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# Hydrophobic Properties of Anticonvulsant Phenylacetanilides. Relationship between Octanol–Water Partition Coefficient and Capacity Factor Determined by Reversed-Phase Liquid Chromatography

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Partition coefficients (octanol–water), $P$, for a series of phenylacetanilides, $\mathrm{PhCH_2CONHC_6H_4}$–$\mathrm{X}_{m,p}$ (I), were determined by the shaking-flask method. The observed $\pi$ values ($\pi = \log P_{(\mathrm{I})} - \log p_{\mathrm{PhCH_2CONHPh}}$) were analyzed in terms of substituent effects to give the relationship $\pi = 0.833\pi_x + 0.788\sigma_x + 0.040$, where $\pi_x = \log_{\mathrm{Phx}} - \log_{\mathrm{PhH}}$.

We also determined and analyzed the capacity factors for these compounds, $k'$, which are regarded as an alternative hydrophobic parameter, by using reversed-phase high-performance liquid chromatography (RP-HPLC) in different $\mathrm{H_2O}$–organic solvent mixtures. It was observed that the substituent effects on the $\log k'$ value are influenced by the organic modifier in the mobile phase. Linear relationships between $\log P$ and $\log k'$ were generally observed but there were some outliers such as OH, CN and $\mathrm{NO_2}$ derivatives.

The deviation of phenolic compounds from the $\log P - \log k'$ linearity can be explained in terms of the difference in partitioning behavior between the octanol–water system and the lipophilic stationary phase–mobile phase system.

**Keywords**——partition coefficient; octanol–water; structure–activity relationship; anticonvulsant activity; *m*-, *p*-substituted phenylacetanilides; hydrophobic parameter; reverse-phase high-performance liquid chromatography; capacity factor; mobile phase; ODS column

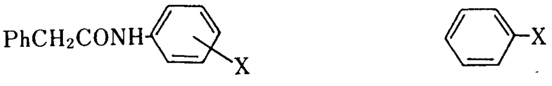
The hydrophobic property of biologically active compounds is an important characteristic in quantitative structure–activity relationship (QSAR) studies. The $\log P$ value ($P$ is the octanol–water partition coefficient) has been most widely used as an index of hydrophobicity. Since the conventional shaking-flask method is laborious and time-consuming, attempts to predict $\log P$ values by calculation have often been made. For example, Fujita has recently proposed equations to evaluate partition coefficients for *meta*- and *para*-disubstituted benzene systems, Y–$\mathrm{C_6H_4}$–$\mathrm{X}_{m,p}$ (Y and X represent fixed and variable substituents, respectively), by taking into account the electronic interactions between X and Y (see Discussion).[1] This approach should be useful in cases where strong X–Y interactions exist.

On the other hand, much effort has recently been directed to establishing practical experimental techniques for determining hydrophobicity by the use of reversed-phase high-performance liquid chromatography (RP-HPLC).[2] In this case, the capacity factor defined by eq. 1 is used as a measure of the hydrophobic property,

$$k' = (t_R - t_0)/t_0 \qquad (1)$$

where, $t_R$ and $t_0$ are the retention times of a substance and a nonretained substance, respectively. A combination of an octanol-coated column (stationary phase) and octanol-saturated water (mobile phase) was previously employed since the results were expected to reflect exactly the partition behavior in the traditional octanol–water system.[2a–c] However, this method has some shortcomings:2e—g) (i) it is difficult to prepare a long-lived coated column, (ii) the use of water as the mobile phase results in a very long retention time for highly lipophilic compounds. For these reasons, commercial ODS columns have become commonly used with water–organic mobile phase systems.2d—f) The problem in utilizing such a system is the choice of a suitable mobile phase which gives a linear relationship with octanol–water partition coefficients.

In connection with our QSAR studies, we found that phenyl acetanilides such as (I) (Chart 1) have pronounced anticonvulsant activity, and we studied their electronic properties on the basis of the carbon 13 nuclear magnetic resonance ($^{13}$C-NMR) spectra.3) The present work was undertaken to determine the hydrophobic parameters (log $P$ and log $k'$) for *m*- and *p*-substituted anilides of phenylacetic acid (I), since hydrophobicity is one of the most important properties in the QSAR of central nervous system (CNS) agents. The measurements were made by the shaking-flask method as well as the HPLC method, and the results were analyzed by applying Fujita's approach. We also discuss how far the HPLC method can be employed in predicting log $P$ values.



