 (−0.03)	1.49 (0.05)	0	1	0	1
Pyrrole (Pyr)	0.58 (−0.06)	−0.279	−0.28 (0.00)	0.76 (−0.01)	0	0	1	1
-2-Et	1.39 (0.03)	0.236	0.19 (0.05)	1.67 (−0.08)	0	0	1	1
-2,5-di-Me	1.27 (0.03)	0.178	0.12 (0.06)	1.57 (−0.10)	0	0	1	1
-2-CO_2Me	1.42 (−0.02)	0.048	0.01 (0.04)	1.34 (−0.07)	1	0	1	1
-2-CO_2Et	1.86 (0.04)	0.331	0.27 (0.06)	1.84 (−0.11)	1	0	1	1
-2-CONHMe	0.44 (0.00)	−0.600	−0.66 (0.06)	0.54 (−0.12)	0	1	1	2
-2-CONHEt	0.80 (−0.01)	−0.362	−0.45 (0.09)	0.96 (−0.16)	0	1	1	2
-2-CONHPr	1.24 (0.01)	−0.086	−0.20 (0.11)	1.45 (−0.20)	0	1	1	2
-2-$CONH_2$	0.13 (0.09)	−0.804	−0.85 (0.05)	0.18 (−0.09)	0	1	1	2
1-Me-pyrrole (1Me-Pry)	1.11 (−0.05)	0.184	0.14 (0.04)	1.23 (−0.08)	0	0	0	0
-2-CO_2Me	2.08 (−0.02)	0.521	0.51 (0.01)	1.83 (−0.03)	1	0	0	0
-2-CONHMe	0.87 (0.01)	−0.303	−0.30 (−0.00)	0.72 (−0.01)	0	1	0	1
-2-CONHEt	1.23 (−0.02)	−0.086	−0.09 (0.00)	1.10 (−0.01)	0	1	0	1
-2-CONHPr	1.75 (−0.10)	−0.169	0.21 (−0.04)	1.55 (0.08)	0	1	0	1
-2-$CONH_2$	0.62 (0.10)	−0.458	−0.45 (−0.01)	0.45 (0.00)	0	1	0	1
Indole (In)	1.92 (−0.02)	0.474	0.50 (−0.03)	2.09 (0.05)	0	0	1	1
-2-Me	2.29 (0.02)	0.708	0.72 (−0.01)	2.50 (0.03)	0	0	1	1
-3-Me	2.55 (−0.05)	0.866	0.87 (−0.00)	2.78 (0.02)	0	0	1	1
-5-Me	2.43 (0.02)	0.797	0.80 (−0.00)	2.66 (0.03)	0	0	1	1
-2-CO_2Me	2.87 (−0.03)	0.849	0.86 (−0.01)	2.75 (0.03)	1	0	1	1
-2-CO_2Et	3.29 (0.06)	1.126	1.11 (0.02)	3.23 (−0.01)	1	0	1	1
-2-CONHMe	1.86 (−0.01)	0.212	0.17 (0.04)	1.97 (−0.07)	0	1	1	2
-2-CONHEt	2.27 (−0.10)	0.393	0.40 (−0.01)	2.29 (0.03)	0	1	1	2
-2-CONHPr	2.73 (−0.14)	0.630	0.67 (−0.04)	2.70 (0.10)	0	1	1	2
-2-$CONH_2$	1.58 (0.11)	0.085	0.00 (0.09)	1.75 (−0.14)	0	1	1	2

TABLE II (*continued*)

