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Reversed-phase high-performance liquid chromatography for evaluating the lipophilicity of pharmaceutical substances with ionization up to log $P_{app} = 8$

Klaus Belsner, Martina Pfeifer and Bob Wilffert

Janssen Research Foundation, Institut für Experimentelle Medizin, D(W)-4040 Neuss 21 (Germany)

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ABSTRACT

The RP-HPLC determination of drug lipophilicity was evaluated for its potency as a substitute for the determination of log P_{Oct-W} values. The relation of log k_w to log P_{app} (= log P_{Oct-W} at pH 7.4) is linear for a testset including protonable nitrogen bases. The method is reproducible on different columns (with the same brand and type of the stationary phases) and needs no recalibration then. Three series of substituted benzene homologues (one hydrogen bond acceptor and two weak donor groups) demonstrate structure–retention effects.

INTRODUCTION

Drug lipophilicity is an interesting molecular property, because in many instances it is correlated with the biological activity of a drug. A good measure of lipophilicity is the octanol–water partition coefficient, but its determination is prone to errors [1] and numerous alternative techniques have been suggested, as reviewed by Kaliszan [2].

Usually chromatographic data for similar organic compounds correlate fairly well with the logarithm of the octanol–water partition coefficient, log $P_{Oct/W}$. The problems associated with the interpretation of the reversal of elution order that sometimes occurs at different contents of organic solvent can be avoided by the so-called "polycratic" elution. Minick and co-workers [3,4] have described this technique and extended it to non-congeners by minimization of solute–silanol interaction. The addition of decylamine and octanol (to mimic the octanol hydrogen bonding activity) to the mobile phase in RP-HPLC resulted in a correlation coefficient of $r = 0.9951$ ($n = 24$) for log $P_{Oct/W}$ versus log k_W. Log k_W is the log k' value extrapolated to zero organic modifier content in the mobile phase. An evaluation of this method seemed reasonable because it offered several interesting advantages: (i) rapid and accurate determination of lipophilicity; (ii) data concerning the hydrogen bonding ability of a drug; and (iii) the influence of partial protonation on the retention of protonable molecules. Additionally, the chromatographic data obtained are useful as a database for the future development of chromatographic methods.

The set of compounds studied included a group of phenones, a group of 4-alkylanilines and a group of alkylbenzenes, to obtain a well characterized control set with different hydrogen bonding properties. With the same intention and to cover a broader range of polarity, a number of non-congeners were taken from the set published by Minick *et al.* [3]. Minick's group [3,4] limited their investigations to relatively simply structured and almost exclusively uncharged molecules in aqueous solution at neutral

Correspondence to: K. Belsner, Janssen Research Foundation, Institut für Experimentelle Medizin, D(W)-4040 Neuss 21, Germany.

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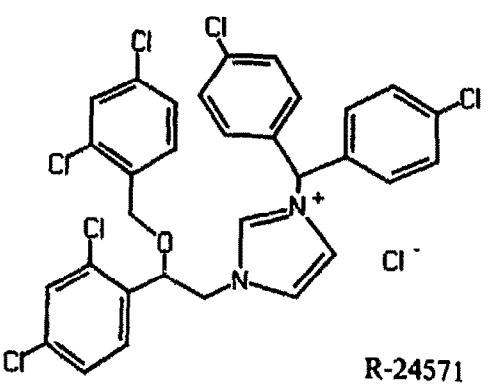
and acidic pH, compatible with silica-based RP-HPLC stationary phases. Although this approach can be extended to alkaline environments using polymer-based supports, with respect to comparability and instrumental requirements it is favourable to use a single column for organic bases and non-protonated compounds. The pH stability of silica supports allows the determination of the exact partition behaviour over the physiological range of pH, which is of major interest in pharmaceutical research. Further, the investigation of some drug molecules with known pK_a and log $P_{Oct/W}$ values should allow a more generalized validation of more complex structure and charge effects. If the results of the reversed-phase HPLC method with a silica-based support are in good agreement with the ion-corrected octanol–water partition coefficients, the problems associated with the use of different columns at different pH values of the eluents can be abolished.

EXPERIMENTAL

Instrumentation and materials

Two Beckmann M114 pumps connected to a system controller were used for solvent delivery and blending of the desired methanol concentration. A 25 mm × 4 mm I.D. cartridge filled with octyl-derivatized silica mounted between the static mixer (Lee Microsystems) and the injection valve served as a presaturator and to prevent column contamination by fines. Samples were injected by a Gilson autoinjector equipped with a Rheodyne Model 7010 injection valve with a 100-μl injection loop. The injection volumes were varied from 1 to 5 μl. A Perkin-Elmer diode-array detector was utilized for solute detection and to obtain low-resolution UV spectra for peak identification. A back-pressure regulator (Altex) was connected to the detector outlet to circumvent bubble formation in the detector cell. A 125 mm × 4 mm I.D. cartridge refilled with Hypersil 3 μm MOS (monooctylsilane) by CS-Chromatographie Service served as the chromatographic column. The autoinjector provided control over the whole chromatographic system via connections to the Beckmann controller and to a Spectra-Physics Model 4270 integrator. Temperature was maintained at 20°C by a laboratory-built Peltier-type column oven connected to a laboratory-built proportional integral differential (PID) regulator.

Methanol (MeOH) of HPLC grade was obtained from Merck (Darmstadt, Germany), octanol and diethylamine of analytical-reagent grade from Baker (Deventer, Netherlands), decylamine of analytical-reagent grade from Fluka (Neu-Ulm, Germany), drug substances (see formulae) from Janssen (Beerse, Belgium) and 3-morpholinopropanesulphonic acid (MOPS) from Sigma (Munich, Germany). The water for HPLC was purified using a Milli-Q water-purification system (Millipore). Mobile phases were filtered through a Millipore Type FH filter (0.5 μm pore size) and vacuum degassed during sonication in a Transsonic TS 540 sonicator (Elma). All other chemicals were of analytical-reagent grade and used as delivered.



