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INFLUENCE OF INTRAMOLECULAR INTERACTIONS ON CHROMATOGRAPHIC BEHAVIOUR OF ARYLALIPHATIC ACIDS

I. COMPARISON OF REVERSED-PHASE THIN-LAYER AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Series of arylacetic acids were subjected both to reversed-phase thin-layer chromatography and to high-performance liquid chromatography using chemically bonded packing materials. In addition to the reference series of arylacetic acids, the dialkoxy and phenylalkoxy derivatives were also studied as their lipophilicities were influenced by intramolecular interactions. The influence of various stationary phases upon changes in lipophilicity of the dialkoxy and phenylalkoxy derivatives was studied through relationships between π and the retention indices (R_M or log k'). It was found that when an aqueous mobile phase containing an organic solvent (50% acetone or 60% methanol) was used, the changes in lipophilicity of the dialkoxy and phenylalkoxy derivatives corresponded to the changes in lipophilicity measured in the reference system *n*-octanol–water. Extrapolation of retention indices to pure water was not advantageous, and negatively influenced the calculation of the π parameters for the dialkoxy and phenylalkoxy derivatives from the corresponding retention indices.

INTRODUCTION

Lipophilicity in quantitative structure–activity relationships (QSAR) is usually characterized by the logarithms of the partition coefficients in the *n*-octanol–water system (log P) or by substituent parameters π[1,2] or fragmental constants f[2,3]. It has been shown[4–6] that the retention indices R_M or ΔR_M from reversed-phase thin-layer chromatography (RP-TLC) are frequently linearly related to log P, π or f. The statistical significance of these relationships is dependent on the character of the partitioning system and on the compounds tested. Such linear relationships have also been observed for a series of closely related compounds with chromatographic systems strikingly different from the *n*-octanol–water reference system.

In connection with our quantitative structure–activity relationship (QSAR) study of arylaliphatic acids we have also used the R_M values from RP-TLC for char-

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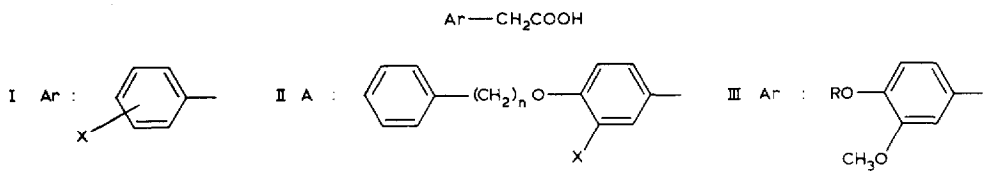
acterizing their lipophilicity[7–10]. Some pitfalls of this approach, due to a non-linearity of the relationship R_M *vs.* π in series with extremely wide lipophilicity ranges, have been described[10]. The relationship between R_M and π can be considered linear provided the lipophilicity range in a series of compounds does not exceed three units of π. It has also been found that silica gel impregnated with a silicone oil and 50% acetone buffered to pH 3.4 is a suitable system for those derivatives of arylaliphatic acids where the lipophilicity is influenced by intramolecular interactions. For example, there is a decline in lipophilicity for 4-benzyloxy derivatives[9,11] of the arylaliphatic acids which could be caused by interaction of both aromatic nuclei. The lower lipophilicity values can be used[11,12] in correlations of biological activities in which a transport process through a biological system prevails. Such a decrease in lipophilicity was also observed in 3,4-dialkoxy derivatives of arylaliphatic acids and the experimental values were suitable for correlations of *in vitro* as well as *in vivo* biological activities[7,13,14].

High-performance liquid chromatography (HPLC) has been used[15–18] for the evaluation of lipophilicity in QSAR. Stationary silica phases, in which the silanol sites are chemically linked to octadecyl residues, are frequently used for this purpose[19]. The remaining silanol groups, which could influence the retention mechanism of compounds, are usually removed by subsequent silylation. Any impregnation[16,17,20] by a suitable solvent (*e.g.* *n*-octanol[16,20] or oleyl alcohol[17]) favours a partition mechanism of separation, although this enhances experimental difficulties. Some commercially available stationary phases are already supplied with a high surface coverage of siliceous material and can be used directly without any pretreatment[21–23]. The mobile phase also affects the retention behaviour. As the use of water tends to increase retention times too much, mixed mobile phases are usually used with methanol, acetonitrile or tetrahydrofuran as the organic modifiers. The influence of these solvents on the polar group selectivity has been studied by Tanaka *et al.*[24] and by Tomlinson and co-workers[25,26]. Maximum differences in the retention of the non-ionic aromatic compounds were found for aqueous methanol while minimum ones were found for aqueous tetrahydrofuran[24]. The strikingly better linear dependence between log *P* for *n*-octanol–water and the retention indices determined with the methanol–water mobile phase shows the preferred use of methanol as the organic modifier. Such a conclusion is further supported by a relationship between the retention indices determined using aqueous methanol (log k'_M) and aqueous tetrahydrofuran (log k'_T). This relationship was calculated from the experimental values for substituted benzenes taken from ref. 24 and is expressed by eqn. 1. The fit is improved by introduction of constants E_W, taken from ref. 27, which correct the effects of hydrogen bonding.

