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THIN-LAYER AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THE EVALUATION OF THE LIPOPHILICITY OF ARYLOXOALKANOIC AND ARYLHYDROXYALKANOIC ACIDS

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SUMMARY

The series of aryloxoalkanoic and arylhydroxyalkanoic acids were subjected to both reversed-phase thin-layer (TLC) and high-performance liquid chromatography (HPLC) using chemically bonded packing materials. The lipophilicity of these acids seems to be dependent on intramolecular interactions between two hydrophilic fragments and was compared chromatographically with the reference group of arylacetic acids. TLC yielded different linear relationships between log P and R_M for the individual groups of the acids. Calculation of the partition coefficients from the log P *vs.* R_M dependence could thus lead to false results for structurally diverse compounds. No such differences were observed, however, in HPLC in the log P *vs.* log k' relationships derived for arylacetic, aryloxoalkanoic and arylhydroxyalkanoic acids.

INTRODUCTION

Chromatographic methods have been widely used for the evaluation of lipophilicity in quantitative structure–activity relationships (QSAR), especially reversed-phase thin-layer chromatography (RP-TLC)[1–4] and recently also high-performance liquid chromatography (HPLC), using chemically modified stationary phases[4–8]. On the assumption of a prevailing partition mechanism, the values of R_M or log k' are directly proportional to the logarithm of the partition coefficient, P_s, determined in the chromatographic system:

$$R_M (\log k') = \log P_s + \text{constant} \quad (1)$$

and can be used as parameters of lipophilicity in biological correlations.

A close similarity of solvation forces in both partitioning systems[9] is a condition of the validity of the Collander relationship[10] (eqn. 2) between the logarithms of the partition coefficients in the reference (P) and the chromatographic system:

$$\log P = a_1 \log P_s + b_1 \quad (2)$$

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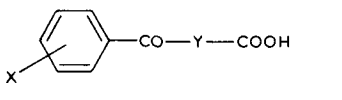
As the nature of the solvation cannot be determined exactly, the extent of the validity of the linear relationships in eqns. 3a and 3b can only be estimated empirically.

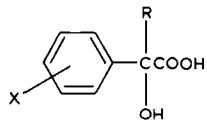
$$\log P = aR_M + b \tag{3a}$$
$$\log P = c\log k' + d \tag{3b}$$

Usually, the stastistical significance of these equations is satisfactory when applied to a series of structurally similar compounds. Increasing structural diversity can lead to deviations from linearity, however, as the conditions of similarity of the solvation effects are not longer fulfilled.

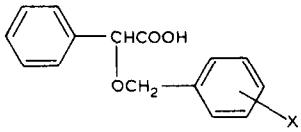
The lipophilicity of the arylaliphatic acids was evaluated[8,11–13] chromatographically on a thin layer of silica gel impregnated with silicone oil with aqueous acetone as the mobile phase. Such a system is similar to the reference system, especially with regard to some intramolecular interactions[8]. This was confirmed in the case of intramolecular hydrophobic interactions in arylalkoxy derivatives of arylaliphatic acids[11,12] and solvation hindrance in 3,4-dialkoxyarylaliphatic acids[8,13]. Similar results were also obtained in the evaluation of lipophilicity by HPLC using chemically bonded stationary phases[8]. Both TLC and HPLC methods were used for the evaluation of the lipophilicity in the series of acids I–VII, where the introduction of a functional group into the connecting chain between the aromatic nucleus and the carboxy group produces so called H/H interactions between the two hydrophilic fragments capable of hydrogen bonding[14]. The acids VIII were used as reference compounds.



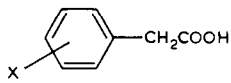
I : Y = $(CH_2)_2$ II : Y = $CH_2CH(CH_3)$
III : Y = $(CH_2)_3$ IV : Y = CH_2CHCH_2 (CH_3)



V: R = CH_3
VI: R = C_2H_5



VII



VIII

Rekker and Nyss[15–17], in their original fragmental method for the calculation of log *P* values (eqn. 4), characterized the influence of these interactions using the p.e. (proximity effect) correction values

$$\log P = \Sigma a_n f_n \tag{4}$$

Later, the intramolecular interactions were generally treated in the modified fragmental system of Leo and co-workers[14,18] by means of the fragmental factors *F*. Log

P wass expressed according to eqn. 5 as the sum of the fragmental constants f and the fragmental factors F.

$$\log P = \Sigma a_n f_n + \Sigma b_m F_m \quad (5)$$

We have used the fragmental method for the calculation of log P values, which were then compared with the experimental values obtained both by shake-flask method in the system octanol–water and by the chromatographic methods.

