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EVALUATION OF LIPOPHILIC PROPERTIES FOR A SERIES OF PHENOLS, USING REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

Experimental R_M^0 and log k^0 values for 48 mono- to penta-substituted phenols were determined using reversed-phase high-performance liquid chromatography and high-performance thin-layer chromatography. Octadecylsilylated silica served as "lipophilic" stationary phase. R_M^0 and log k^0 values were calculated by extrapolation of the plot of the methanol percentage composition of the mobile phase (methanol–0.01 *N* aqueous HCl mixtures) *versus* R_M or log k values to the intercept with the *y*-axis (*i.e.*, 0% methanol).

R_M^0 and log k^0 values obtained under these standardized conditions are correlated significantly to the log *P* values reported for shake-flask partition experiments. Substituent constants derived from the chromatographic lipophilicity data, in accordance to π, lead to values that can be used to estimate the hydrophobic properties of other compounds.

INTRODUCTION

The hydrophobic or lipophilic character of chemicals is one of the most important parameters in the analysis of relationships between biological activity and chemical structure, as has been shown for numerous examples[1]. Lipophilic parameters, however, are not only of interest in biological, pharmacological or medical sciences, they may also be used to describe the environmental behaviour of chemicals. For example, the bioconcentration or bioaccumulation of compounds[2–5] and their adsorption to soil[6] are highly correlated to their lipophilicity. Therefore the newly released "Chemikaliengesetz" of the Federal Republic of Germany[7] demands the measurement of physico-chemical properties, among them the partition coefficient in an octanol–water system[8,9], of chemicals newly brought onto the market.

Besides the shake-flask methods of obtaining partition coefficients, chromatographic methods can be used to quantitate lipophilic properties. Reversed-phase material, either alkane- or silicon-coated silica[10–14] or octadecylsilylated silica[15–19] have

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been used, the latter in some cases being additionally coated with octanol to imitate the octanol–water shake-flask partition[20–22].

To standardize the results from chromatographic measurements, Biagi *et al.*[11,12] introduced R_M^0 values, *i.e.*, R_M^0 values calculated by extrapolation to 100% water. We extended this standardization to high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) for the evaluation of lipophilic properties for a series of phenols using octadecylsilylated silica. We chose this group of compounds because phenols are an important sector of synthetic chemicals[23].

MATERIAL AND METHODS

Equipment

The HPLC system comprised: pumps, Type 5200, gradient mixer, Type 9100, UV photometer, Type 8500; and oven, Type 6000, all from H. Knauer (Berlin, G.F.R.). Knauer columns (25 cm × 4.6 mm I.D.) were packed with 10-μm LiChrosorb RP-18 (E. Merck, Darmstadt, G.F.R.). Retention times were measured with a Varian integrator CDS 111.

A Camag Nanomat (Camag, Berlin, G.F.R.) was used to spot the compounds on RP-18 F_{254} plates for HPTLC (E. Merck). They were developed with a Camag linear developing chamber.

Phenols were obtained from E. Merck, Fluka (Neu-Ulm, G.F.R.), EGA (Steinheim, G.F.R.) and Sigma (München, G.F.R.). They were used without further purification.

Conditions

HPLC measurements were performed at 30°C with a flow-rate of 2 ml/min (*ca.* 100 bar) using different solvent mixtures of methanol–0.01 *N* aqueous HCl (80–50% methanol, in 5% steps). The injected amount was *ca.* 3 μg of phenol in 20 μl of solvent. The detection wavelength was 220 nm.

For HPTLC, 200 nl of solutions of phenols in methanol were spotted on the plates with the Nanomat. Both sides of a plate were used, developing them from the ends to the centre in a horizontal mode with the linear developing chamber. Solvent mixtures of methanol–0.01 *N* aqueous HCl (90–75% methanol, in 2.5% steps) were used. The phenols were detected under UV light (254 nm).

As phenols are weak to strong acids, pentachiorophenol being the most acidic phenol investigated with a pK_a of 5.3 (ref. 24), the solvent has to be strongly acidic if the hydrophobic properties of the undissociated phenols are to be described by chromatographic parameters. If 0.01 *N* aqueous HCl is used instead of water, the undissociated fraction for all phenols investigated is less than 1%, which is negligible because the value for the difference of pK_a − pH exceeds 2[25].