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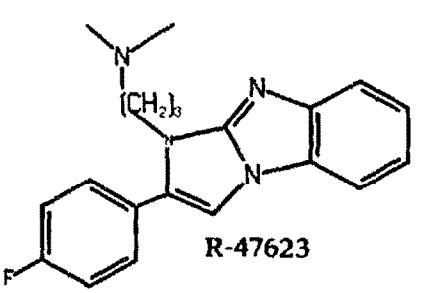

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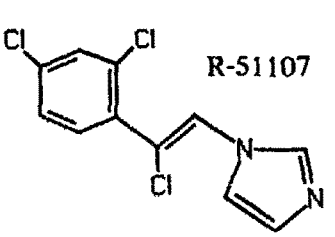

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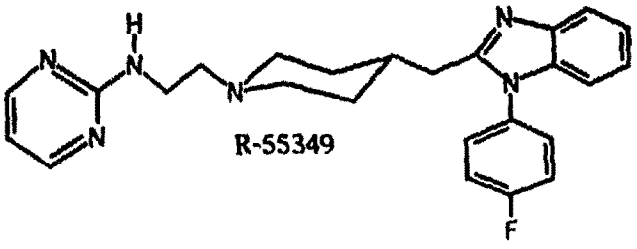

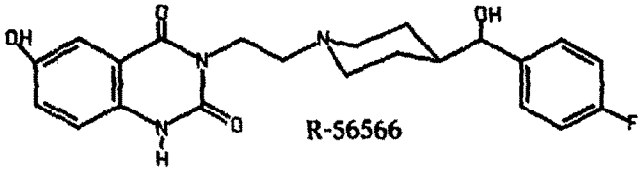

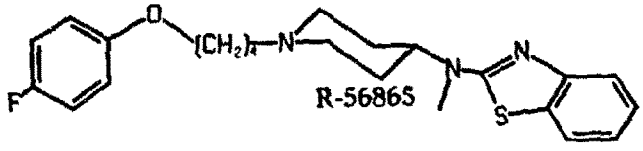

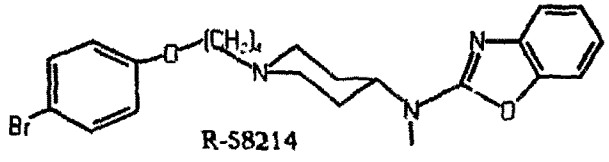

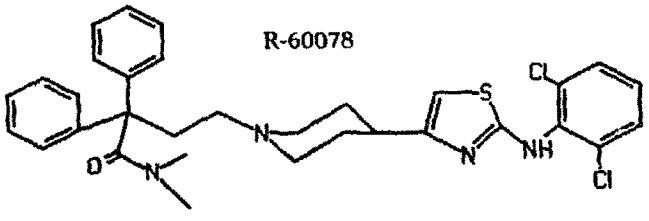

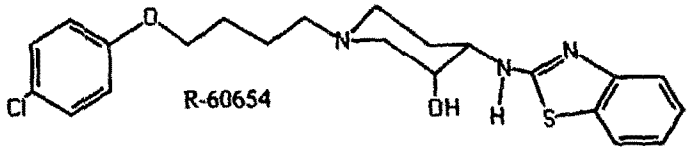

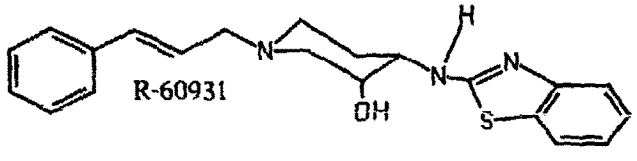

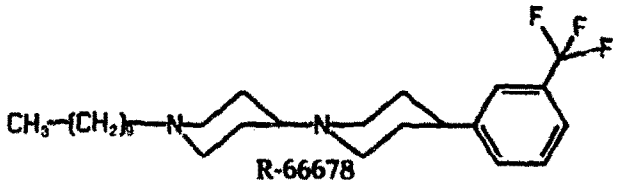

Methods

The mobile phases were a slightly modified version of those described by Minick *et al.* [3]: (A) 20 m*M* MOPS buffer in water, containing 0.15% (v/v) *n*-decylamine, pH 7.4 (adjusted with 1 *M* NaOH); (B) 94.75% (v/v) MeOH–5% (v/v) diethylamine stock solution, containing 3% (v/v) diethylamine in water, pH 7.4 adjusted with acetic acid–0.25% (v/v) *n*-octanol.

The solutes were eluted isocratically with several different mobile phase compositions and the corresponding retention indices were determined. A regression was calculated on the basis of a linear dependence of log k' on the methanol content of the mobile phase, φ_{MeOH}. The log k' values were extrapolated to a mobile phase without methanol. The resulting abscissa is log k_W and the slope is S. This procedure is "polycratic" elution as outlined by Minick *et al.* [3].

After some initial experiments the following conditions were formulated. A sufficient set of data had to contain at least four different concentrations of methanol and one k' value larger than 12. An exception was made only for adenine, with $k' < 12$ at 20% B. The concentration of methanol ranged from 92.5% (95% B) to 18% (v/v) (20% B). The concentration steps were 95%, 92.5%, 90% and then 10% increments of B.

At each methanol concentration a large set of compounds were chromatographed, thereby reducing the time for equilibration. Every injection was repeated twice. The flow-rate was 1.0 ml/min in all experiments. During elution the eluents were gently purged with helium to prevent redissolution of gases.

The void volumes of the columns were determined by injection of urea or deuterated water at each eluent composition and monitored at 205 nm. All regression calculations were performed using the natural logarithms of the k' values.