Compound	Log k_w (Δ^b) calcd.[a]	Log k'_{M50} obsd.	Log k'_{M50} (Δ^d) calcd.[c]	Log P (Δ^e) calcd.[c]	HB_E	HB_{AM}	HB_H	HB_D
-3-CO_2Me	2.67 (−0.13)	0.589	0.74 (−0.15)	2.29 (0.28)	1	0	1	1
-3-CO_2Et	3.12 (−0.05)	0.865	1.01 (−0.15)	2.78 (0.27)	1	0	1	1
-3-CONHMe	1.24 (0.11)	−0.163	−0.20 (0.04)	1.31 (−0.06)	0	1	1	2
-3-CONHEt	1.60 (0.11)	0.053	0.01 (0.04)	1.69 (−0.07)	0	1	1	2
1-Me-indole (1Me-In)	2.54 (0.06)	0.967	0.98 (−0.01)	2.61 (0.03)	0	0	0	0
Benzene (Bz)	2.05 (−0.06)	0.766	0.69 (0.08)	2.26 (−0.13)	0	0	0	0
-Me	2.59 (0.00)	1.109	1.01 (0.10)	2.86 (−0.17)	0	0	0	0
-CO_2Me	2.38 (−0.04)	0.706	0.69 (0.02)	2.15 (−0.03)	1	0	0	0
-CO_2Et	2.91 (−0.01)	1.006	1.00 (0.01)	2.68 (−0.01)	1	0	0	0
-CONHMe	1.05 (−0.05)	−0.240	−0.20 (−0.04)	0.83 (0.07)	0	1	0	1
-CONHEt	1.42 (−0.13)	−0.047	0.02 (−0.07)	1.17 (0.11)	0	1	0	1
-CONHPr	1.84 (−0.14)	0.200	0.26 (−0.06)	1.61 (0.12)	0	1	0	1
-$CONH_2$	0.80 (0.06)	−0.377	−0.34 (−0.04)	0.59 (0.05)	0	1	0	1

[a] Calculated by eqn. 14.

[b] Difference between observed and calculated log k'_w values by eqn. 14.

[c] Calculated by eqn. 21.

[d] Difference between observed and calculated log k'_{M50} values by eqn. 21.

[e] Difference between observed and calculated log P values by eqn. 21.

$$\log k_w = 0.918 \log P + 0.186$$

$$n = 28, r = 0.990, s = 0.111, F = 1306 \qquad (9)$$

$$\log k_w = 0.976 \log P + 0.181 HB_{AM} - 0.020$$

$$n = 28, r = 0.994, s = 0.089, F = 1026 \qquad (10)$$

Addition of the corresponding compounds of system H produced eqns. 11 and 12:

$$\log k_w = 0.895 \log P + 0.166$$

$$n = 45, r = 0.987, s = 0.129, F = 1555 \qquad (11)$$

$$\log k_w = 0.957 \log P + 0.173 HB_{AM} - 0.140 HB_H + 0.010$$

$$n = 45, r = 0.995, s = 0.081, F = 1327 \qquad (12)$$

Again, the coefficients in eqn. 12 are very close to those in eqns. 4 and 10.

As the pre-analyses gave very stable correlations, we attempted analyses with the combined data set including all the compounds studied and obtained an excellent correlation (eqn. 14), which was much improved in comparison with eqn. 13:

$$\log k_w = 0.950 \log P + 0.145$$

$$n = 61, r = 0.981, s = 0.165, F = 1478 \qquad (13)$$

$$\log k_w = 0.960 \log P + 0.340 HB_E + 0.178 HB_{AM} - 0.147 HB_H + 0.008$$

$$n = 61, r = 0.996, s = 0.077, F = 1759 \qquad (14)$$

As shown in Table II and Fig. 2, the log k_w values calculated by eqn. 14 agreed well with the observed values.

Relationship between the S parameter and log P

Several investigators [9,20] have found that the S parameter also correlates well with log P. The relationship between S and log P is shown in Fig. 3 for all the compounds studied. The correlation was poor ($r = 0.84$). It should be noted that the ester and amide derivatives behaved differently from the parent compounds in such a manner that esters and amides gave greater S values than parent compounds under the equivalent log P values. Chen *et al.* [20] have investigated in detail the properties of the S parameter. They demonstrated that the S value for a given solute is nearly constant regardless of the differ-