R_M and log k values (k = capacity ratio) obtained for each solvent mixture and each phenol are the mean of from five to seven HPLC or HPTLC measurements.

Calculations

R_M and log k were calculated according to

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \text{ (refs. 10 and 26)} \quad (1)$$

and

$$k = \frac{t_R - t_0}{t_0} \text{ (ref. 27)} \quad (2)$$

where R_F is the ratio of the distances travelled by the substance and the solvent front in thin-layer chromatography, and t_R and t_0 are the retention times of a retained or an unretained peak respectively[27] for HPLC.

R_M^0 and log k^0 values were calculated by extrapolation of the plot of the methanol percentage composition *versus* R_M or log k to the intercept with the y-axis, *i.e.*, 0% methanol, using least-squares regression analysis[28].

RESULTS AND DISCUSSION

R_M^0 and log k^0 values

Taking R_M or log k values obtained for a compound with different solvent mixtures, an extrapolation to 100% water as a standard solvent is possible by applying regression analysis. This extrapolation, originally carried out by Biagi *et al.*[11,12] for R_M values from reversed-phase thin-layer chromatography (TLC) experiments with silicone-coated silica, results in lipophilicity constants that are standardized and easily comparable. It has been shown that this approach is theoretically and experimentally correct[29].

We applied it to the measurement of R_M (HPTLC) and log k (HPLC) data using octadecylsilylated silica as reversed-phase material for a series of phenols; the R_M^0 and log k^0 values obtained are listed in Table I.

The calculation of lipophilicity data from chromatographic results using only one solvent mixture may lead to an over- or under-estimation of the lipophilicity. This holds even for a distinct group of substances such as phenols, as may be seen from the different slopes of 2-nitrophenol and 4-iodophenol for the dependence of their log k values on the solvent composition (see Fig. 1a). This also holds for the R_M values obtained in HPTLC experiments (see Fig. 1b). For example, using only the solvent mixture methanol–0.01 *N* aqueous HCl (90:10) to quantify the hydrophobic properties of 2-nitro- and 4-iodophenol by HPLC could lead to the misapprehension that they have the same lipophilicity. As can be seen from the log k^0 values listed in Table I, however, and in accordance with log P values (P = partition coefficient) given in the literature[30], 4-iodophenol is *ca.* six times more lipophilic than 2-nitrophenol.

The log P data listed by Leo *et al.*[30] are in good agreement with our log k^0 and R_M^0 values. This is demonstrated in Figs. 2 and 3, and by a correlation analysis:

$$\log k^0 = 0.8478\ (0.0336) \log P_{\text{oct.}} - 0.3112\ (0.0737) \quad (3)$$
$$n = 29,\ r = 0.9794,\ s = 0.1490$$
$$F = 636.37,\ \alpha < 0.1\%$$

and

$$R_M^0 = 1.0988\ (0.0599) \log P_{\text{oct.}} - 0.2426\ (0.1335) \quad (4)$$
$$n = 28,\ r = 0.9634,\ s = 0.2500$$
$$F = 336.09,\ \alpha < 0.1\%$$

Standard deviations for the regression coefficients are given in parentheses; r is the correlation coefficient, n the number of compounds, s the standard error of estimate, F the overall F-test for the regression, and α the level of significance.