Calculation of apparent partition coefficients, log P_{app}

To correlate the lipophilicity parameter log k_W achieved by HPLC at pH 7.4 with the corresponding octanol–water partition coefficient of the neutral molecule, the apparent octanol–water distribution coefficients at pH 7.4 were calculated [5] on the basis of published ion-corrected water–octanol partition coefficients [5–12]:

$$\log P_{app} = \log P_{Oct/W} - \log (1 - 10\, pK_a - pH) \quad (1)$$

Eqn. 1 is the relationship between the pK_a of a monoprotic base and pH after introduction of the Henderson–Hasselbach equation into the distribution equilibrium to calculate the free base concentration. It is valid with the assumption that despite the fact that the distribution of a protonatable molecule is the sum of the distributions of the charged and the neutral species, the partition coefficient of the neutral molecule is orders of magnitude greater than that of the charged molecules. Diprotic bases can be treated similarly by consideration of their second pK_a.

Even higher protonation numbers can be handled in the same way, with correction terms for the resulting polyvalent ions. For completion additional terms to overcome the limits of the underlying Arrhenius law can be introduced. Concentrations can be replaced with activities (which decrease with increasing ionic charge) involving the influence of the ionic strength I according to Davies (see ref. 13).

$$\log f = KZ^2\left(0.2I - \frac{\sqrt{I}}{1 + \sqrt{I}}\right) \quad (2)$$

$$a(BH_i) = f(BH_i)[BH_i] \quad (3)$$

where a_i is the activity of the ion i and $K \approx 0.5$ for water at 25°C. This can be the starting point for a thorough calculation of concentration corrections as published by Poncelet *et al.* [14]. A calculation without ion strength refinement was applied to R-55349, a base with three potential protonation sites (pK_a = 2.00, 4.85, 8.32). The result is log P_{app} = 3.02 at pH 7.4, if all protonation steps are included in the correction. The calculation using eqn. 1 and treating R-55349 as a monoprotic base with pK_a = 8.32 gives log P_{app} = 3.00 (see Table III). Hence for polyprotic bases some simplifications are applicable: (a) pK_a values lower than 2 units below the pH of the aqueous phase contribute less than 1% to the distribution coefficient and can be neglected; and (b) in dilute solutions with low ionic strength as used in our method the activity coefficient nearly equals 1.

The P_{app} values of all solutes with more than one basic nitrogen were calculated with these simplifications. For weak acids such as catechol or amphoteric molecules such as ketanserin with a weak acidic group with a $pK_a > 9.4$ the contribution to the octanol–water distribution at pH 7.4 was also neglected.

Hydrogen bonding characteristics

As published by Minick *et al.* [4], the slope of the regression of log k' over φ_{MeOH}, S, was analysed for its hydrogen bonding dependence.

RESULTS

The investigations were started using the same eluents as described by Minick *et al.* [3]. These were prepared without diethylamine (DEA) in eluent B. At high percentages of B (≥90%) the long-chain 4-alkylanilines eluted as broad peaks. In this concentration range the effective concentration of *n*-decylamine is <0.015%. Unfortunately, this long-chain alkylamine is not suficiently soluble in water to prepare a concentrated solution at pH 7.4 which could then be added instead of DEA to the organic phase. A high concentration of the amine stock solution is necessary to keep the content of water in B as low as possible. The addition of DEA effectively reduced the polar surface interactions of 4-alkylanilines at percentages of B ≥90% and led to reasonable peak shapes for these compounds. The linear increase in log k' with decreasing φ_{MeOH} for tetradecylaniline and decylaniline (r = 0.9996 and 0.9998), which were chromatographed at methanol concentrations ranging from 78.4 to 90.25% in five steps, and those for propylaniline (r = 0.9997) or acetophenone (r = 0.997) with seven or nine steps down to a methanol concentration of 68.9% indicates the absence or at least the indepence of silanol–solute interactions over that methanol concentration range.

The correct determination of the void volume of the column is essential for the linear correlation of log k' values with the methanol content of the mo-

TABLE 1

CALIBRATION SETS

Compounds of the homologous series used for structure–retention investigation and the calibration set used for the regression calculation wit values of log k_W, $-S$ and log $P_{Oct/W}$. Ion-corrected value for the free base calculated according to ref. 5. Log $P_{Oct/W}$ from ref. 6 unless stated otherwise.

Substance	Log k_W	$-S$	Log $P_{Oct/W}$	Substance	Log k_W	$-S$	Log $P_{Oct/W}$
Alkylbenzenes				*Others*			
Methyl-	5.42	6.84	2.73	Pyrazole[a]	−0.07	2.14	0.26
Ethyl-	6.56	7.87	3.15	Diltiazem	5.65	7.13	2.8 [8]
n-Propyl-	7.79	8.96	3.68	Verapamil	4.30	5.72	3.27 [9]
n-Butyl	9.06	10.09	4.26	Propranolol[a]	1.52	2.62	3.56
n-Pentyl-	10.15	11.02		Lidoflazine	11.09	12.83	5.60 [7]
n-Hexyl	11.36	12.08	5.52	Flunarizine[a]	12.44	13.62	5.78 [7]
n-Heptyl	12.76	13.36		Lidocaine	3.07	4.00	2.26
sec.-Butyl-	8.70	9.76		Anthracene	8.72	9.94	4.45
tert.-Butyl-	8.35	9.45	4.11	Quinoline[a]	3.40	5.29	2.03
Phenones				Adenine[a]	−1.30	1.30[b]	2.27
Aceto-	2.59	4.40	1.73	4-Acetylpyridine[a]	0.15	2.06	0.54
Propio-	3.73	5.38	2.19	9-Anthracene carbaldehyde	8.07	9.85	
Butyro-	5.12	6.75		Benzothiazole	3.24	5.07	2.01
Valero-	6.44	7.97		Phenol	1.98	3.72	1.46
Hexano-	7.71	9.10		Resorcinol	0.99	4.56	0.80
Octano-	10.27	11.40		Hydroquinone	−0.06	3.42	0.55
Decano-	12.63	13.49		Acridine	5.87	7.72	3.40
Lauro-[a]	14.91	15.46		Sabeluzole	8.31	9.98	4.61 [7]
Myristo-[a]	17.46	17.76		R-56865	8.86	9.84	5.37 [7]
4-Alkylanilines				Cinnarizine	11.27	12.25	5.77 [7]
Methyl-	2.49	4.49	1.39	Mioflazine	11.24	13.64	5.26 [7]
Ethyl-	3.790	5.66		9-Anthracenemethanol	6.44	8.33	
Propyl-	5.24	7.03					
Butyl-	6.59	8.29					
Pentyl-[a]	7.895	9.45					
Heptyl-	10.28	11.55					
Octyl-	11.26	12.34					
Decyl-	13.67	14.50					
Tetradecyl-[a]	18.36	18.63					