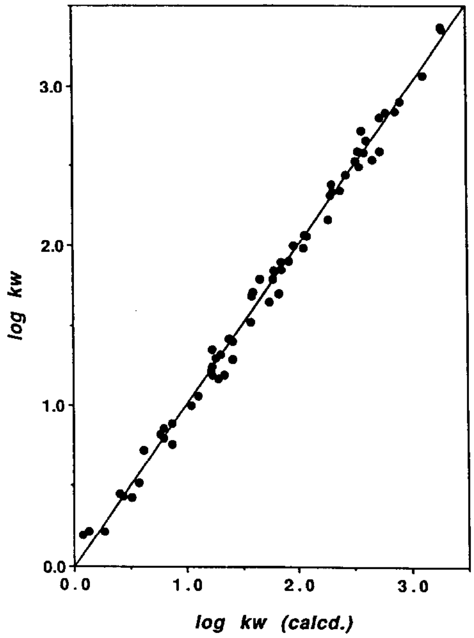

Fig. 2. Relationship between log k_w and the calculated log k_w values (eqn. 14).

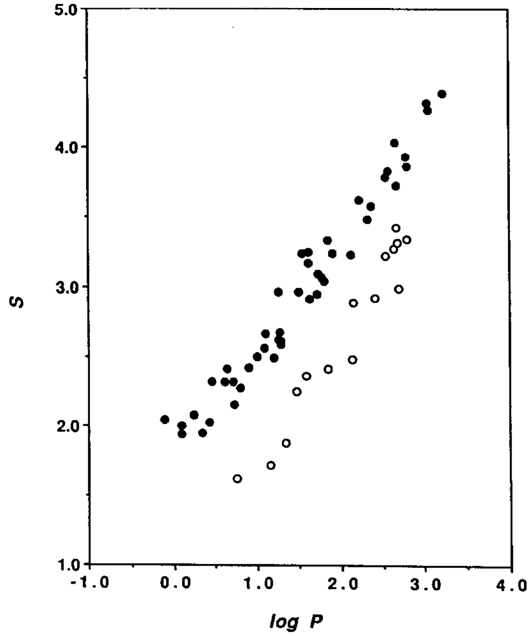

Fig. 3. Relationship between the S parameter (eqn. 1) and log P for all compounds given in Table I. ○ = Parent compounds; ● = ester and amide derivatives.

ent C_{18} packing agents, and concluded that the S value is determined mainly by the interaction between the solute and the mobile phase, meaning that the S value derived from the same mobile phase system reflects the nature of the solute. To confirm this point in our case, we determined the S values for the parent and ester compounds (31 compounds) with a Chemcopac ODS-H column and obtained results that agreed well with the S values in Table I with an average deviation of ±0.06. The features shown in Fig. 3 indicate that the retention of esters and amides is more dependent on the mobile phase composition, in other words, the hydrogen-bond ability of ester and amide groups is more sensitive to changes in the surrounding medium.

Relationship between isocratic log k′ and log P

The results presented above strongly suggest that the log k' *vs.* log P relationship varies with the methanol content in the mobile phase. Therefore, isocratic retention data were similarly analysed at 30, 50 and 70% methanol concentrations (M30, M50 and M70). Excellent correlations corresponding to eqn. 14 were obtained, as shown by eqns. 16, 18 and 20 in Table III.

It is of interest that the HB_E term was found to be insignificant in the correlation in the M50 eluent (eqn. 18), while the HB_{AM} term was found to be insignificant in the M30 eluent (eqn. 16). As the coefficients of the HB_{AM} and HB_H terms agreed within a 95% confidence interval in eqn. 18, we attempted to combine both terms into a common hydrogen-donor parameter HB_D:

$$\log k_{M50} = 0.561 \log P - 0.198 HB_D - 0.503$$

$$n = 61, r = 0.995, s = 0.055, F = 2898 \qquad (21)$$

where HB_D ($= HB_H + HB_{AM}$) takes the values 0, 1 and 2 depending on the number of H-donating sites contained in the molecule, that is $HB_D = 1$ for compounds with –CONH– or ring-N*H*, $HB_D = 2$ for those with both –CONH– and ring-N*H*, and $HB_D = 0$ for the others. As shown in Fig. 4 and Table II, eqn. 21, the simplest correlation obtained in this work, could predict the log P values satisfactorily. This treatment may be rationalized by the reason given later.