EXPERIMENTAL

TLC

Silanized Kieselgel 60 F_{254} (E. Merck, Darmstadt, F.R.G.) was used as the stationary phase. Impregnation was carried out by washing the glass plates (20 × 10 cm) with a 5% ethereal solution of silicone oil Lukoil 100 (VChZ Kolín, Czechoslovakia); the volatile components were evaporated within 16 h at 20°C. Solutions (1%) of the acids I–VIII in methanol were prepared and 5-μl samples were applied to the plate 3 cm from the lower edge. After evaporating the methanol at 20°C, ascending one-dimensional TLC was carried out using a citrate buffer (pH 3.4) containing 50% acetone as the mobile phase. The chromatographic chamber was equilibrated with the mobile phase for 16 h at 20°C. After migration for 15 cm, the plates were removed and, after the remaining mobile phase had been evaporated, the acids were detected under UV light (254 nm). Each chromatogram contained six compounds, two acids serving as reference samples. In the individual chromatograms the R_F values of the standards did not differ by more than 0.02.

HPLC

Experiments were carried out using a liquid chromatograph assembled from a Model 6000 A pump, a U6K injector, a 440-nm fixed wavelength detector and an M 730 data module (Waters Assoc., Milford, MA, U.S.A.). A commercial μBondapak C_{18} column (30 cm × 3.9 mm I.D.) (Waters Assoc.) was used as the stationary phase. Mixtures of 0.0025 M aqueous phosphate buffer (pH 3.0) with 40% acetonitrile or 50% methanol respectively were used as mobile phases. Doubly distilled water filtered through 0.45-μm Millipore filters was used throughout, and methanol was of LiChrosolv quality (E. Merck). The eluent flow-rate was 1 ml/min. Detection was performed by UV absorption at 254 μm, range 0–0.01 a.u. The retention time of sodium nitrate (0.2% solution) was taken as t_0 and the capacity factor, k', was evaluated from the retention time of the solute, t_R, by the relationship $k' = (t_R - t_0)/t_0$.

Determination of partition coefficients

Partition coefficients, P_{exp}, were determined by the shake-flask method[19] in an octanol–water system at 20°C, with both phases pre-saturated with the other. To eliminate the effect of dissociation, the aqueous phase employed was an acetate buffer (pH 3.4). The concentrations of the acids in the two phases were determined spectrophotometrically and the partition coefficients, P, were calculated as the ratio of concentrations in the octanol and aqueous phases ($P = C_0/C_w$).

Sample preparation

To prepare the acids I–IV we used the Friedel–Crafts reaction of anhydrides of dicarboxylic acids with the appropriately substituted aromatic compounds[20]. The acids V and VI were obtained[21] from the esters of aryloxocarboxylic acids by reaction with methylmagnesium iodide and subsequent hydrolysis. The acids VII were obtained[21] by reaction of 2-phenyl-2-hydroxyacetate with the corresponding benzyl chlorides in the presence of sodium hydride, followed by hydrolysis. The arylacetic acids VIII were prepared[22] by the Wilgerodt reaction or by the hydrolysis of the corresponding arylacetonitriles.

Calculations

Log P values of unsubstituted acids I–V and VII were calculated by the modified fragmental method[18]. Log P values of the substituted acids I–IV were calculated using the parameters π derived[23] for the substituted benzoic acids. For the acids V–VII the parameters π derived for the substituted benzyl alcohols were used, and for the acids VIII the parameters π were taken from those derived for the arylacetic acids[23]. The sum of the π parameters for the 3-chloro-4-alkoxy derivatives was reduced by 0.23, in accordance with the results of partition chromatography of those derivatives of arylaliphatic acids[11,13,22].