[a] See Conclusions.
[b] See also Table II.

bile phase. The misinterpretation of a peak of a very polar solute instead of the refractive index as the "void volume peak" leads to a curve rather than a straight line of the graph of log k' *versus* φ_{MeOH}. The deviation from linearity can be exponential if this contaminant itself elutes without additional ionic interaction or more complex if ion-pair formation or exclusion are effective. Therefore, it is desirable to obtain void volumes independent of the marker. With urea and deuterated water this was achieved after analysis of the spectra of the void volume peaks with the diode-array spectrophotometer. Urea and deuterated water are only detectable with a UV spectrophotometer by the refractivity change of the solvent and therefore a UV spectrum registered at the maximum of the resulting peak in the chromatogram only reflects the wavelength dependence of the refractive index, a monotonic decline with increasing wavelength, in contrast to peaks of polar contaminants washed out behind the urea or D_2O peak with UV maxima between 220 and 260 nm. These peaks are higher than than the refractiv-

ity peak and their retention is dependent on φ_{MeOH} (volume percentage of methanol in the mobile phase), but can be discriminated by this qualitative analysis.

Table I lists the set of compounds that were chosen to obtain a representative group for the comparison of lipophilicity measured by HPLC with the corresponding apparent octanol–water data. In Table II the log k_W and the calculated apparent log $P_{Oct/W}$ ($\equiv$ log P_{app}) values [5] at pH 7.4 are given together with their pK_a values.

The result of the regression calculation for the values of log k_W and log P_{app} is shown in Fig. 1. For substances without a protonable nitrogen log $P_{Oct/W}$ is identical with log P_{app}.

$$\log P_{app} = 0.4197 \ln k_W + 0.5136 \quad (4)$$
($n = 29$; $r = 0.988$)
(S.D.: coefficient = 0.0124, intercept = 0.266)

Verification

A comparison of the the apparent log P_{app} calculated from the reported log $P_{Oct/W}$ and the values of

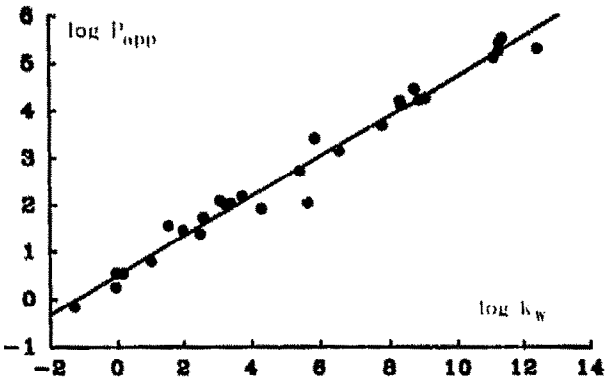

Fig. 1. Relationship between measured log $P_{Oct/W}$ or calculated log P_{app} and log k_W determined by HPLC for a series of noncongeners without separation into groups of high or low hydrophobicity.

TABLE II

APPARENT LOG $P_{Oct/W}$ AND LOG k_W OF THE CALIBRATION SET

Log k_W *versus* the apparent log $P_{Oct/W}$ at pH 7.4 $\equiv$ log P_{app} of members of the calibration set with protonatable nitrogen atoms. All calculations as described in ref. 5.

Substance	Log k_W	Log P_{App}	pK_a
4-Acetylpyridine	0.15	0.54	4.96
Acridine	5.87	3.40	5.58
Adenine	−1.30	−0.16	4.12, 9.83[a]
Hydroquinone	−0.06	0.55	10.35
Lidocaine	3.07	2.09	7.90
Propranolol	1.52	1.56	9.32
Phenol	1.98	1.46	9.89
Quinoline	3.40	2.03	4.90
Resorcinol	0.99	0.80	9.81
p-Toluidine	2.49	1.39	5.08
Flunarizine	12.44	5.30	7.71
Verapamil	4.30	1.92	8.73
Diltiazem	5.65	2.05	8.06
Cinnarizine	11.27	5.43	3.50, 7.47
Lidoflazine	11.09	5.10	2.60, 7.74
Mioflazine	11.24	5.24	4.25, 6.10
R-56865	8.86	4.22	3.33, 8.52
Sabeluzole	8.31	4.20	3.40, 7.60

[a] The value for log $P_{Oct/W}$ published in ref. 4 was measured at pH 7.4 and taken.

log P_{calc} derived from the HPLC data using eqn. 4 further exemplifies the usefulness of the reported method. The group of substances selected for this purpose combines drugs and some other commonly available chemicals with known pK_a values and octanol–partition coefficients are listed in Table III. The means of the corresponding log P_{app} and log P_{calc} were calculated (abcissa in Fig. 2) and plotted together with the deviation from this mean value. The averaged absolute deviation from the mean was 0.13 ($n = 28$). The systematic deviation (bias) was 0.059 and can be neglected. The standard deviation (S.D.) from the log P_{app} to log P_{calc} group is 0.26, which is corresponds to the S.D. of the calibration set. This demonstrates the applicability of the regression results of the control group for the substances in Table III.