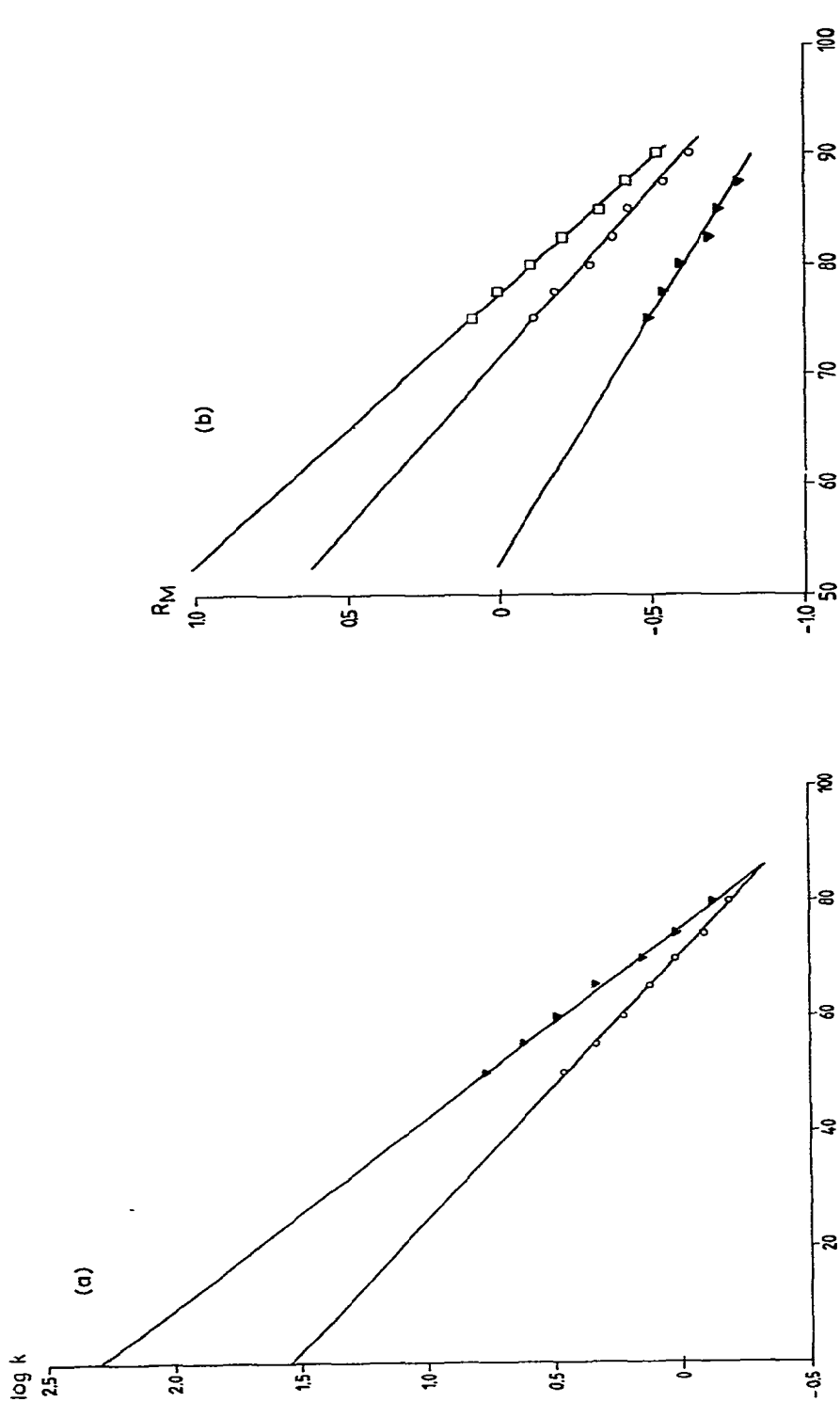

Fig. 1. (a). Relationship between log k values of phenols and the concentration of methanol in the mobile phase. ▼ = 4-Iodophenol; ○ = 2-nitrophenol. (b), Relationship between R_M values of phenols and the concentration of methanol in the mobile phase. □ = 2,4-Dichlorophenol; ○ = 4-bromophenol; ▼ = 4-methoxyphenol.