I II

Chart 1

## Experimental

**Materials**——The preparation of most of the compounds studied has already been described.3) The synthesis of the others will be reported elsewhere. The samples were purified by recrystallization.

**Measurement of *P***——Partition coefficients of (I) were measured by the conventional shaking-flask method.4) The concentration in the water phase was determined spectrophotometrically (Hitachi 220 spectrometer). Determinations were performed at three different concentrations at least.

**HPLC Procedure**——HPLC was performed on a Shimadzu LC5A liquid chromatograph equipped with a Model 7125 valve loop injector (Rheodyne), and SPD-2A UV (Shimadzu) and Shodex SE-31 refractive index (Shoden) detectors. Retention times were determined by using the C-R2AX Chromatopac (Shimadzu). A commercial ZORBAX ODS column (4.6 × 250 mm, Dupont) was used without further treatment. Samples were dissolved in methanol and 1—2 $\mu$l of solution was injected. The amount of sample had no effect on the retention time. The peak of methanol was used to determine $t_0$ value. The reproducibility of log $k'$ was about 0.01.

## Results and Discussion

### Analysis of log *P*

The newly determined log $P$ values of phenylacetanilides (**1**—**28**) are listed in Table I together with their $\pi$ values defined by

$$\pi = \log P_{X/PhY} - \log P_{PhY}$$

where $P_{X/PhY}$ and $P_{PhY}$ are the partition coefficients of the substituted (**2**—**28**) and reference (**1**) compounds, respectively. It can be seen that some $\pi$ values considerably differ from the $\pi$ values of monosubstituted benzene with the same substituent X (PhX), $\pi_x$,5) indicating that the electronic interactions between the fixed substituent Y (PhCH$_2$CONH) and X cause variations in the hydrogen-bonding solvation of substituents, and therefore the partition coefficient is not an additive property in the present system.

For the purpose of making appropriate corrections for such electronic interactions, Fujita has recently proposed eq. 2 for *m*- and *p*-disubstituted benzenes with fixed Y and variable X substituents,1)

TABLE I. Values of log $P$ (Octanol–Water) of Substituted Phenylacetanilides (I)

| No. | X | log $P$ | $\pi_{\mathrm{obsd}}$ | $\pi_{\mathrm{calcd}}$[a] | $\Delta\pi$[b] | $\sigma$ |
|---|---|---|---|---|---|---|
| **1** | H | 2.70 | 0.00 | 0.04 | −0.04 | 0.00 |
| **2** | *m*-Me | 3.14 | 0.44 | 0.45 | −0.01 | −0.07 |
| **3** | *m*-Et | 3.47 | 0.77 | 0.83 | −0.06 | −0.07 |
| **4** | *m*-F | 3.10 | 0.40 | 0.42 | −0.02 | 0.34 |
| **5** | *m*-Cl | 3.61 | 0.91 | 0.92 | −0.01 | 0.37 |
| **6** | *m*-Br | 3.77 | 1.07 | 1.07 | 0.00 | 0.39 |
| **7** | *m*-I | 3.91 | 1.21 | 1.25 | −0.04 | 0.35 |
| **8** | *m*-$CF_3$ | 3.84 | 1.14 | 1.11 | 0.03 | 0.43 |
| **9** | *m*-OH | 2.22 | −0.48 | −0.42 | −0.06 | 0.12 |
| **10** | *m*-$NH_2$ | 1.59 | −1.12 | −1.11 | −0.01 | −0.16 |
| **11** | *m*-NHMe | 2.31 | −0.39 | −0.59 | 0.20 | −0.30 |
| **12** | *m*-NHEt | 2.59 | −0.11 | −0.08 | −0.03 | −0.24 |
| **13** | *m*-OMe | 2.86 | 0.16 | 0.12 | 0.04 | 0.12 |
| **14** | *m*-CN | 2.75 | 0.05 | 0.01 | −0.04 | 0.56 |
| **15** | *m*-$NO_2$ | 2.93 | 0.23 | 0.37 | −0.14 | 0.71 |
| **16** | *m*-COMe | 2.62 | −0.08 | −0.12 | 0.04 | 0.38 |
| **17** | *m*-OAc | 2.37 | −0.33 | −0.19 | −0.14 | 0.39 |
| **18** | *m*-OEt | 3.23 | 0.53 | 0.44 | 0.09 | −0.10 |
| **19** | *m*-$OSO_2Me$ | 2.32 | −0.38 | −0.39 | 0.01 | 0.39 |
| **20** | *p*-Me | 3.05 | 0.35 | 0.37 | −0.02 | −0.17 |
| **21** | *p*-F | 2.86 | 0.16 | 0.20 | −0.04 | 0.06 |
| **22** | *p*-Cl | 3.54 | 0.84 | 0.81 | 0.03 | 0.23 |
| **23** | *p*-Br | 3.70 | 1.00 | 0.94 | 0.06 | 0.23 |
| **24** | *p*-OH | 1.82 | −0.88 | −0.81 | −0.07 | −0.37 |
| **25** | *p*-OMe | 2.49 | −0.21 | −0.19 | −0.02 | −0.27 |
| **26** | *p*-CN | 2.82 | 0.12 | 0.09 | 0.03 | 0.66 |
| **27** | *p*-$NO_2$ | 3.18 | 0.48 | 0.42 | 0.06 | 0.78 |
| **28** | *p*-COMe | 2.75 | 0.05 | −0.02 | 0.07 | 0.50 |