The differences in the retentions of reference compounds after 2000 injections caused by column ageing were in the range of the usual chromatographic inaccuracy. Thereafter the column was routinely exchanged to prevent a sudden loss of performance. Temperature-dependent variations of the retention times were successfully counteracted by the thermostating Peltier unit attached to the column. The measured temperature fluctuations in the column compartment were <0.1 K.

Structure–retention correlation

The parameter S of a solute, which is the slope of the line obtained by the linear least-squares fit of the

TABLE III

VERIFICATION WITH A TEST SET

Results of the calculation of log P_{calc} derived from log k_W data using eqn. 4 and comparison with the log P_{app} values at pH 7.4 derived from log $P_{Oct/W}$ using eqn. 1 (n = 28).

Substance	Log k_W	Log P_{calc}	Log P_{app}	Log $P_{Oct\,W}$	pK_a
Alprenolol	1.45	1.12	0.79[a]		9.50
Anisaldehyde	2.52	1.57	1.76	1.76	
Anisole	3.80	2.11	2.08	2.08	
Azobenzene	8.14	3.93	3.82	3.82	
Bemoridan	1.17	1.00	2.39	2.39	
Benzene	3.94	2.17	2.15	2.15	
Benzyl acohol	1.54	1.16	1.15	1.15	
Catechol	1.41	1.11	1.01[a]		9.85
Diazepam	5.94	3.01	2.86	2.86	3.30
Imipramine	3.87	2.14	2.52	4.62	9.50
Ketanserine	5.57	2.85	2.88	3.29	10.80
					7.60
Loperamide	5.23	2.71	3.66	5.13	8.86
Lorcainide	3.21	1.86	2.12	4.16	9.44
Naphthalene	6.41	3.20	3.37	3.37	
Nebivolol	5.47	2.81	2.91	4.04	8.50
Nebivolol (*SRR*)	5.51	2.83	3.01	4.05	8.40
Nebivolol (*RSS*)	5.5	2.82	3.092	4.06	8.40
Nimodipine	7.67	3.73	4.43	4.43	2.00
Nortryptyline	2.8	1.69	1.71[a]		9.70
Pimobendan	5.05	2.63	2.39	2.39	
Promethazine	6.35	3.18	3.02	4.73	9.10
Quinidine	2.47	1.55	1.11	3.71	5.40
					10.00
Quinine	2.74	1.66	1.41	3.71	5.07
					9.70
Ritanserine	8.74	4.18	4.34	5.20	0.90
					8.20
R-24571	11.98	5.54	5.37	5.37	
R-51107	7.97	3.86	4.07	4.07	5.13
R-55349	4.92	2.58	3.00	3.97	2.00
					4.85
					8.32
R-56566	3.39	1.94	1.92	2.62	8.00
					9.00

[a] The published value was determined at pH 7.4.

respective log k' *versus* φ_{MeOH}, is linearly correlated with log k_W in the three series of homologues in this study. Figure 3 presents the plots of S and log k_W for these groups. S is discussed as a lipophilicity measure and at least partially as a hydrogen bond activity indicator [3].

The results of the statistical evaluation are shown in Table IV. The slopes for phenones and alkylbenzenes are each outside their counterparts' 95% confidence intervals [15]. The slope calculated for the 4-alkylanilines compared with the phenones is also outside the 95% confidence interval. In comparison with the alkylbenzenes, the slope for the 4-alkylanilines is clearly inside the 95% confidence interval. The intercepts of the phenones and alkylbenzenes group are inside of each others' 95% confidence region but with respect to this parameter the 4-alkylaniline group is clearly outside the 95% interval. Hence all three regression lines are different with respect to their 95% confidence intervals. The lines

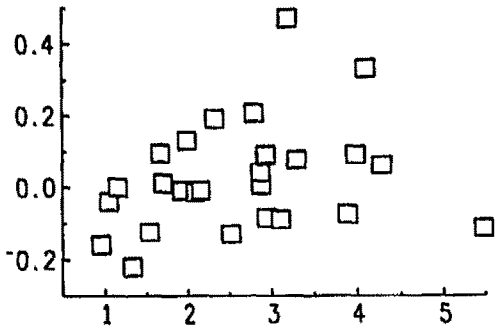

Fig. 2. Statistical evaluation of the test group (Table III). On the abscissa the mean of the corresponding log P_{app} and log P_{calc} and on the ordinate the deviation from this mean value are plotted. The averaged absolute deviation from the mean value of corresponding pairs was 0.130 (n = 28). The systematic deviation (bias) was 0.059. The most prominent deviations are found for nimodipine and loperamide.

for phenones have different slopes to those for benzenes and anilines. The lines for the anilines and the benzenes are nearly parallel but have different intercepts. The regression yielded nearly identical intercepts for the benzenes and phenones.

To answer the question of whether S or log k_W is the better choice for lipophilicity determinations, nineteen substances were randomly chosen and chromatographed on two columns containing different batches of the same stationary phase (Table V). Both columns were routinely refilled one time by the same supplier. The criteria for the choice of the eluent composition were the same as for the group of analytes in the reference group, so at the extreme positions acetylpyridine, hydroquinone and adenine were chromatographed in buffer A with 20% B and flunarizine with a maximum content of 95%B ≡ 92.5% methanol. The resulting S (Fig. 4) and log k_W values (Fig. 5) for both columns