TABLE III

COEFFICIENTS FOR CORRELATIONS OBTAINED BY EQN. 2 AT EACH MOBILE PHASE COMPOSITION

Log $k' = a \log P + b_E HB_E + b_{AM} HB_{AM} + b_H HB_H + c$.

Mobile phase	Term					n	r	s	F	Eqn. No.
	Log P	HB_E	HB_{AM}	HB_H	c					
M0[a]	0.950				0.145	61	0.981	0.165	1478	13
	0.960	0.340	0.178	−0.147	0.008	61	0.996	0.077	1759	14
M30	0.743				−0.372	61	0.983	0.121	1675	15
	0.729	0.167	(0)[b]	−0.163	−0.332	61	0.997	0.053	2959	16
M50	0.604				−0.746	61	0.967	0.140	838	17
	0.549	(0)	−0.229	−0.174	−0.476	61	0.996	0.052	2117	18
	0.466				−1.072	61	0.925	0.166	351	19
M70	0.399	−0.119	−0.335	−0.187	−0.696	61	0.989	0.065	653	20

[a] Figures after M represent the volume-% of methanol

[b] Statistically insignificant.

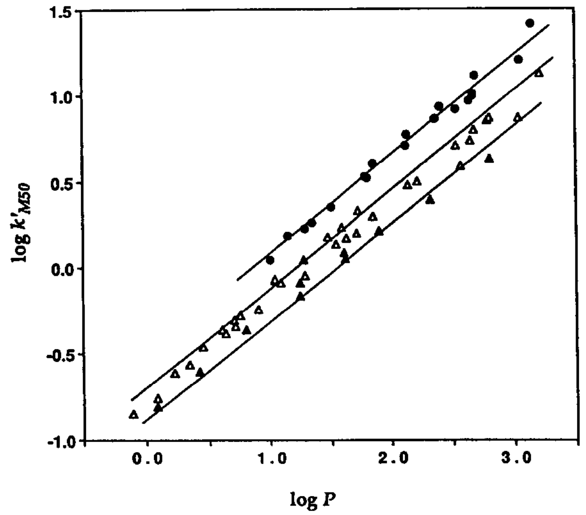

Fig. 4. Relationship between log k' and log P by eqn. 21. ● = parent compounds ($HB_D = 0$); △ = compounds with $HB_D = 1$; ▲ = compounds with $HB_D = 2$.

DISCUSSION

It is known that an HPLC system using an alkyl-bonded stationary phase often discriminates among solutes according to the hydrogen-bond properties. Usually, log k'–log P plots give a good linear relationship among the same congeners, but tend to give separate correlations for different groups of congeners [6,7,9,21–24]. The procedure employed in this work is based on this fact. Our results revealed that the relationship between hydrohobicity indices derived from RPLC and octanol–water systems can be excellently expressed, as a first approximation, by the general eqn. 2 as far as the compounds studied are concerned. This means that the hydrogen-bond effects attributed to ester, ring-N*H* and amide groups are approximately additive. The fact that discrete type parameters, *HB*, worked well suggests that the same type of substituents (*e.g.*, CO_2R) have comparable hydrogen bond abilities regardless of the kind of aromatic system to which they are attached. This reasoning may be rationalized by considering that H-acceptor abilities, β, for $ArCO_2R$ are approximately equivalent [25].

When the corresponding coefficients of each term were compared as a function of the mobile phase composition, systematic changes were observed. The coefficients of log P, HB_E and HB_{AM} terms were seen to decrease linearly with increase in methanol concentration. Especially the plot of the coefficient of log P against the volume fraction of methanol showed perfect linearity with a correlation coefficient better than 0.999, yielding an intercept of 0.97, which is very close to unity. A similar plot with the *c* values gave an intercept close to zero. These results demonstrate not only that the log k_w parameter can be a direct indication of the log *P* value for non-hydrogen bonders, but also that the hydrophobic component (log *P* term) can be successfully separated from other correction terms ($\Sigma\, b_i HB_i$), if required, supporting the validity of eqn. 2.