TABLE I

R_M^0 AND log k^0 VALUES COMPARED WITH log $P_{oct.}$ VALUES FOR PHENOLS

No.	*Substituent(s)*	R_M^0	*s**	*log* k^0	*s**	*log* $P_{oct.}$**
0	Unsubstituted	1.61	(0.11)	0.93	(0.03)	1.47***
1	2-Bromo-	2.04	(0.09)	1.52	(0.04)	2.35
2	3-Bromo-	2.32	(0.10)	1.80	(0.03)	2.63
3	4-Bromo-	2.44	(0.10)	1.80	(0.02)	2.59
4	2,4,6-Tribromo-	4.13	(0.12)	2.96	(0.03)	—
5	2-Chloro-	2.11	(0.06)	1.44	(0.03)	2.17***
6	3-Chloro-	2.43	(0.13)	1.66	(0.04)	2.49***
7	4-Chloro-	2.53	(0.12)	1.70	(0.03)	2.42***
8	2,3-Dichloro-	2.52	(0.12)	2.03	(0.05)	—
9	2,4-Dichloro-	3.12	(0.11)	2.30	(0.05)	—
10	2,5-Dichloro-	3.06	(0.17)	2.16	(0.02)	—
11	2,6-Dichloro-	2.34	(0.05)	1.85	(0.03)	—
12	3,4-Dichloro-	2.86	(0.03)	2.20	(0.03)	—
13	3,5-Dichloro-	3.68	(0.23)	2.56	(0.02)	—
14	2,3,4-Trichloro-	3.51	(0.16)	2.68	(0.04)	—
15	2,3,6-Trichloro-	3.46	(0.16)	2.49	(0.04)	—
16	2,4,6-Trichloro-	3.76	(0.19)	2.65	(0.05)	3.38***
17	2,3,5-Trichloro-	4.56	(0.15)	2.94	(0.03)	—
18	3,4,5-Trichloro-	4.27	(0.13)	3.28	(0.10)	—
19	2,3,4,5-Tetrachloro-	5.05	(0.06)	3.35	(0.06)	—
20	2,3,5,6-Tetrachloro-	4.88	(0.08)	3.20	(0.03)	—
21	Pentachloro-	5.46	(0.03)	3.77	(0.06)	5.01
22	2-Methyl-	1.83	(0.22)	1.53	(0.03)	1.95
23	3-Methyl-	1.94	(0.10)	1.52	(0.02)	2.00***
24	4-Methyl-	2.03	(0.14)	1.54	(0.03)	1.93***
25	2-Methoxy-	1.25	(0.09)	1.02	(0.05)	—
26	3-Methoxy-	1.25	(0.19)	0.99	(0.06)	1.58
27	4-Methoxy-	1.33	(0.09)	0.78	(0.05)	1.34
28	2-Cyano-	1.65	(0.13)	1.10	(0.02)	—
29	3-Cyano-	1.73	(0.13)	1.03	(0.03)	1.70
30	4-Cyano-	1.81	(0.10)	0.97	(0.04)	1.60
31	2-Carboxy-	1.90	(0.17)	1.65	(0.02)	2.24***
32	3-Carboxy-	1.50	(0.21)	1.04	(0.03)	1.50
33	4-Carboxy-	1.15	(0.12)	0.86	(0.03)	1.58
34	2-Ethyl-	2.64	(0.10)	1.80	(0.03)	—
35	3-Ethyl-	2.59	(0.15)	1.76	(0.02)	2.40
36	4-Ethyl-	2.37	(0.05)	1.79	(0.02)	—
37	2-Fluoro-	—		1.30	(0.02)	—
38	3-Fluoro-	1.82	(0.10)	1.53	(0.05)	1.93
39	4-Fluoro-	1.94	(0.27)	1.39	(0.02)	1.77
40	4-Iodo-	2.63	(0.13)	2.30	(0.04)	2.91
41	2-Amino-	—		0.54	(0.04)	—
42	2-Ammonium-[§]	—		−0.12	(0.01)	—
43	2-Nitro-	2.29	(0.08)	1.54	(0.02)	1.76***
44	3-Nitro-	1.74	(0.09)	1.34	(0.02)	2.00
45	4-Nitro-	1.72	(0.11)	1.23	(0.03)	1.91
46	2-Hydroxy-	1.10	(0.09)	0.51	(0.03)	0.95***
47	3-Hydroxy-	0.36	(0.08)	0.25	(0.03)	0.79***
48	4-Hydroxy-	—		−0.12	(0.02)	0.55***

* Standard deviation of the intercept.

** As compiled by Leo *et al.*[30].

*** Mean, if more than one value is listed.

[§] Using methanol–buffer (pH 6.5) mixtures instead of methanol–0.01 *N* aqueous HCl.