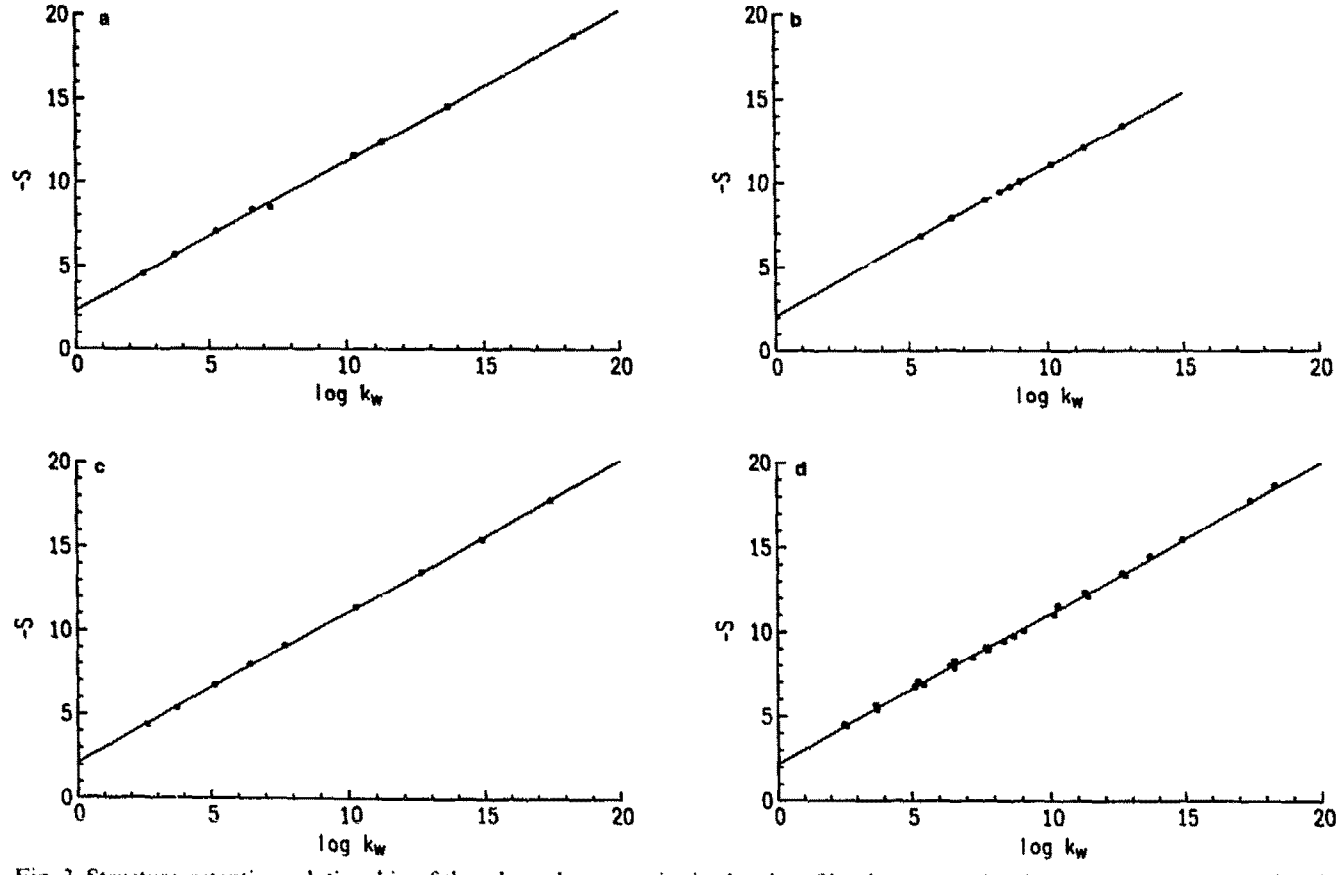

Fig. 3. Structure–retention relationship of three homologous series in the plot of log k_W *versus* Δlog k_W. $\Delta\varphi_{MeOH} \equiv S$. (a) 4-*n*-Alkylanilines; (b) alkylbenzenes; (c) phenones; (d) a plot of all three groups to illustrate the linearity and close resemblance of the regression results despite their significant differences (Table IV).

TABLE IV

STRUCTURE AND RETENTION

Statistical evaluation of the regression of log k_W and $-S$ for three series of substituted benzenes for structure–retention elucidation.

Class	Slope with 95% confidence	Abscissa with 95% confidence	r
Alkylbenzenes	0.884	2.061	0.9999
	0.876–0.892	1.989–2.134	(5)[a]
4-Alkylanilines	0.887	2.385	0.9999
	0.876–0.898	2.278–2.493	(6)[a]
Phenones	0.898	2.124	0.9999
	0.888–0.908	2.022–2.226	(7)[a]
Combined	0.891	2.191	0.9992
			(8)[a]

[a] Numbers in parentheses are the numbers of the regression equations.

were statistically analysed. A linear regression yielded $r = 0.979$, intercept 0.249 and slope = 0.9625 for S and $r = 0.996$, intercept 0.182 and slope = 0.980 for the log k_W values. The difference from unity of the slope is greater for the S than for the log k_W values and the same is observed for the corresponding intercepts. The mean difference between the two series is -0.0797 for log k_W and 0.05 for S. The corresponding S.D. values are 0.38 and 0.68. Hence 95% of all measured log k_W values will tend to appear in the region of two standard deviation ± 0.76 on the log k_W scale of the mean value of two determinations with different columns, hence use of log k_W is favored in measuring lipophilicity by RP-HPLC.

The time consumption for the determination of log k_W and $-S$ values depends to some extent on the problems associated with the solubility of the compounds of interest in organic solvents. For simplicity dissolving the sample in methanol was tried, thereafter another solvent was chosen that does not interfere with the chromatographic process. With the equipment described under Experimental one person can easily measure the HPLC data of 20–30 new substances in one week.

CONCLUSIONS

Every HPLC method based on the use of retention indices is sensitive to errors in the determination of the column void volume. Care must be taken not to detect polar and UV-absorbing contaminants as the void volume peak, otherwise the interpretation of the alteration of log k' with the methanol content may be complicated by artificial non-linearities. Contaminant peaks co-eluting with the void volume at high methanol concentrations can be misinterpreted as an increase in void volume when the methanol content is decreased and the contaminants are slightly retained. The use of a diode-array detector to identify the contaminants was helpful in this instance. Hence our results are independent of the void volume test compound.