*a*) Calculated from eq. 3. *b*) $\Delta\pi = \pi_{\mathrm{cald}} - \pi_{\mathrm{obsd}}$.

$$\pi = a\pi_{\mathrm{X}} + \rho_{\mathrm{Y}}\sigma_{\mathrm{X}} + \rho_{\mathrm{X}}\sigma_{\mathrm{Y}}(\mathit{meta}\text{ or }\mathit{para}) + c \qquad (2)$$

where $\pi_{\mathrm{x}}$ is the hydrophobic substituent constant for monosubstituted benzenes, $\sigma_{\mathrm{x}}$ and $\sigma_{\mathrm{Y}}$ are the Hammett's constants of X and Y, respectively, and $\rho_{\mathrm{x}}$ and $\rho_{\mathrm{Y}}$ are constants which represent the susceptibility to hydrogen-bonding solvation at the X and Y substituent positions, respectively. The coefficients $a$, $\rho_{\mathrm{x}}$, $\rho_{\mathrm{Y}}$ and the intercept $c$ can be determined by the least-squares method. We applied this equation to our compounds and obtained an excellent correlation, eq. 3,

$$\begin{array}{l} \pi = 0.833\pi_{\mathrm{x}} + 0.788\sigma_{\mathrm{x}} + 0.040 \\ \quad (0.043) \quad (0.089) \quad (0.033) \end{array} \qquad (3)$$

$$n = 28,\ r = 0.993,\ \mathrm{S.D.} = 0.072$$

where $n$ is the number of compounds used for calculation, $r$ is the correlation coefficient, S.D. is the standard deviation, and the figures in parentheses are the 95% confidence intervals. Preliminary analyses using $\rho_{\mathrm{x}}$ values available in the literature and those estimated from data for closely related substituents showed that the $\rho_{\mathrm{x}}\sigma_{\mathrm{Y}}$ term is insignificant in eq. 3, which suggests that the $\sigma_{m,p}$ values of the Y-substituent ($PhCH_2CONH$) are fairly small. The calculated $\pi$ values and the difference between the observed and the calculated values are included in Table I. From eq. 3 we can predict accurately log $P$ of phenylacetanilides except for a few with polar substituents.

It is of interest to note that the coefficient of $\pi_x$ is smaller than that of acetanilides ($a$= 0.989).[1)] This appears to mean that the extent of "iceberg" formation produced by the introduction of the X-substituent in the monosubstituted benzene ring (Ph–Y) becomes lower as the fixed substituent (Y) becomes of larger size relative to the residual part of the molecule ($-C_6H_4X$).