	n	r	s	F	
$\log k'_T = 1.045 \log k'_M + 0.300$	16	0.957	0.142	151.2	(1)
$\log k'_T = 0.841 \log k'_M - 0.185 E_W + 0.574$	16	0.981	0.098	167.3	(2)

The utility of HPLC retention indices for the evaluation of lipophilicity in QSAR has been verified by a number of authors. A comparison of thin-layer chromatography (TLC) and HPLC retention indices showed a satisfactory agreement for the series of penicillins[28,29] and phenols[30].



The present paper deals with the retention behaviour of the arylacetic acids I–III as determined by both TLC and HPLC. Our attention was aimed at those derivatives where the intramolecular interactions among the substituents could lead to a failure of the additivity principle. The use of different stationary phases made it possible to estimate their influence on a decrease in lipophilicity of the arylalkoxy derivatives (II) and the 3,4-dialkoxy derivatives (III), compared with the values computed from the tabulated π parameters. The influence of the organic modifiers was studied in the selected systems by extrapolating the retention indices to pure water. The results from different chromatographic systems were compared with the corresponding changes in lipophilicity for the *n*-octanol–water system.

EXPERIMENTAL

TLC

Three systems with different stationary phases were used.

System A. The stationary phase was prepared by shaking 25 g of silica gel GF_{254} for 90 sec with a mixture of 5% of silicone oil Lukoil 100 (VChZ Kolín, Czechoslovakia) with 6 ml of acetone and diluting with dioxane to 50 ml. The glass plates (20 × 10 cm) were covered with a 0.25-mm layer of a slurry of the support using standard equipment. The volatile components of the impregnating solution were evaporated within 16 h at 20°C.

System B. Silanized Kieselgel 60 F_{254} (E. Merck, Darmstadt, F.R.G.) was used as stationary phase. Impregnation was carried out by washing the glass plates (20 × 10 cm) with a 5% ethereal solution of silicone oil Lukoil 100; the volatile components were evaporated within 16 h at 20°C.

System C. Silanized Kieselgel 60 F_{254} was used as stationary phase without any pretreatment.

For all three systems, 1% solutions of the acids I–III in methanol were prepared, and 5-μl samples were applied to the plate 3 cm from the lower edge. After evaporating off the methanol at 20°C, ascending one-dimensional TLC was carried out using a citrate buffer (pH 3.4) containing various percentages of acetone as the mobile phase. A 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 off, the acids were detected under UV light (λ = 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 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 in System E. The other columns were custom-made (25 cm × 4 mm I.D.), slurry-packed with 5-μm Spherisorb ODS (in System D) and Partisil 5-ODS* (in System F). A mixture of methanol and 0.0025 *M* aqueous phosphate buffer (pH 3.0) was used as the mobile phase. Double-distilled water filtered through 0.45-μm Millipore filters was used throughout, and methanol was Lichrosolv quality (E. Merck). Water determinations were carried out by Karl Fischer titration, with a dead-stop end-point indication. The eluent flow-rate was 1 ml/min. Detection was performed by UV absorption at 280 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_{SF}, were determined by the shake-flask method[31] in a *n*-octanol–water system at 20°C, with both phases being presaturated 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 *n*-octanol and aqueous phase ($P = C_0/C_w$).

Sample preparation

The arylacetic acids I–III were prepared[9,11] by the Wilgerodt reaction or by the hydrolysis of the corresponding arylacetonitriles; the alkoxy derivatives were obtained by alkylation of the methyl esters of the corresponding 4-hydroxyarylacetic acids and subsequent hydrolysis.

Calculations

The π parameters derived[1] for arylacetic acids were used for calculation of the $\Sigma\pi_{tab}$ values for compounds I III. The π parametes for the alkoxy and for the higher alkyl groups were calculated using the following increments[31]: $\Delta\pi = 0.5$ for aliphatic CH_2, 0.41 for cyclic CH_2, −0.2 for branching and −0.3 for a double bond. 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[7,9–11].