The test set used in this investigation covers a broad range of lipophilicity from log $P_{Oct/W} = -0.16$ to far above 6. The observed high correlation of log $P_{Oct/W}$ and log k_W found in these experiments (eqn. 4) confirms the results and conclusions presented by Minick and co-workers [3,4]. The addition of diethylamine to the mobile phase effectively extends the upper range of lipophilicity to a log P_{app} of *ca.* 8.3 (see tetradecylaniline, Table I), if a linear extrapolation to this region is allowed. This offers the possibility of avoiding the manual determination of partition coefficients of such highly lipophilic substances in a water–octanol solvent system. Especially for these compounds the log $P_{Oct/W}$ determination is prone to errors. Although the test sets chosen by Minick and co-workers [3,4] cover a broad range of polarity, they are mainly restricted to relatively simply structured and small molecules. For use in pharmaceutical drug research, the method had to be improved to accommodate molecules as large as flunarizine or larger and containing at least partially protonated nitrogen atoms. In Table III some examples with known pK_a and log $P_{Oct/W}$

TABLE V

COLUMN TO COLUMN COMPARABILITY

A test set of nineteen different substances measured with two different columns (1 and 2) containing the same stationary phase (see Experimental). The linear regression coefficients (r) for log k' over φ_{MeOH} are given.

Compound	Column	Log k_w	$-S$	r
Acetylpyridine	1	0.0953	2.0634	−0.9903
	2	0.1538	2.0634	−0.9996
Acridine	1	4.7136	6.1018	−0.9920
	2	5.8706	7.7216	−0.9999
Adenine	1	−0.9204	2.8303	−0.9732
	2	−1.2934	1.2996	−0.9283
Acetophenone	1	2.4268	4.1200	−0.9910
	2	2.5930	4.3976	−0.9977
Anthracene	1	8.5610	9.8772	−0.9992
	2	8.7721	9.9431	0.9997
9-Anthracenemethanol	1	5.9545	7.9085	−0.9972
	2	6.4421	8.3274	−0.9996
9-Anthracenecarbaldehyde	1	8.0584	10.0452	−0.9999
	2	8.0656	9.8516	−0.9999
Flunarizine	1	12.4954	13.8210	−0.9997
	2	12.4364	13.6210	−0.9998
Hydroquinone	1	−0.3587	2.5703	−0.9501
	2	−0.0663	3.4241	−0.9999
Nebivolol	1	5.2604	7.0185	−0.9899
	2	5.5104	7.2056	−0.9961
Phenol	1	2.1297	4.3362	−0.9929
	2	1.9817	3.7273	−0.9999
Quinoline	1	3.4570	5.7043	−0.9976
	2	3.3971	5.2897	−0.999
Resorcinol	1	0.7201	3.7864	−0.9850
	2	0.9938	4.5588	−0.999
R-47623	1	3.8262	4.5694	−0.9959
	2	3.8858	4.6099	−0.9959
R-58214	1	10.5948	11.7092	−0.9909
	2	9.8161	10.6899	0.9990
R-60078	1	6.9630	8.6186	−0.9972
	2	6.8968	8.3050	−0.9956
R-60654	1	7.34	8.6543	−0.9981
	2	7.2653	8.7267	−0.9948
R-60931	1	6.5418	8.2845	−0.9973
	2	6.6194	8.2337	−0.9963
R-66678	1	11.3287	11.6564	−0.9971
	2	11.2134	11.3988	−0.9996

values demonstrate the applicability of the method to this group of substances. When the apparent partition coefficient for the octanol–water system, log P_{app}, is taken into account, the data for organic bases correlate well with the retention log k_w or log P_{calc}, which can be calculated from log k_w using eqn. 4. The small deviations of the log P_{app} values from their counterparts' log P_{calc} in Table III indicate that the retention in this experimental set-up is favourably due to the relative amount of the free base or of the ion with the lowest positive charge, as for R-24571 with its quaternary nitrogen. Acidic compounds suffer from ion-pairing effects (data not shown), but can be chromatographed with mobile phases without basic modifiers.

For further use, one must remember that without an exact value for pK_a, the results of HPLC experiments only reflect the partition coefficient of a substance in an environment with the pH of the mobile phase. The good correlation of log P_{app}, derived

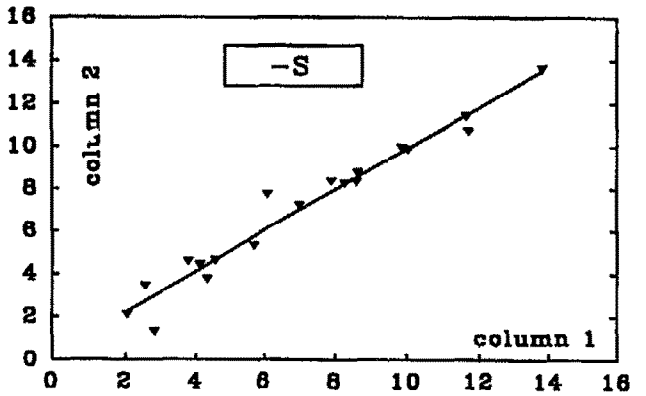

Fig. 4. Reproducibility of the chromatographic retention parameter S on two columns refilled with the same stationary phase but manufactured from diffent batch numbers of the latter.

from log $P_{Oct/W}$ for the actual pH, with the corresponding log P_{calc}, calculated from log k_W, seems to justify the application of the latter as a lipophilicity parameter. It will be easy to model closely the dependence of the partition and the pH over the (patho)physiological range when additional measurements at various pH values are performed.

The close correlation of log P_{calc} and log P_{app} strengthens the evidence for the similarity between the factors governing the retention mechanism in this HPLC environment and the factors of the octanol–water equilibrium. In dilute solution these are mainly hydrophobicity, hydration effects, charge distribution and hydrogen bond affinity. This is reflected by the results in Tables II and III. A differentiation according to hydrogen bond acid and other structure effects of molecules seems possible and will be part of future work.