As is clear in eqn. 14, the log k_w value involves a positive contribution from the ester group (H-acceptor) and a negative contribution from the ring N*H* group (H-donor), leading to overestimated log *P* values for esters and underestimated values for compounds with the ring-N*H* group, if we estimate log *P* values from their log k_w values. The amide group (amphiprotic group) made a small positive contribution for the reason described below. The overall hydrogen-bond effect of amides could be treated as the sum of effects of –C(=*O*)*N*H– (H-acceptor) and –C(=O)N*H*– (H-donor) moieties. It is interesting that the sum of the coefficients of HB_E and HB_H terms is approximately equivalent to the coefficient of the HB_{AM} term in all eluents. This finding led us to hypothesize that the H-accepting ability of –C(=*O*)*O*R is similar to that of –C(=*O*)*N*H– and also the H-donating ability of the ring N*H* is similar to that of –C(=O)N*H*–. Thus, the fact that the HB_{AM} term is insignificant at 30% MeOH concentration, as shown in eqn. 16, would be explained by considering that the H-acceptor and H-donor abilities of the amide group are compensated around this eluent composition (note that the coefficients of HB_E and HB_H are similar values of opposite signs). In more water-rich eluents, the H-acceptor effect probably exceeds the H-donor effect in the amide group, judging from the HB_E and HB_H values for M0, thereby yielding a positive coefficient of the HB_{AM} term. This is the case observed in eqn. 14.

As for the HB_H term, the change in coefficient was very small and fairly constant, and the more important is that the coefficient of the HB_H term was negative in all the equations obtained. This is a phenomenon usually observed [7,9,16,24,26, 29] and thought to be inevitable so long as alkyl-bonded stationary phases are used because H-donors are more hydrophobic relative to non-H-donors in the octanol–water system than the RPLC system owing to the higher basicity of octanol than methanol and stationary phases. An explanation regarding this problem was given in detail in a previous paper [11].

As mentioned above, the M50 eluent hardly differentiated H-acceptors from non-hydrogen bonders. Under this condition, the amide group is thought to behave as an H-donor. This is the reason why the two parameters HB_H and HB_{AM} could be reasonably unified into HB_D. We have already reported [11,13] analogous findings that H-acceptors and non-hydrogen bonders give a single log *k*′–log *P* linearity in the M50 eluent. The utility of this mobile phase composition was further verified by applying eqn. 21 to other H-acceptors not included in Table II. The compounds tested and the results of analyses are summarized in Table IV.

Pyrazine has two strong H-accepting sites in the aromatic nucleus whereas the $CONMe_2$ substituent is a stronger H-acceptor than CO_2R [25,27,28]. It is clearly shown that the log *P* values calculated by eqn. 21 agreed with the log *P* values fairly well whereas their log k_w values gave overestimated values. Several other investigators [29–32] have reported that isocratic log *k*′ data determined in eluents containing around 50% MeOH yield a better correlation with log *P* than the log k_w data do.

The ester effect was observed in a similar manner when we compared octanol–water log *P* values (log P_{oct}) of monosubstituted diazines with those determined from the chloroform–water partition system (log P_{CL}) [15]. The plot of log P_{CL} against log P_{oct} (Fig. 1 in ref. 15) presents a good linear relationship through the points for substituents such as H, alkyl, OR, NMe_2, SMe with CO_2R as positive deviants, presenting a feature which is very similar to the corresponding log *k*′–log *P* plot in water-rich eluents (Fig. 1 in ref. 11). We have explained this result in terms of stronger H-accepting ability of CO_2R groups than other well behaved substituents and of the number of hydrogen-bondable sites [15]. We tried the same treatment on a furan series consisting of furan, alkylated furans