The coefficients in the regression equations were calculated from the experimental results by multiple regression analysis. The statistical significances of the regression equations were tested by the standard deviation (s), the coefficient of multiple correlation (r) and the Fischer–Snedecor criterion (F).

RESULTS AND DISCUSSION

The experimental R_F and R_M values of acids I–IV are summarized in Table I. The relationship between the logarithms of partition coefficients and the R_M values is expressed by the equation

$$\log P = 2.715\, R_M + 2.127$$
$$n = 22,\ r = 0.994,\ s = 0.105,\ F = 1676.8 \qquad (6)$$

Comparison of the logarithms of the experimental partition coefficients with the values computed by the fragmental method[14,18] (see Table II) shows that the corrections used describe well the H/H interactions and their effect on the lipophilicity. Eqn. 6 was applied to the acids I–IV with different connecting chains Y and that with different interactions between both hydrophilic fragments. From this equation it can be inferred that the corresponding changes in lipophilicity are similarly reflected both in the chromatographic and in the octanol–water reference system. Eqn. 6 does not include the 4-phenyl derivatives Ii, IIc, IIId and IVc as their lipophilicities differ substantially from the log P values in the octanol–water system.

The differences, however, do not prevent the use of R_M values as parameters of lipophilicity in the correlation with the collagen-induced aggregation of platelets. The equivalence of the log P and R_M values is evident from eqns. 7 and 8.

TABLE I

TLC CHARACTERISTICS OF ω-ARYLOXOALKANOIC ACIDS I–IV

No.	*X*	*Log P**	R_F	R_M	*Log* $(1/C_{exp})$**	*Log* $(1/C_c)$***
Ia	H	1.30*	0.655	−0.28	2.376	2.395
Ib	4-CH_3O	1.38*	0.655	−0.28	2.541	2.395
Ic	3-Cl-4-CH_3O	1.98	0.533	−0.055	2.602	2.648
Id	4-*i*-C_3H_7O	2.18	0.49	0.02	2.640	2.707
Ie	4-Br	2.28	0.517	−0.03	–	–
If	4-*i*-C_3H_7	2.70	0.40	0.18	–	–
Ig	3-Cl-4-*i*-C_3H_7O	2.78	0.365	0.24	2.785	2.803
Ih	4-*i*-C_4H_9	3.20	0.31	0.35	2.836	2.810
Ii	4-C_6H_5	3.20 * (2.70)§	0.38	0.21	2.780	2.797
Ik	4-cyclo-C_6H_{11}	3.76	0.207	0.585	2.775	2.731
Il	4-*n*-$C_6H_{13}O$	3.88	0.167	0.70	–	–
Im	3-Cl-4-cyclo-C_6H_{11}	4.36	0.14	0.79	2.500	2.557
IIa	H	1.62*	0.587	−0.155	2.240	2.352
IIb	4-*i*-C_3H_7O	2.50	0.393	0.19	2.699	2.593
IIc	4-C_6H_5	3.40* (3.13)§	0.30	0.37	2.638	2.610
IId	3-Cl-4-*i*-C_4H_9O	3.60	0.21	0.58	–	–
IIe	4-cyclo-C_6H_{11}	4.08	0.16	0.72	2.417	2.430
IIIa	3-Cl-4-CH_3O	2.17*	0.483	0.03	3.009	3.041
IIIb	4-*i*-C_3H_7	2.89	0.345	0.28	3.167	3.137
IIIc	4-*i*-C_4H_9	3.39	0.234	0.515	3.071	3.095
IIId	4-C_6H_5	3.35* (2.97)§	0.33	0.31	3.185	3.139
IIIe	4-cyclo-C_6H_{11}	3.95	0.172	0.685	2.975	2.986
IVa	3-Cl-4-CH_3O	2.60*	0.414	0.15	2.896	2.907
IVb	3-Cl-4-*i*-C_3H_7O	3.40	0.241	0.50	2.936	2.904
IVc	4-C_6H_5	3.82 (3.43)§	0.248	0.48	2.839	2.912
IVd	4-cyclo-C_6H_{11}	4.38	0.147	0.76	2.762	2.719

* Values marked with asterisks were determined in octanol–buffer (pH 3.5) by the shake-flask method and the others were calculated using the respective π values (see Experimental).

