Journal of Chromatography, 449 (1988) 391–401
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 705

ENTHALPY–ENTROPY COMPENSATION IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF SERIES OF ARYLOXOALKANOIC AND ARYLHYDROXYALKANOIC ACIDS

M. KUCHAŘ*, E. KRAUS, V. REJHOLEC and V. MILLER
Research Institute for Pharmacy and Biochemistry, 17 Kouřimská, 13060 Prague 3 (Czechoslovakia)
(First received March 16th, 1988; revised manuscript received May 27th, 1988)

SUMMARY

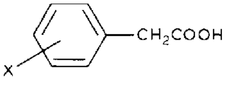
The capacity factors of various arylacetic (I), aryloxoalkanoic (II), arylhydroxyalkanoic (III) and substituted benzyloxyphenylacetic (IV) acids have been determined at various temperatures on a μBondapak C_{18} reversed-phase system with buffered aqueous methanol, acetonitrile or tetrahydrofuran (pH 3.0) as the mobile phases. Three groups of acids were evaluated, the lipophilicity and retention behaviour of which were affected by intramolecular interactions. A series of acids I, used for comparison, showed a linear correlation between the logarithms of capacity factors, log k', and the corresponding changes in enthalpy, $-\Delta H^0$. Interactions of two hydrophilic groups (so-called H/H interactions) affect the lipophilicity and retention properties of the acids II, III in a similar way. Thus it is possible to calculate log P values from the linear dependence log P *vs.* log k', regardless of the modifier used. These interactions did not show in the enthalpy–entropy compensation of the systems containing methanol or tetrahydrofuran as a modifier. Thus, the linear relationships between log k' and ΔH^0 are valid for the whole group of acids I–III. An effect of intramolecular hydrophobic interactions upon the retention properties of acids IV in 50% methanol was accompanied by a deviation from the isokinetic relationship derived for the acids I–III. This type of interaction did not occur in 40% tetrahydrofuran and the entropy–enthalpy compensation operated over the whole group of acids I–IV. The enthalpy changes were minimal in the system with 40% acetonitrile as the mobile phase and thus the isokinetic relationship was affected by a large error.

INTRODUCTION

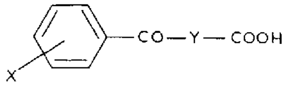
Partition chromatography is widely used for the evaluation of the lipophilicity of bioactive compounds[1–6]. Statistically highly significant regression equations were obtained[7,8] for series of arylalkanoic acids, in which logarithms of the partition coefficients were correlated with the chromatographic parameters R_M or log k'. Silica gel impregnated with a silicone oil or silanized silica gel were used as the stationary phases in thin-layer chromatography (TLC). High-performance liquid chromato-

0021-9673/88/$03.50

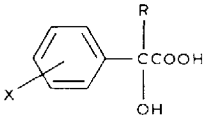
graphy (HPLC) using various C_{18} silanized packings[7] was also employed without any change in statistical significance of the regression equations. The best results were obtained with buffered water (pH 3.5) as the mobile phase in a mixture with a suitable organic solvent; acetone for TLC or methanol for HPLC gave[8] a linear dependence of log *P* and log *k*′ for groups of acids containing arylacetic acids (I), aryloxoalkanoic acids (II) and arylhydroxyalkanoic acids (III). Introduction of an hydrophilic group in the vicinity of the carboxyl in acids II and III results[9] in so-called H/H interactions[10] which influence the lipophilicity.



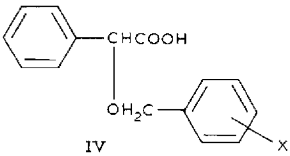
I



II



III



IV

For the groups of acids I–IV, the dependence of the retention characteristics on temperature was studied as well as the enthalpy–entropy compensation in systems with different modifiers in the mobile phase, *i.e.*, methanol, acetonitrile, tetrahydrofuran. The equation derived by Melander *et al.*[11]

$$\log k_T' = -\frac{\Delta H^0}{2.3R}\left(\frac{1}{T}-\frac{1}{\beta}\right) - \frac{\Delta G_\beta^0}{2.3R\beta} + \log\varphi \tag{1}$$