TABLE IV

APPLICATION OF EQN. 21 TO OTHER COMPOUNDS CONTAINING STRONG H-ACCEPTORS

Compound	Log P[a]	Log k_w[b]	Δ[c]	Log k'_{M50} obsd.	Log k'_{M50} (Δ[e]) calcd.[d]	Log P (Δ[f]) calcd.[d]	HB_D
FR-2CONMe$_2$	0.41	0.92	0.51	−0.311	−0.27 (−0.04)	0.36 (0.05)	0
FR-3CONMe$_2$	0.24	0.81	0.57	−0.380	−0.37 (−0.01)	0.24 (−0.00)	0
BF-2CONMe$_2$	1.85[g]	2.42	0.57	0.535	0.54 (−0.01)	1.85 (−0.00)	0
In-2CONMe$_2$	1.96[g]	2.40	0.44	0.518	0.40 (0.12)	2.16 (−0.21)	1
In-3-OAc	2.03[g]	2.30	0.27	0.494	0.44 (0.05)	2.12 (−0.09)	1
1Me-In-2CO$_2$Me	3.37[g]	–[h]	–	1.447	1.39 (0.06)	3.45 (−0.08)	0
BZ-CONMe$_2$	0.62	1.42	0.80	−0.026	−0.16 (0.13)	0.86 (−0.24)	0
BZ-Ac	1.58	1.78	0.20	0.347	0.38 (−0.03)	1.52 (0.06)	0
Pyrazine (Pr)	−0.26	0.15	0.41	−0.590	−0.65 (0.06)	−0.13 (−0.13)	0
Pr-Me	0.21	0.49	0.28	−0.383	−0.39 (0.01)	0.24 (−0.03)	0
Pr-Et	0.69	1.02	0.33	−0.084	−0.12 (0.04)	0.76 (−0.07)	0

[a] Taken from refs. 5 and 13 unless indicated otherwise.
[b] Derived by eqn. 1.
[c] Difference between log k_w and log P values.
[d] Calculated by eqn. 21.
[e] Difference between observed and calculated log k'_{M50} values.
[f] Difference between observed and calculated log P values.
[g] This work.
[h] Not obtained because the retention time was too long in the eluent containing 30% MeOH.

and ester derivatives. Here again, esters and non-hydrogen-bonding substituents gave separate plots, reflecting the difference in association of the CO_2R moiety with octanol relative to that with $CHCl_3$ (not shown). These results confirm that the partition of ester derivatives, being strong H-acceptors, varies more sensitively with the change in the partitioning system. All those described above led us to conclude that the outstanding behaviour exerted by the ester group is ascribed to the solute–solvent interaction and not phenomena characteristic of the chromatographic retention process. The fact that the parameters S for esters are larger relative to the parent compounds confirms this argument.

The log k_w parameter is preferred by most investigators in predicting log P [6,9,21,33,34]. The merits pointed out are that the selective solute–solvent interactions can be eliminated [6] and the hydrogen-bond effects are reduced by using log k_w [23]. On the other hand, other results demonstrate that isocratic log k' data yield improved correlations. We can obtain an insight into this problem from the present results. Inspection of Table III reveals that the optimum mobile phase composition would vary depending on the hydrogen-bond properties of solutes. If a data set contains no hydrogen-donor components, an isocratic method with the use of an eluent containing about 50% methanol, yielding a single linear relationship, would be recommended. On the other hand, for a data set without H-acceptors, the log k_w method would improve the correlation because the contribution of the hydrogen-donor effect becomes minimal at 0% methanol concentration. As amphiprotic solutes can be treated as the sum of H-donor and H-acceptor components, the overall effect should depend on the relative hydrogen-bond ability of H-donating and H-accepting sites in the substituent. There would be cases where the log k_w parameter can be a good indication of log P

without corrections. This is probably because an over-estimating effect of H-accepting site(s) is almost cancelled by an under-estimating effect of H-donating site(s) in the log k_w value. Such examples can be found in practice in this work. When the difference between two opposite effects is not so large, the log k_w method might apparently work.

If the data set consists of solutes with different hydrogen-bond properties, our systematic studies dealing with simple O- and N-containing aromatic systems revealed that an eluent containing around 50% methanol, in which amphiprotic solutes are expected to behave only as H-donors, would give the simplest correlation with log P, permitting reliable log P values to be derived, provided that the hydrogen-donating components are treated separately as shown by eqn. 21. Further investigations will be needed to elucidate the hydrogen-bond effects of amphiprotic solutes on the correlation between chromatographic hydrophobicity and octanol–water partition coefficients. Our future work will deal with this problem.

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