** The anti-aggregating activity was measured by the Born's method[24] and expressed by the concentration C (mol l^{-1}) that produced a 50% inhibition of aggregation.

*** Calculated from eqn. 8.

§ Calculated from eqn. 6.

$$\log (1/C) = 0.869 \log P - 0.144 (\log P)^2 + 0.145 E_S + 0.328 I_L + 1.839 \quad (7)$$
$$n = 22, r = 0.973, s = 0.064, F = 75.3$$
$$\log (1/C) = 0.739 R_M - 1.152 R_M^2 + 0.159 E_S + 0.328 I_L + 1.839 \quad (8)$$
$$n = 22, r = 0.972, s = 0.065, F = 73.2$$

Similarity of the solvation forces in the two systems cannot be overestimated, however. The common chromatographic evaluation of selected acids I–VI and VIII yielded the R_M values summarized in Table II. Eqn. 9 and Fig. 1 illustrate the relationship between log P and R_M for all acids.

$$\log P = 2.495 R_M + 2.476 \quad (9)$$
$$n = 21, r = 0.972, s = 0.198, F = 329.7$$

Systematic deviations of the individual structurally different series of acids indicate that these compounds cannot be merged into a single regression equation. In

TABLE II

CHROMATOGRAPHIC BEHAVIOUR OF ACIDS I–VI AND VIII

No.	*X*	*Log P**	R_M	*Log k'***	*Log k'****
Ia	H	1.30* (1.24)§	−0.365	0.201	0.107
Ic	3-Cl-4-CH_3O	1.98	–	0.610	0.365
Id	4-*i*-C_3H_7O	2.18	−0.03	0.815	0.504
Ie	4-Br	2.28	−0.015	0.675	0.445
If	4-*i*-C_3H_7	2.70	0.18	1.039	0.683
Ii	4-C_6H_5	3.20*	–	–	0.795
Ik	4-cyclo-C_6H_{11}	3.76	–	–	1.190
Ih	4-*i*-C_4H_9	3.20	0.30	–	–
IIa	H	1.62* (1.65)§	−0.225	0.437	0.268
IIc	4-C_6H_5	3.40*	–	–	0.964
IIe	4-(2',4'-$F_2C_6H_3$)	3.68	–	–	1.068
IIIa	3-Cl-4-CH_3O	2.17* (2.10)§§	−0.02	0.819	0.452
IIId	4-C_6H_5	3.35*	–	–	0.901
IVa	3-Cl-4-CH_3O	2.60* (2.51)§§	0.145	–	0.650
IVb	3-Cl-4-*i*-C_3H_7O	3.40	–	1.532	–
IVc	4-C_6H_5	3.82*	–	–	1.068
Va	H	0.80* (0.91)§	−0.715	−0.040	−0.144
Vb	4-CH_3O	0.81	−0.71	−0.030	−0.144
Vc	3-Cl-4-CH_3O	1.26	−0.51	–	–
Vd	4-*i*-C_4H_9	2.75*	0.06	1.152	0.653
Ve	4-C_6H_5	2.71*	−0.05	–	0.514
Vf	4-cyclo-C_6H_{11}	3.26	0.215	–	–
Vg	4-C_2H_5	1.78	−0.33	–	–
VIa	4-*i*-C_4H_9	3.37*	0.20	1.432	0.898
VIb	4-C_6H_5	3.37	–	–	0.747
VIIIa	H	1.45*	−0.44	0.226	0.120
VIIIb	4-Cl	2.15	−0.205	0.591	0.379
VIIIc	4-C_2H_5	2.43	−0.03	0.819	0.524
VIIId	4-*i*-C_3H_7	2.85	0.12	1.086	0.710
VIIIe	4-*t*-C_4H_9	3.13	0.26	1.297	0.857
VIIIf	4-*n*-$C_5H_{11}O$	3.46	0.335	1.541	1.052
VIIIg	4-cyclo-C_6H_{11}	3.91	–	–	1.199

* Values marked with asterisks were determined in octanol–buffer (pH 3.5) by the shake-flask method and the others were calculated using the respective π values (see Experimental).