### Capacity Factor. Relationship between $\log P$ and $\log k'$

The capacity factors, $k'$, of phenylacetanilides were measured by using $MeOH-H_2O$ mobile phases: $M_1$, $MeOH-H_2O$ (50:50); $M_2$, $MeOH-H_2O$ (70:30), by volume. Table II summarizes the results together with those for the corresponding available monosubstituted benzenes determined under the same conditions. Plots of $\log k'$ in mobile phase $M_1$ against $\log P$ values showed fairly good linear relationships as shown in (Fig. 1). The correlations obtained by the least-squares method are given in Table III. It can be seen that points corresponding to some substituents such as OH (**9**, **24**), OAc (**17**) and NHEt (**12**) deviate from linearity beyond the range of experimental error. Departures from linearity are also ob-

Table II. Capacity Factors and Related Quantities for Phenylacetanilides (Amide (I)) and Monosubstituted Benzenes (Ph–X(II))

| No. | X | Amide (I) | | | | | | Ph–X (II) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\log k'_{M_1}$[a)] | $\log k'_{M_2}$[b)] | $\log k'_{M_3}$[c)] | $\Delta\kappa'_{M_1}$[d)] | $\Delta\kappa'_{M_2}$[e)] | $\Delta\kappa'_{M_3}$[f)] | No.[g)] | $\log k'_{M_1}$[a)] | $\log k'_{M_2}$[b)] | $\log k'_{M_3}$[c)] |
| **1** | H | 0.91 | 0.11 | 0.97 | −0.10 | −0.07 | −0.12 | **1′** | 0.96 | 0.36 | 1.03 |
| **2** | *m*-Me | 1.22 | 0.31 | 1.23 | −0.03 | −0.03 | −0.08 | **2′** | 1.30 | 0.61 | 1.38 |
| **3** | *m*-Et | 1.48 | 0.49 | 1.54 | −0.01 | 0.00 | −0.02 | **3′** | 1.62 | 0.81 | 1.71 |
| **4** | *m*-F | 1.11 | 0.23 | 1.25 | −0.05 | −0.03 | −0.03 | **4′** | 0.98 | 0.33 | 1.07 |
| **5** | *m*-Cl | 1.40 | 0.44 | 1.53 | −0.02 | 0.00 | −0.02 | **5′** | 1.32 | 0.58 | 1.42 |
| **6** | *m*-Br | 1.50 | 0.50 | 1.63 | 0.00 | 0.00 | 0.00 | **6′** | 1.42 | 0.65 | 1.51 |
| **7** | *m*-I | 1.60 | 0.57 | 1.74 | 0.00 | 0.00 | 0.00 | **7′** | 1.57 | 0.76 | 1.69 |
| **8** | *m*-$CF_3$ | 1.62 | 0.51 | 1.79 | 0.09 | 0.07 | 0.11 | **8′** | 1.43 | 0.55 | 1.56 |
| **9** | *m*-OH | 0.46 | −0.28 | 0.60 | −0.02 | −0.01 | −0.03 | **9′** | 0.17 | −0.32 | 0.33 |
| **10** | *m*-$NH_2$ | 0.34 | −0.34 | 0.39 | 0.00 | 0.01 | 0.02 | **10′** | 0.13 | −0.32 | 0.16 |
| **11** | *m*-NHMe | 0.71 | −0.08 | 0.75 | 0.04 | 0.04 | 0.09 | **11′** | 0.64 | 0.06 | 0.65 |
| **12** | *m*-NHEt | 0.98 | 0.11 | 1.00 | 0.11 | 0.07 | 0.11 | **12′** | 0.87 | 0.25 | 0.92 |
| **13** | *m*-OMe | 1.00 | 0.15 | 1.04 | −0.05 | −0.05 | −0.07 | **13′** | 0.94 | 0.33 | 0.98 |
| **14** | *m*-CN | 0.93 | 0.06 | 1.02 | 0.03 | 0.03 | 0.06 | **14′** | 0.54 | −0.07 | 0.50 |
| **15** | *m*-$NO_2$ | 1.12 | 0.23 | 1.29 | 0.00 | 0.01 | 0.02 | **15′** | 0.75 | 0.15 | 0.82 |
| **16** | *m*-COMe | 0.90 | 0.04 | 0.87 | 0.02 | 0.00 | −0.03 | **16′** | 0.60 | 0.03 | 0.53 |
| **17** | *m*-OAc | 0.90 | 0.01 | 0.89 | −0.02 | −0.03 | −0.06 | **17′** | 0.64 | 0.01 | 0.59 |
| **18** | *m*-OEt | 1.28 | 0.33 | 1.29 | — | — | — | | | | |
| **19** | *m*-$OSO_2Me$ | 0.76 | −0.14 | 0.87 | — | — | — | | | | |
| **20** | *p*-Me | 1.20 | 0.29 | 1.22 | −0.01 | −0.02 | −0.05 | | | | |
| **21** | *p*-F | 1.01 | 0.16 | 1.11 | −0.05 | −0.02 | −0.04 | | | | |
| **22** | *p*-Cl | 1.38 | 0.43 | 1.52 | 0.02 | 0.02 | 0.03 | | | | |
| **23** | *p*-Br | 1.50 | 0.50 | 1.63 | 0.05 | 0.04 | 0.08 | | | | |
| **24** | *p*-OH | 0.33 | −0.39 | 0.43 | 0.03 | 0.03 | 0.04 | | | | |
| **25** | *p*-OMe | 0.89 | 0.06 | 0.90 | −0.02 | −0.03 | −0.02 | | | | |
| **26** | *p*-CN | 0.90 | 0.06 | 0.86 | −0.03 | 0.00 | −0.15 | | | | |
| **27** | *p*-$NO_2$ | 1.17 | 0.28 | 1.38 | 0.02 | 0.03 | 0.08 | | | | |
| **28** | *p*-COMe | 0.94 | 0.03 | 1.03 | 0.01 | −0.06 | 0.07 | | | | |