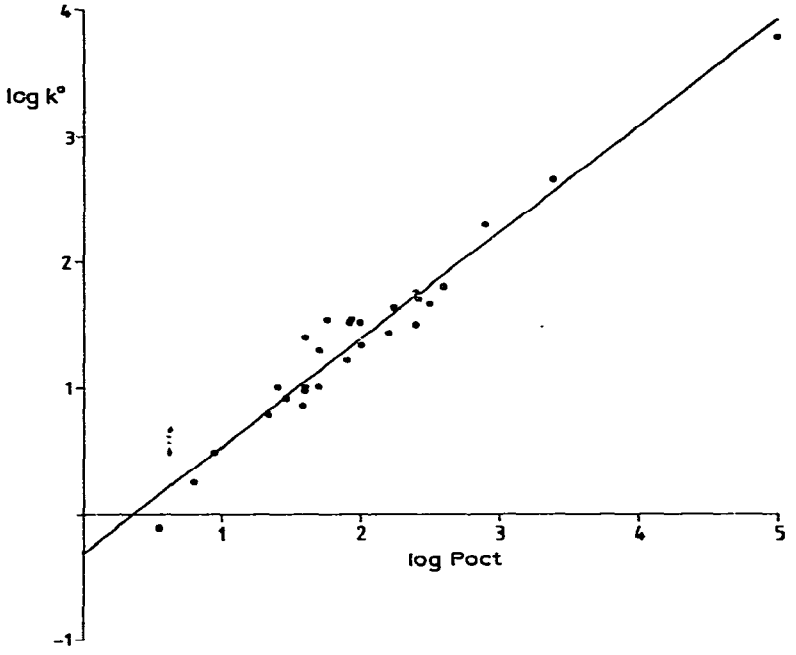

Fig. 2. Comparison of log $P_{oct.}$ with log k^0 evaluated by HPLC. For correlation analysis, see eqn. 3.

The slopes of eqns. 3 and 4 are not far from unity, and the intercept is not far from zero. This supports the hypothesis that the octadecylsilylated silica phase, as used in the HPLC and HPTLC experiments, has nearly the same properties in respect to phenols as does octanol. Thus the suggestion[31] that chromatographic methods should be used to measure lipophilicity, as an alternative to octanol–water partition, may be carried out without changing the properties of the lipophilic phase drastically. Furthermore, using HPLC, it is possible to extend the application range of the octanol–water partition method, especially to compounds of higher lipophilicity[16].

On the other hand, there may be problems with HPTLC when evaluating the lipophilic properties of rather hydrophilic compounds, because solvent mixtures containing more than 40–50% water do not wet the sorbent, thus making it impossible to develop the plates. Similar problems were reported for reversed-phase TLC with alkane-coated silica. On this layer, sulphonic acids and sulphonamides behaved as if it did not contain the organic phase, whereas lipophilic compounds followed the reversed-phase partition mechanism[32]. As HPLC does not show the limitations mentioned above, it should preferably be used to quantitative lipophilic properties, even though the equipment is more expensive.

ΔR_M^0 and $\Delta \log k^0$ values

In addition to the log k^0 and R_M^0 values reported above, it is possible to derive substituent constants (with reference to π), defining them as:

$$\Delta \log k^0 = \log k^0_{R-X} - \log k^0_{R-H} \quad (5)$$

and

$$\Delta R_M^0 = R^0_{M_{R-X}} - R^0_{M_{R-H}} \quad (6)$$

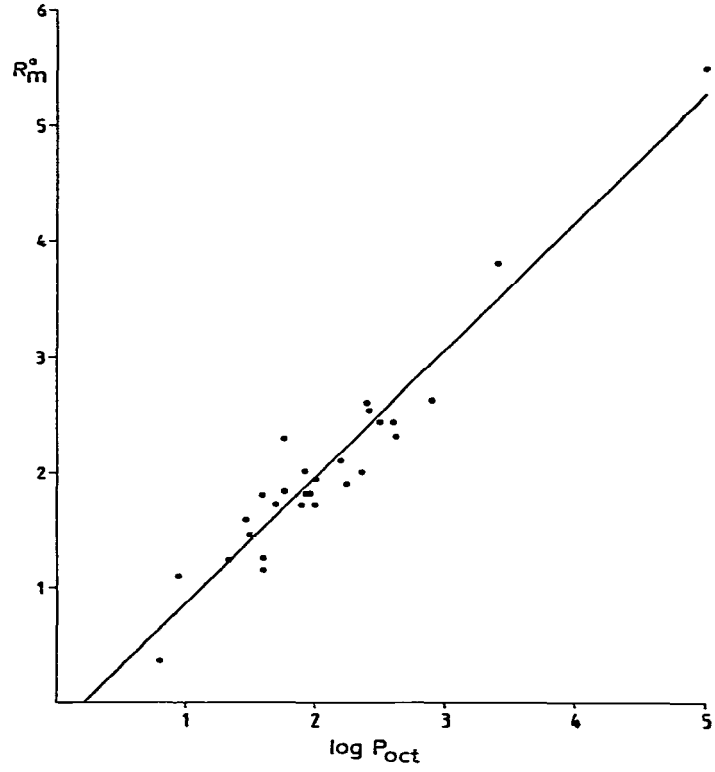

Fig. 3. Comparison of log $P_{oct.}$ with R_M^0 evaluated by HPTLC. For correlation analysis, see eqn. 4.