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 values of the retention indices for the acids I–III are sum-

* Spherisorb was kindly donated by Dr. M. J. Holdoway (Phase Separations, Hauppage, NY, U.S.A.) and Partisil was gained through the kindness of Dr. T. E. Beasley (Whatman, Clifton, NJ, U.S.A.).

TABLE I

TLC R_M VALUES FOR ARYLACETIC ACIDS I–III

Compound No.	*Substituents*	*R_M values***						
		A	B_{45}	B_{50}	B_{55}	B_{60}	B_0	*C*
Ib	3-Cl-4-CH_3O	−0.38	−0.14	−0.225	−0.275	−0.31	0.351	−0.345
Ic	4-Cl	−0.33	−0.05	−0.13	−0.21	−0.275	0.627	−0.275
Id	4-*iso*-C_3H_7O	−0.36	0.03	−0.055	−0.165	−0.22	0.80	−0.165
Ie	4-C_2H_5	−0.25	0.09	0.015	−0.105	−0.155	0.86	−0.10
If	3-Cl-4-CH_2=$CHCH_2O$	−0.22	0.21	0.115	−0.055	−0.085	1.155	−0.015
Ig	3-Cl-4-*iso*-C_3H_7O	−0.19	0.25	0.165	0	−0.055	1.224	0.03
Ih	4-*iso*-C_3H_7	−0.14	0.29	0.21	0.055	−0.02	1.273	0.10
Ii	4-*tert.*-C_4H_9	−0.055	0.47	0.315	0.18	0.06	1.69	0.225
Il	4-*n*-$C_5H_{11}O$	0.06	0.68	0.52	0.315	0.155	2.287	0.34
Im	4-*cyclo*-C_6H_{11}	0.14	0.92	0.76	0.485	0.25	3.003	0.52
In	4-*n*-$C_6H_{13}O$	0.20	1.00	0.82	0.535	0.305	3.154	0.60
IIa	4-$C_6H_5CH_2O$	−0.14	0.265	0.205	0.10	−0.04	1.46	0.12
IIb	3-CH_3O-4-$C_6H_5CH_2O$	−0.20	0.235	0.10	−0.065	−0.10	1.30	0
IIc	3-Br-4-$C_6H_5CH_2O$	0.02	0.68	0.50	0.285	0.09	2.473	0.375
IId	3-Cl-4-$C_6H_5CH_2O$	−0.015	0.63	0.41	0.20	0.07	2.312	0.33
IIe	3-Cl-4-$C_6H_5(CH_2)_2O$	0.055	–	0.53	0.31	0.09	2.693	0.435
IIf	3-Cl-4-$C_6H_5(CH_2)_3O$	0.185	1.00	0.80	0.52	0.27	3.267	0.60
IIIa	3-CH_3O-4-*iso*-C_3H_7O	−0.425	−0.22	−0.27	−0.32	−0.35	0.128	−0.37
IIIb	3-CH_3O-4-*n*-$C_6H_{13}O$	0.01	0.64	0.49	0.25	0.14	2.22	0.315
IIIc	3-CH_3O-4-*cyclo*-$C_6H_{11}CH_2O$	0.015	0.66	0.50	0.275	0.14	2.27	0.355

* Subscript at *B* denotes percentage of acetone in the mobile phase; column B_0 gives the extrapolated values.