** methanol was used as a modifier in the mobile phase.

*** Acetonitrile was used as a modifier in the mobile phase.

§ Values were calculated using the fragmental constants from ref. 18.

§§ Values were calculated by a combination of the fragmental method[18] (unsubstituted compound) and π parameters from ref. 23.

such a case an attempt to use the equation for the computation of the partition coefficients for structurally diverse compounds could lead to false results. It is evident also from the different linear relationships between log P and R_M for aryloxoalkanoic acids (eqn. 10), arylhydroxyalkanoic acids (eqn. 11) and arylacetic acids (eqn. 12).

$$\log P = 2.765\, R_M + 2.267 \quad (10)$$

$$n = 8,\ r = 0.995,\ s = 0.067,\ F = 565.9$$

$$\log P = 2.632\, R_M + 2.682 \quad (11)$$

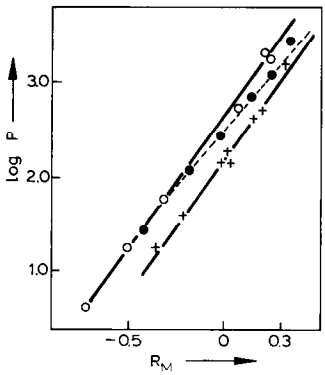


Fig. 1. Relationship between log P and R_M for arylacetic (●), aryloxoalkanoic (+) and arylhydroxyalkanoic (○) acids.

$$n = 7,\ r = 0.998,\ s = 0.066,\ F = 1619.9$$
$$\log P = 2.463\ R_M + 2.562 \qquad (12)$$
$$n = 6,\ r = 0.996,\ s = 0.077,\ F = 445.9$$

The capacity factors of selected acids I–VI and VIII are given in Table II. In contrast to previous results there are no significant differences among the individual series of acids, regardless of the modification of the mobile phase by acetonitrile or methanol. The common linear relationships between log P and log k' values are expressed by eqns. 13 and 14.

$$\log P = 2.310 \log k'\ (CH_3CN) + 1.177 \qquad (13)$$
$$n = 25,\ r = 0.993,\ s = 0.106,\ F = 1716.9$$
$$\log P = 1.657 \log k'\ (CH_3OH) + 0.949 \qquad (14)$$
$$n = 17,\ r = 0.992,\ s = 0.114,\ F = 903.9$$

Eqn. (13) also contains the biphenylyl analogues If, IIc, e, IIId and IVc. These results indicate that for these particular systems the Collander rule holds for all three groups of acids, distiguished by considerable structural changes.

TABLE III

CHROMATOGRAPHIC BEHAVIOUR OF ACIDS VII

No.	*X*	R_M	*Log k'**	*Log k'***	Log P_{exp}***	*Log P_{calc}*		
						A§	*B*§§	*C*§§§
VIIa	H	−0.25	0.875	0.584	2.60	2.71	2.40	2.53
VIIb	3-Cl-4-CH_3O	−0.01	1.158	0.791	2.45	3.32	2.87	3.00
VIIc	4-Cl	−0.09	1.264	0.847	2.60	3.57	3.04	3.13

* Methanol was used as a modifier in the mobile phase.
** Acetonitrile was used as a modifier in the mobile phase.
*** Determined in octanol–buffer (pH 3.5) by the shake-flask method.
§ Calculated by the fragmental method.
§§ Calculated from eqn. 14 using log $k'(CH_3OH)$.
§§§ Calculated from eqn. 13 using log $k'(CH_3CN)$.

The lipophilicity of three derivatives of 2-benzyloxyphenylacetic acid (VII) was also evaluated by HPLC (see Table III). Similarly to the benzyloxy arylaliphatic acids, there was a decrease in the experimental values of log P compared with the values calculated by the fragmental method. This may be due to the intramolecular hydrophobic interaction of both aromatic nuclei connected by a sufficiently flexible three-atom chain. The retention characteristics of these acids do not correspond to the lipophilicity measured in the octanol–water system. The values of log P calculated from eqns. 13 and 14 are considerably higher than those of log P_{exp} (see Table III).

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