The $\Delta\log k^0$ and ΔR_M^0 values for the *meta*- and *para*-substituted phenols (*ortho*-phenols were omitted because of the strong interaction of the hydroxyl group with the *ortho* substituent) show a significant correlation to the hydrophobic substituent constant π (see Table II).

Correlation analysis gives the following results:

$$\Delta\log k^0 = 0.7029\ (0.0831)\ \pi + 0.2572\ (0.0530) \quad (7)$$
$$n = 20,\ r = 0.8938,\ s = 0.2178$$
$$F = 71.48,\ \alpha < 0.1\%$$

and

$$\Delta R_M^0 = 0.8036\ (0.1250)\ \pi + 0.0674\ (0.0797) \quad (8)$$
$$n = 20,\ r = 0.8345,\ s = 0.3276$$
$$F = 41.30,\ \alpha < 0.1\%$$

The use of these substituent constants, ΔR_M^0 and $\Delta\log k^0$, to estimate the lipophilic properties of substituted phenols, leads to rather good approximations. For example, the measured and calculated log k^0 value of 2,4,6-tribromophenol: log k^0 (calculated), 2.98 ($\log k^0_{\text{phenol}}$: 1.61 + 2 · $\Delta\log k^0_{2-\text{Br}}$: 2 · 0.59 + $\Delta\log k^0_{4-\text{Br}}$: 0.87); log k^0 (measured), 2.96.

TABLE II

ΔR_M^0 AND Δ log k^0 SUBSTITUENT CONSTANTS DERIVED FROM PHENOLS COMPARED WITH π

Substituent	ΔR_M^0	Δ log k^0	π*
3-Br	0.71	0.87	0.86
4-Br	0.83	0.87	0.86
3-Cl	0.82	0.73	0.71
4-Cl	0.92	0.77	0.71
3-CH_3	0.33	0.59	0.56
4-CH_3	0.42	0.61	0.56
3-OCH_3	−0.36	0.06	−0.02
4-OCH_3	−0.28	−0.15	−0.02
3-CN	0.12	0.10	−0.57
4-CN	0.20	0.04	−0.57
3-COOH	−0.15	0.11	−0.32
4-COOH	−0.46	−0.07	−0.32
3-C_2H_5	0.98	0.83	1.02
4-C_2H_5	0.76	0.86	1.02
3-F	0.21	0.60	0.33
4-F	0.33	0.46	0.33
4-I	1.02	1.37	1.12
3-NO_2	0.13	0.41	−0.28
4-NO_2	0.11	0.30	−0.28
3-OH	−1.25	−0.68	−0.67

* As compiled by Seydel and Schaper[1].

For highly substituted phenols, however, rather large deviations are possible. A calculation of the log k^0 value for pentachlorophenol by adding the substituent constants for chlorine (Δlog $k^0_{2\text{-Cl}}$: 0.51; Δlog $k^0_{3\text{-Cl}}$: 0.73, Δlog $k^0_{4\text{-Cl}}$: 0.77) to the log k^0 value of phenol gives a value of 4.18 instead of the measured value of 3.77. Presumably this is the result of fewer water molecules being clustered around each chlorine atom in pentachlorophenol than in the monochlorophenols, as already reported by Leo[30]. From this difference between calculated and measured lipophilicity, it may be concluded that substituent constants are only applicable for an approximate orientation and do not replace an exact evaluation.

CONCLUSIONS

The advantages of the bonded reversed-phase HPLC and HPTLC techniques over shake-flask methods for the evaluation of lipophilicity constants are their simplicity, speed and wide application range. Furthermore, only small amounts of substance are needed, and they need not necessarily be of high purity. If log k and R_M values are measured at various solvent mixtures, then log k^0 and R_M^0 standardized parameters can be calculated.

As already pointed out by Tomlinson[14], chromatographic methods, though being dynamic ones, give values for the hydrophobic properties of many drugs that correlate even better with biological or biochemical data than do the log P parameters. Therefore the methods should be considered at least equivalent to the use of partition coefficients.

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