*a)* Mobile phase, $MeOH-H_2O$ (50:50). *b)* Mobile phase, $MeOH-H_2O$ (70:30).
*c)* Mobile phase, $MeOH-THF-H_2O$ (40:5:55).
*d—f)* Deviation from the predicted values calculated using eqs. 18—20.
*g)* Compounds *n* and *n′* have the same substituent X.

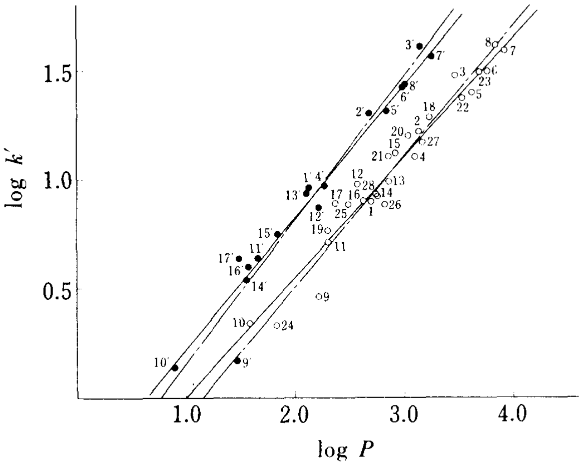


Fig. 1. Plot of $\log k'$ against $\log P$ in MeOH–$H_2O$ (50:50)

○, phenylacetanilides; ●, monosubstituted benzenes. — - —, slope obtained with all compounds; ——, slope calculated by omitting phenolic compounds (**9'**, **9**, **24**).

The compounds are numbered as in Table II.

TABLE III. Linear Correlations between $\log P$ and $\log k'$ for Phenylacetanilides (Amide (I)) and Monosubstituted Benzenes (Ph–X (II))

$$\log P = a \log k' + c \qquad (4)$$

| Mobile[a] phase | $a$ | $c$ | $n$[b] | $r$[c] | S.D.[d] | Eq. No. | $a$ | $c$ | $n$[b] | $r$[c] | S.D.[d] | Eq. No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Amide (I) | | | | | | Amide (I)[e] | | | |
| $M_1$ | 1.669<br>(0.143)[f] | 1.151<br>(0.159) | 28 | 0.978 | 0.125 | (5) | 1.791<br>(0.143) | 1.001<br>(0.164) | 26 | 0.983 | 0.106 | (8) |
| $M_2$ | 2.214<br>(0.203) | 2.548<br>(0.062) | 28 | 0.975 | 0.134 | (6) | 2.383<br>(0.208) | 2.497<br>(0.063) | 26 | 0.979 | 0.116 | (9) |
| $M_3$ | 1.561<br>(0.125) | 1.142<br>(0.150) | 28 | 0.981 | 0.119 | (7) | 1.591<br>(0.144) | 1.100<br>(0.177) | 26 | 0.978 | 0.120 | (10) |
| | | | Ph–X (II) | | | | | | Ph–X(II)[e] | | | |
| $M_1$ | 1.502<br>(0.175) | 0.782<br>(0.181) | 17 | 0.978 | 0.150 | (11) | 1.628<br>(0.112) | 0.632<br>(0.120) | 16 | 0.993 | 0.087 | (14) |
| $M_2$ | 1.936<br>(0.276) | 1.642<br>(0.122) | 17 | 0.968 | 0.182 | (12) | 2.111<br>(0.224) | 1.559<br>(0.100) | 16 | 0.983 | 0.132 | (15) |
| $M_3$ | 1.423<br>(0.105) | 0.775<br>(0.116) | 17 | 0.991 | 0.097 | (13) | 1.467<br>(0.091) | 0.716<br>(0.102) | 16 | 0.994 | 0.078 | (16) |