In Table III nimodipine and loperamide are obviously far less retarded in HPLC than could be expected from their log $P_{Oct/W}$ values. Both seem to be examples for the influence of steric effects. These molecules have a stiff central region and polar substituents at the molecular periphery, which can be covered by water molecules. Hence their central lipophilic parts are shielded and cannot not interact with hydrocarbon chains, which are fixed to the support surface, in contrast to liquid–liquid partition where neither of the bulk phases is ordered.

The analysis of the data found for the three different butylbenzenes shows a decrease in log $P_{Oct/W}$ from *n*-butylbenzene to *tert.*-butylbenzene corresponding to the decrease in log k_W. Interestingly, the change in log k_W is proportional to the change in S (Fig. 3). A plot of log k_W against the side-chain carbon number reveals as expected that *sec.*- and *tert.*-butylbenzene are not members of the *n*-alkyl series. On the other hand, the three straight-chain homologues (Fig. 6) show a step-width in log k_W for each chain increment of 1.22 (S.D. = 0.01).

The responsibility of proton donor or acceptor effects for the position relative to similar compounds in the log k_W *versus* S plot [3] can only be interpreted qualitatively. Each series of homologues represents a single straight line. The weak hydrogen bond donors (alkylbenzenes, alkylanilines) are found on parallel lines and the line for acceptors (alkylphenones) has the same S for log k_W = 0 as

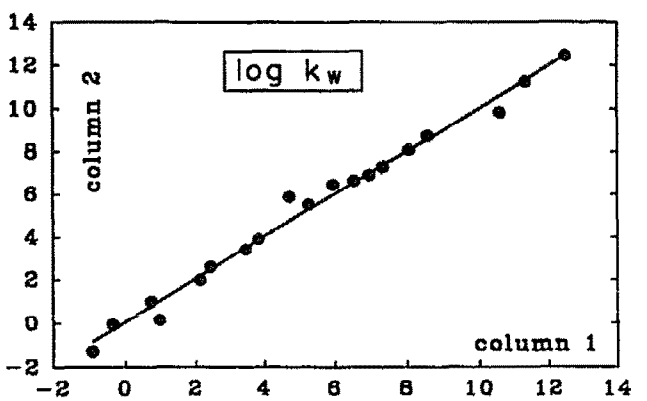

Fig. 5. Reproducibility of the chromatographic retention parameter log k_W on two columns with the same stationary phase but manufactured from diffent batch numbers of the latter.

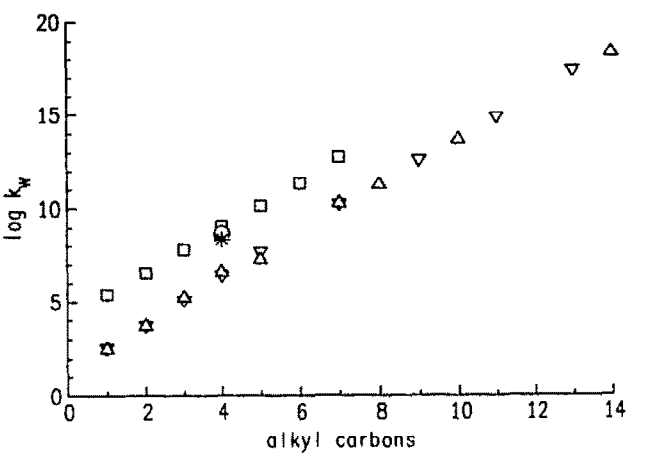

Fig. 6. Relationship between log k_W and carbon chain length and structure of (□) *n*-alkylbenzenes, (▽) *sec.*-butylbenzene, (*) *tert.*-butylbenzene, (△) phenones and (△) 4-*n*-alkylanilines.

the other monosubstituted phenyl derivatives but a different slope. The variations of both the intercept and slope between these groups are significant but small. Additional data sets containing more donor and acceptor series are needed for quantitative calculations. For example, the S values can be calculated using eqn. 5 and the log k_W and the difference of the calculated value to the measured S can be used to construct a ΔS scale as a parameter in structure–activity investigations or as a measure for hydrogen bonding affinity [3]. Therefore, the proposal of Braumann *et al.* [16] to use chromatographic data in QSAR studies gains further support. Possibly this will give access to protein binding constants [4,17].

The question of whether log k_W or S is the better choice for the comparison of data from different columns (with the same stationary phase) without recalibration is answered in favour of log k_W. The reproducibility of log k_W on different columns (with the same stationary phase) allows the calculation of log P_{calc} with deviations smaller than or equal to those found for octanol–water partition coefficient data in the literature and which can be qualified as "reliable" [deviations lower than 0.3 in compliance with good laboratory practice (GLP) guidelines]. The control group mentioned above can serve to minimize the differences and as a quality assurance.

Small structure effects can be substantiated by chromatography of binary mixtures of solutes in the described manner. This is possible with shake-flask techniques only, when an additional chromatographic step is added [18] or if molecules labelled with two different isotopes are synthesized.

Therefore we would like to recommend chromatography as a substitute for the log $P_{Oct/W}$ determination. For routine use a reduced set of compounds for calibration is sufficient, containing some of the polar, lipophilic and intermediate members of the current test set. In order not to lose the effects of more complex molecular structures, solutes such as the dihydropyridines can be included. A possible set of compounds are marked *a* in Table I. This will reduce the inevitable standardization overhead. A conversion to the octanol–water scale to establish comparability with literature data can easily be done at any time.

Another prospect is chromatography at different pH values to model more closely the behaviour of the solutes under (patho)physiological conditions and to obtain information concerning the approximate pK_a data for basic organic molecules, especially because the aproximate elution times can be roughly proposed using a first-guess pK_a. Interlaboratory comparability of the data is possible if the same packing material and the same control group are used.

Questions concerning the correlation of the results obtained with different stationary phases will be the subject of a future publication.

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