was used where k_T' is the capacity factor of a solute at temperature T, β is the compensation temperature, ΔH^0 is the change in standard enthalpy, ΔG_β^0 is the Gibbs energy of a process at temperature β and φ is the phase ratio of the column. The value of ΔH^0 for the transfer of the solute from the mobile phase to the stationary phase can be calculated from the slope of the linear relationship between log k' and $1/T$

$$\log k' = -\frac{\Delta H^0}{2.3R}\cdot\frac{1}{T} + \frac{\Delta S^0}{2.3R} + \log\varphi \tag{2}$$

where ΔS^0 is the corresponding change in standard entropy. This method of verification of the isokinetic relationship was used for a series of arylacetic acids[12] including derivatives the lipophilicity of which was affected by the intramolecular hydrophobic interaction of the aromatic nuclei and by steric hindrance of solvation. A difference in the slope of the linear equation 1 was found in both cases in comparison with the standard series of arylacetic acids.

EXPERIMENTAL

HPLC

Experiments were carried out using a liquid chromatograph assembled from a Model 6000 A pump, an U6K injector, a 440 fixed-wavelength detector and a M 730 data module (Waters Assoc., Milford, MA, U.S.A.). To maintain the temperature (± 0.1°C), a water-jacketed column connected to a water-circulating bath was used. A μBondapak C_{18} column (300 mm × 3.9 mm) was obtained from Waters Assoc. 0.0025 *M* Aqueous phosphate buffer (pH 3.0) modified with 50% (v/v) methanol, 40% (v/v) acetonitrile and 40% (v/v) tetrahydrofuran, respectively, was used as a mobile phase. Doubly distilled water, filtered through 0.45-μm Millipore filters, was used throughout. The eluent flow-rate was 1 ml/min. Detection was performed by UV absorption at 200 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, t_R, of the solute using the relationship $k' = (t_R - t_0)/t_0$.

Determination of partition coefficients

Partition coefficients, P_{exp}, of acids Ia, IIa, f, IIIc, d, IVa, b, c, were determined by the shake-flask method[13] in an octanol–water system at 20°C, with both phases presaturated with the other. To eliminate the effect of dissociation of the acids, 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, $P_{\mathrm{exp}} = c_O/c_W$.

Sample preparation

The arylacetic acids (I) were prepared[14] by the Wilgerodt reaction or by hydrolysis of the corresponding arylacetonitriles. To prepare aryloxoalkanoic acids (II) we used the Friedel–Crafts reaction of anhydrides of dicarboxylic acids with appropriate aromatic acids[15]. The acids III were obtained[16] from the esters of aryloxocarboxylic acids by reactions with methylmagnesium iodide and subsequent hydrolysis. The acids IV were obtained[16] by reaction of phenylhydroxyacetate with the corresponding benzyl chlorides in the presence of sodium hydride, followed by hydrolysis.

Calculations

The log P values of the remaining acids I were calculated using the parameters π derived[17] for the arylacetic acids. For the acids II the parameters π were derived[17] for the substituted benzoic acids, and for the acids III and IV the parameters π were taken from those derived for the substituted benzyl alcohols. The sums of the parameters π for the 3-chloro-4-alkoxy derivatives were reduced by 0.23, in accordance with the results[14,18] of partition chromatography of those derivatives of arylaliphatic acids. The log P value of the unsubstituted acid IVa was calculated by the fragmental method according to the equation:

$$\begin{aligned}\log P\,(\mathrm{IVa}) &= 2f(C_6H_5) + f(CH_2) + f(CH) + f^{1R}(COOH) + f^{1R}(\text{–O–}) + \\ &\quad + 4F_b + F_{gBr} - 0.42\,[f^{1R}(COOH) + f^{1R}(\text{–O–})] \\ &= 3.80 + 0.66 + 0.43 - 1.03 - 1.52 - 0.48 - 0.22 + 1.07 \\ &= 2.71\end{aligned}$$

where fragmental constants *f* and fragmental factors *F* were taken from refs. 10 and 19. 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 capacity factors, *k'*, of the series of acids I–IV determined in the temperature range of 22–57°C are given in Tables I–III. The corresponding regression relationships between log *P* and log *k'* are summarized in Table IV; the log *P* values introduced in Table I were used in the calculations. Eqns. 3–7 hold for the system, where methanol was used as a modifier of the mobile phase. Eqns. 8–12 are valid for acetonitrile and eqns. 13–17 for tetrahydrofuran. The statistical significance of the linear relationships log *P* *vs.* log *k'* for the groups of acids I–III is not influenced by a change of the modifier or by the temperature. The slope,