*a*) See Table II. *b*) Number of compounds used for correlations.
*c*) Correlation coefficients. *d*) Standard deviations.
*e*) Correlations obtained by omitting the OH-derivatives **9**, **24** and **9'**.
*f*) The 95% confidence intervals.

served for the same substituents in the monosubstituted benzene system. In particular, the deviations of OH compounds are remarkable and, in fact, the use of the $\log P - \log k'$ calibration line would underestimate $\log P$ values of OH-compounds by 0.2—0.5 log unit. The omission of these compounds from the calculations improved the correlations (Table III). Separate analyses for hydrogen bonders and non-bonders did not improve the correlations. Although the use of $k_0'$ values ($k_0'$ refers to the $k'$ value obtained by extrapolation to 0% methanol) would give a better correlation than the use of $k'$ at any other concentration,[2d,f,7] it was difficult to obtain appropriate retention times at lower methanol concentrations (<30%), even on a shorter column (10 cm).

In order to obtain a better linear relationship between $\log P$ and $\log k'$ for a variety of substituents including the OH substituent, other mobile phases were examined. Preliminary tests with $CH_3CN$–$H_2O$ (35:65) mixture showed that the retention behavior in this mobile

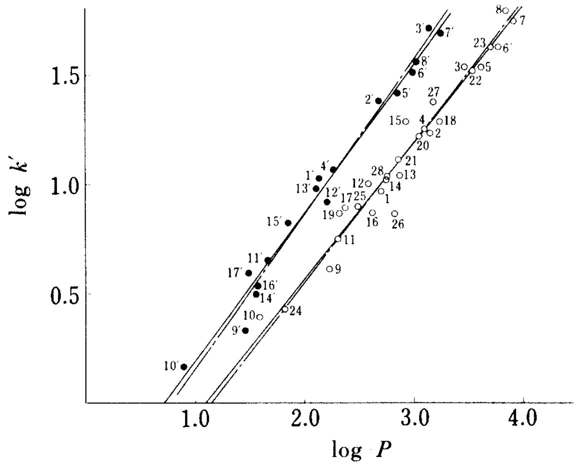


Fig. 2. Plot of log$k'$ against log$P$ in MeOH–THF–$H_2O$ (40:5:55)

○, phenylacetanilides; ●, monosubstituted benzenes. ——–, slope obtained with all compounds; ——, slope calculated by omitting phenolic compounds (**9′**, **9**, **24**).
The compounds are numbered as in Table II.

phase is very similar to that in mobile phase $M_1$. Tetrahydrofuran (THF), however, was found to behave differently. Several workers have already reported that phenols are retarded in THF–$H_2O$ mixtures relative to MeOH–$H_2O$.$^{6)}$ With this functional group selectivity in mind, we examined a ternary system containing THF: $M_3$, MeOH–THF–$H_2O$ (40:5:55). The composition was chosen so as to give a slope and retention times similar to those in mobile phase $M_1$. The results are plotted in Fig. 2, and the log$k'$ values and derived correlations are included in Tables II and III. The change in the retention behavior resulting from a small addition of THF to the MeOH–$H_2O$ binary system appears to resemble that observed in going from the MeOH–$H_2O$ system to the THF–$H_2O$ system. It was observed$^{6)}$ that nitro, cyano and phenolic compounds are specifically retarded in THF–$H_2O$ relative to in MeOH–$H_2O$, while methylated compounds are accelerated. In our case, the same features were observed for nitro, alkyl and phenolic compounds. However, the *p*-cyano derivative exhibited a large acceleration instead of retardation. As a result, the deviation from the log$P$−log$k'$ linearity decreased for OH and alkyl derivatives (**9**, **24**, **3**) but increased for CN and $NO_2$ derivatives (**26**, **15**, **27**), leading to a slightly better correlation in the mobile phase $M_3$ (eq. 7) than in the mobile phase $M_1$ (eq. 5).