TABLE II

HPLC RETENTION INDICES FOR ARYLACETIC ACIDS I–III

Compound No.	*Substituent*	$\Sigma\pi_{tab}$	*log k' values**						
			D_{50}	D_{58}	D_{60}	$D_{67.5}$	D_0	E	F
Ia	H	0	0.145	−0.246	−0.280	−0.683	2.495	−0.293	0.005
Ib	3-Cl-4-CH_3O	0.46	0.419	0.031	−0.020	−0.402	2.752	−0.093	0.189
Ic	4-Cl	0.70	0.492	0.116	0.090	−0.298	2.732	−0.008	0.297
Id	4-*iso*-C_3H_7O	0.81	0.609	0.220	0.181	−0.198	2.897	0.070	0.386
Ie	4-C_2H_5	0.98	0.718	0.344	0.250	−0.068	2.959	0.213	0.499
If	3-Cl-4-CH_2=$CHCH_2O$	1.16	0.818	0.412	0.332	−0.039	3.258	0.222	0.528
Ig	3-Cl-4-*iso*-C_3H_7O	1.26	0.870	0.473	0.411	0.016	3.301	0.269	0.607
Ih	4-*iso*-C_3H_7	1.40	0.971	0.572	0.490	0.131	3.362	0.364	0.703
Ii	4-*tert.*-C_4H_9	1.68	1.178	0.753	0.613	0.287	3.705	0.536	0.878
Ik	4-*iso*-C_4H_9	1.90	1.275	0.900	0.765	0.390	3.822	–	–
Il	4-*n*-$C_5H_{11}O$	2.01	1.340	0.970	0.845	0.466	3.854	–	–
Im	4-*cyclo*-C_6H_{11}	2.46	1.655	1.239	1.095	0.676	4.471	0.912	1.270
In	4-*n*-$C_6H_{13}O$	2.51	1.675	1.263	1.099	0.709	4.457	0.972	1.334
IIa	4-$C_6H_5CH_2O$		1.059	0.642	0.542	0.156	3.637	0.399	0.735
IIb	3-CH_3O-4-$C_6H_5CH_2O$		0.854	0.449	0.361	−0.039	3.404	0.231	0.550
IIc	3-Br-4-$C_6H_5CH_2O$		1.408	0.939	0.785	0.406	4.273	0.659	0.990
IId	3-Cl-4-$C_6H_5CH_2O$		1.301	0.861	0.720	0.337	4.061	0.585	0.921
IIe	3-Cl-4-$C_6H_5(CH_2)_2O$		1.534	1.064	0.899	0.508	4.474	0.768	1.120
IIf	3-Cl-4-$C_6H_5(CH_2)_3O$		1.720	1.311	1.112	0.712	4.636	0.985	1.352
IIIa	3-CH_3O-4-*iso*-C_3H_7O		0.442	0	−0.040	−0.452	2.900	−0.104	0.203
IIIb	3-CH_3O-4-*n*-$C_6H_{13}O$		1.509	1.043	0.882	0.502	4.392	0.757	1.119
IIIc	3-CH_3O-4-*cyclo*-$C_6H_{11}CH_2O$		1.535	1.076	0.917	0.542	4.378	0.792	1.153

* Subscript at D denotes percentage of methanol in the mobile phase; column D_0, gives the extrapolated values.

marized in Tables I and II. The derivatives without intramolecular interactions and those containing a chloro group and an alkoxy group were included in series I. The relationships between the tabulated $\Sigma\pi$ values and the retention indices of acids I from six different chromatographic systems A–F are given in Table III. As is evident from eqns. 3–8, all the chromatographic systems are comparable to the *n*-octanol–water reference system. Even with the alkoxy derivatives, Id, f, g, l and n, which have a tendency to hydrogen bonding[32], the systems remain regular. A possible criterion for the suitability of the retention indices R_M or log k' (indicated as Y in Table III) for the evaluation of lipophilicity is the slope of the linear relationship between $\Sigma\pi$ and Y. The lower the slope, the larger is the range of retention index corresponding to the same range of lipophilicity, while the selectivity of hydrophobic retention of a separation system increases. From such a viewpoint, a silanised silica gel impregnated with a silicone oil (in System B), is the optimum stationary phase. A similar advantage holds for all three chemically bonded carriers in HPLC.

These chromatographic systems were also examined with regard to their capacity to reflect any influence of intramolecular interactions on the total lipophilicity of a solute. Using the retention indices of the acids II and III, the corresponding $\Sigma\pi$ values were calculated from the regression equations (see Table IV). The $\Sigma\pi_{tab}$ values calculated from the tabulated π parameters and the values of $\Sigma\pi_{exp}$ determined from log P_{SF} (*n*-octanol–water) are given in Table IV for purposes of comparison. The fall in lipophilicity for groups II and III is obvious; the reasons for such a decline are discussed elsewhere[14,33]. The values of $\Sigma\pi$ calculated from the R_M values indicate that the decrease in lipophilicity of the acids II and III in the systems A–C corresponds to the change in lipophilicity in the reference system *n*-octanol–water. Similar changes in lipophilicity are evident also in the HPLC systems D–F; however, the decrease is not so striking as in the TLC systems, especially for the dialkoxy derivatives III.