Thus, the fact that the functional group selectivity varies from one mobile phase to another is troublesome from the viewpoint of determining log$P$ values by HPLC.

## Comparison between Octanol–Water Partition Behavior and Chromatographic Partition Behavior

In order to compare the chromatographic partition behavior with the octanol–water partition, we intended to apply eq. 2 to analyze log$k'$ values. In this case, the equation equivalent to eq. 2 is as follows,

$$\kappa' = a\kappa'_x + \rho_Y\sigma_X + \rho_X\sigma_Y + c \qquad (17)$$

where $\kappa' = \log k'_{X/PhY} - \log k'_{PhY}$ and $\kappa'_x$ is the corresponding difference for monosubstituted benzenes.

Since $\rho_x$ values are unknown, calculations were made, as a first approximation, by assuming that the $\rho_x\sigma_Y$ term is insignificant or negligible due to the small $\sigma_{m,p}$ values of the $PhCH_2CONH$ substituent. Good correlations, eqs. 18—20, were obtained.

$$\begin{array}{ll} M_1: & \kappa' = 0.753\kappa'_x + 0.354\sigma_x + 0.100 \qquad (18) \\ & \qquad\quad (0.044) \quad (0.057) \quad (0.022) \\ & \qquad (n=26,\ r=0.992,\ \text{S.D.}=0.046) \end{array}$$

$$\mathrm{M_2:}\quad \kappa'=0.717\kappa'_{\mathrm{x}}+0.280\sigma_{\mathrm{x}}+0.070 \qquad (19)$$
$$(0.045)\quad(0.046)\quad(0.018)$$
$$(n=26,\ r=0.991,\ \mathrm{S.D.}=0.037)$$

$$\mathrm{M_3:}\quad \kappa'=0.740\kappa'_{\mathrm{x}}+0.477\sigma_{\mathrm{x}}+0.117 \qquad (20)$$
$$(0.063)\quad(0.088)\quad(0.033)$$
$$(n=26,\ r=0.985,\ \mathrm{S.D.}=0.070)$$

Though more complete analyses using eq. 17 could not be done, the observation of acceptable correlation coefficients and standard deviations suggests that we can discuss the significant difference between two hydrophobic indexes, $\log P$ and $\log k'$, on the basis of eqs. 18—20. The deviations of $\kappa'$ from the correlation ($\Delta\kappa'$) are included in Table II.

The retention behavior on RP-HPLC is complex, because the organic modifier is enriched in the stationary phase as a result of hydrophobic expulsion from the aqueous mobile phase.7) THF is known to be extracted to a much larger extent than MeOH.7) Hence, in the case of the mobile phase $M_3$, the extent of interactions between solutes and THF in the stationary phase should be higher than that in the mobile phase.

It can be seen from eqs. 18—20 that the higher content of water in the mobile phase is associated with a higher $\rho_Y$ value, which is not unexpected since hydrogen bonding effects become more important in more polar solutions. The $\rho_Y$ value was still increased in the mobile phase $M_3$. This is attributed to the fact that THF, being a stronger base than methanol, works more effectively as a hydrogen bond acceptor than methanol. This mobile phase, $M_3$, produces somewhat greater deviations from the correlation ($\Delta\kappa'$) as compared with $M_1$ and $M_2$. Additional electronic interactions which cannot be described by eq. 20 might be responsible for such deviations.

It is noteworthy that the unsubstituted phenylacetanilide (**1**) displays a significant specific acceleration (negative $\Delta\kappa'$) relative to disubstituted derivatives in all solvents studied. No rational explanation can be found for this at the present stage. The trifluoromethyl derivative (**8**) shows a large retardation. An anomalous retention mechanism for this group was reported by Braumann *et al.*8)

It has already been noticed by several workers,6a) that phenolic compounds exhibit a downward deviation from the $\log k'$ against $\log P$ calibration line, as observed in this study. Since the $\kappa'$ values for **9** and **24** are well described by eqs. 18—20, the gap cannot be attributed to the anomalous retention behavior of the solutes. Rather, the hydrogen-donating capability of phenols is expected to account for this situation. Phenols undergo hydrogen bonding with the solvent as follows: $\mathrm{ArOH}\cdots\underset{\mathrm{H(R')}}{\mathrm{O}}\mathrm{-R}$ (type A). For this type of association, octanol is favored over methanol because the former is more basic than the latter. This factor would make phenols more hydrophobic in the octanol–water system ($\log P$) than in the lipophilic stationary phase –MeOH–$H_2O$ mobile phase system ($\log k'$), and hence we would have a higher ratio of $\log P/\log k'$ (or $\pi/\kappa'$) for phenols than for other compounds lacking hydrogen bond donor. This is presumably the reason why OH derivatives deviate from the straight line determined by using non-hydrogen donors. When THF is added to MeOH–$H_2O$ mixtures, the extent of hydrogen bonding of type A increases (in the stationary phase) because THF is a stronger base than methanol. The $k'$ value of phenol will, therefore, increase as the THF content is increased. The approach of OH-derivatives (**9**, **24**) to the straight line on addition of a small amount of THF to MeOH–$H_2O$ mixtures (Fig. 2) can be understood on this basis.

Examination of the $^{13}$C-NMR chemical shifts of phenol in these solvents would be useful to investigate the hydrogen bonding behavior. Table IV summarizes the $^{13}$C chemical shifts of phenol in solvents necessary for the present discussion. The data were taken from our unpublished results. The assumption was made that the inherent solvent effect on chemi-

TABLE IV. C-13 Carbon Shifts for Phenol[a)]

| Solvent | Phenol | | | $C_B$[b)] | *p*-CSB[c)] |
|---|---|---|---|---|---|
| | $C_o$ | $C_m$ | $C_p$ | | |
| MeOH | 116.27 | 130.38 | 120.48 | 129.31 | −8.82 |
| THF | 115.91 | 129.89 | 119.67 | 128.97 | −9.30 |
| Octanol | 115.77 | 129.24 | 119.23 | 128.42 | −9.19 |
| $H_2O$[d)] | 117.34 | 131.87 | 122.50 | 130.41 | −7.91 |
| $M_1$[e)] | 116.19 | 130.61 | 121.13 | 129.27 | −8.15 |
| $M_3$[e)] | 116.15 | 130.58 | 120.96 | 129.27 | −8.30 |

*a*) In ppm downfield from TMS. *b*) Chemical shift of benzene.
*c*) *p*-CSB $=C_p-C_B$. *d*) *tert*-BuOH was used as an internal standard.
*e*) Mobile phases used for determining $\log k'$, see Table II.
The accuracy of the results is ±0.03 ppm. The concentration of the solutions was 0.01—0.02 M.

cal shift could be cancelled out by comparing the shifts from benzene in the same solvent, CSB. The CSB value for the *para* position, *p*-CSB, is thought to be the best measure. The benzene ring of a phenol would be shielded with an increase in the extent of hydrogen bonding solvation of type A, which would result in a highfield shift of cabon-13 resonances. In THF as well as octanol, *p*-CSB is more negative than in MeOH, indicating a greater amount of type A association with THF or octanol than with MeOH. The least negative *p*-CSB value in water seems to mean that water is the strongest acid among the solvents studied.

## Conclusion

It has been shown that $\pi$ values for a series of *m*- and *p*-substituted phenylacetanilides(I) in an octanol–water system can be accurately predicted by eq. 3. The capacity factor ($\log k'$), an alternative hydrophobic index obtainable by RP-HPLC, was found to be linearly dependent on the $H_2O$–octanol partition coefficient, $\log P$, ($\log P = a \log k' + b$). The correlations are fairly good but there are some outliers, for example, phenolic compounds in MeOH–$H_2O$ ($M_1$), and nitro and cyano derivatives in MeOH–THF–$H_2O$ ($M_3$). The correlation derived with all compounds is better in $M_3$ (eq. 7) than in $M_1$ (eq. 5) whereas the omission of OH-derivatives gave a better result with $M_1$ (eq. 8) than with $M_3$ (eq. 10). Therefore care is necessary in the choice of mobile phase when determining the hydrophobic parameters by HPLC. When we need to estimate $\log P$ values for a series of compounds with a variety of substituents, MeOH–$H_2O$ mixtures rich in water seem to be convenient for practical use as long as we treat compounds carrying a strong hydrogen bond donor, such as OH or $CO_2H$, as outliers.

In order to establish a universal chromatographic system which is directly proportional to the standard system (octanol–water system), it will be necessary to make a systematic study of substituent effects (including hydrophobic and polar group selectivity) in terms of mobile phase effects as well as bonded phase effects.

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