Retention indices obtained by linear extrapolation of the mobile phase to pure water are frequently used to express lipophilicity[6,17,32,34,35]. It is necessary to work at several different concentrations of the modifier; however the results are considered more reliable compared with those obtained in a mobile phase containing an organic modifier[17,32,34]. Thus, a dependence of the retention indices on the concentration of

TABLE III

RELATIONSHIPS BETWEEN $\Sigma\pi$ AND RETENTION INDICES Y IN THE SERIES OF ARYLACETIC ACIDS I

$\Sigma\pi = aY + b$

System	Y	a	b	n	r	s	F	*Eqn. No.*
A	R_M	3.501	1.871	11	0.993	0.101	724	3
B_{50}	R_M	1.990	0.949	11	0.998	0.048	2047	4
C	R_M	2.222	1.218	11	0.997	0.053	1679	5
D_{60}	log k'	1.802	0.519	13	0.999	0.030	7931	6
E	log k'	1.959	0.664	11	0.998	0.053	2133	7
F	log k'	1.849	0.095	11	0.998	0.058	1808	8
B_0	R_M	0.797	0.317	11	0.991	0.097	495	9
D_0	log k'	1.172	−2.639	13	0.985	0.135	371	10

TABLE IV

LIPOPHILICITY PARAMETERS OF PHENYLALKOXYARYLACETIC ACIDS (II) AND 3-METHOXY-4-ALKOXYARYLACETIC ACIDS (III)

Compound No.	*Substituent*	*log* P_{SF}*	$\Sigma\pi_{tab}$	$\Sigma\pi_{exp}$**	$\Sigma\pi_{calc}$***							
					A§	B_{50}	B_0	C	D_{60}	D_0	E	F
IIa	4-$C_6H_5CH_2O$	2.85	1.91	1.40	1.38	1.36	1.38	1.48	1.49	1.62	1.45	1.45
IIb	3-CH_3O-4-$C_6H_5CH_2O$	2.59	1.95	1.14	1.16	1.15	1.26	1.22	1.17	1.35	1.12	1.11
IIc	3-Br-4-$C_6H_5CH_2O$	3.51	2.77	2.06	1.94	1.95	2.12	2.05	1.93	2.37	1.96	1.93
IId	3-Cl-4-$C_6H_5CH_2O$	3.43	2.59	1.98	1.82	1.77	2.00	1.95	1.82	2.12	1.81	1.80
IIe	3-Cl-4-$C_6H_5(CH_2)_2O$	3.53	3.09	2.08	2.06	2.00	2.28	2.18	2.14	2.60	2.17	2.17
IIf	3-Cl-4-$C_6H_5(CH_2)_3O$	3.90	3.59	2.45	2.52	2.54	2.64	2.55	2.53	2.79	2.59	2.59
IIIa	3-CH_3O-4-*iso*-C_3H_7O	1.75	0.85	0.30	0.38§§	0.41	0.41	0.40	0.46	0.76	0.46	0.47
IIIb	3-CH_3O-4-*n*-$C_6H_{13}O$	3.30	2.55	1.85	1.90§§	1.93	1.93	1.92	2.13	2.51	2.15	2.16
IIIc	3-CH_3O-4-*cyclo*-$C_6H_{11}CH_2O$	3.35	2.51	1.90	1.92	1.95	1.97	2.01	2.18	2.50	2.22	2.23

* P_{SF} values are the true partition coefficients of un-ionized species measured by the shake-flask method.

** For calculation of $\Sigma\pi_{exp}$, the value of log P_{SF} = 1.45 for phenylacetic acid[31] was used.

*** Calculated values from retention indices (R_M, log k', respectively) using the corresponding equations (eqns. 3–10).

§ The values 1.31, 1.10, 1.81 and 1.91 for IIa, IIb, IId and IIIc, respectively, were determined by the same method[9] as in this paper.

§§ The values 0.41 and 1.80 were determined[7] by the same method for the same derivatives of 2-methyl-3-phenylpropionic acid.

the organic solvent was evaluated in the systems B and D, and extrapolated values of R_M and log k' were calculated. The experimental results are given in Tables I and II, while the regression equations 9 and 10 derived for the extrapolated values of the retention indices are given in Table III. An increase in selectivity of the hydrophobic retention is documented by a decrease in the slopes. At the same time, however, the statistical significance of both equations is slightly diminished. The use of extrapolated values of R_M and log k' negatively influenced the calculation of the $\Sigma\pi$ values of derivatives II and III, respectively, so that these values did not correspond to the $\Sigma\pi_{exp}$ values for the *n*-octanol–water reference system. Particularly significant changes were found in the HPLC extrapolated system D_0 where the lipophilicities of both groups of derivatives II and III increased.

It may be concluded that the chromatographic systems A–F which contain an organic solvent (50% acetone and 60% methanol, respectively) in the mobile phase correspond with the *n*-octanol–water reference system. Such a similarity is expressed by a linear relationships between the retention indices and π, and by a similar decrease in lipophilicity, probably due to intramolecular interactions. Extrapolation to pure water does not bring any substantial advantages. Moreover, different influences of intramolecular interactions on lipophilicity were observed.

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