TABLE I

LOGARITHMS OF CAPACITY FACTORS (log *k'*) OF ACIDS I–IV AT VARIOUS TEMPERATURES

50% Methanol was used as the mobile phase.

Compound			*log P***	*Temperature (°C)*				
No.	*X*	*Y (R)**		*22*	*30*	*40*	*50*	*58*
				1/T ($K^{-1} \cdot 10^3$)				
				3.390	*3.300*	*3.195*	*3.096*	*3.030*
Ia	H		1.45***	0.226	0.154	0.060	0.028	−0.034
Ib	4-Cl		2.15	0.591	0.516	0.397	0.325	0.290
Ic	4-C_2H_5		2.43	0.819	0.751	0.631	0.565	0.510
Id	4-*iso*-C_3H_7		2.85	1.086	0.994	0.877	0.796	0.724
Ie	4-*tert.*-C_4H_9		3.13	1.297	1.198	1.076	0.983	0.908
If	4-*n*-$C_5H_{11}O$		3.46	1.541	1.435	1.288	1.184	1.108
IIa	H	CH_2CH_2	1.23***	0.201	0.139	0.021	−0.004	−0.040
IIb	3-Cl-4-CH_3O	CH_2CH_2	1.91	0.610	0.516	0.394	0.321	0.247
IIc	4-*iso*-C_3H_7O	CH_2CH_2	2.11	0.815	0.727	0.614	0.543	0.435
IId	4-Br	CH_2CH_2	2.21	0.675	0.589	0.482	0.413	0.344
IIe	4-*iso*-C_3H_7	CH_2CH_2	2.63	1.039	0.952	0.827	0.729	0.641
IIf	H	$CH_2CH(CH_3)$	1.62***	0.437	0.373	0.260	0.221	0.180
IIh	3-Cl-4-*iso*-C_3H_7O	$CH_2CH(CH_3)CH_2$	3.40	1.532	1.416	1.272	1.158	1.075
IIIa	H	CH_3	0.80	−0.040	−0.104	−0.206	−0.232	−0.300
IIIb	4-CH_3O	CH_3	0.81	−0.030	−0.095	−0.241	−0.249	−0.289
IIIc	4-*iso*-C_4H_9	CH_3	2.75***	1.152	1.052	0.923	0.829	0.751
IIId	4-*iso*-C_4H_9	C_2H_5	3.37***	1.432	1.327	1.184	1.083	1.007
IVa	H		2.00***	0.875	0.772	0.641	0.543	0.471
IVb	3-Cl-4-CH_3O		2.45***	1.158	1.038	0.876	0.753	0.687
IVc	4-Cl		2.60***	1.264	1.149	0.991	0.880	0.801

* Y applies to the acids II and R to the acids III.

** Calculated, *cf.*, Experimental.

*** Determined in octanol–buffer (pH 3.5) by the shake-flask method.

TABLE II

LOGARITHMS OF CAPACITY FACTORS (log k') OF ACIDS I–IV AT VARIOUS TEMPERATURES

40% Acetonitrile was used as the mobile phase.

No.	*Temperature (°C)*				
	22	*30*	*40*	*50*	*55*
	1/T ($K^{-1} \cdot 10^3$)				
	3.390	*3.300*	*3.195*	*3.096*	*3.049*
Ia	0.102	0.066	0.013	−0.030	−0.071
Ib	0.348	0.304	0.248	0.193	0.165
Ic	0.483	0.441	0.391	0.339	0.312
Id	0.656	0.616	0.563	0.509	0.475
Ie	0.795	0.754	0.701	0.845	0.609
If	0.975	0.932	0.875	0.812	0.778
IIa	0.089	0.053	0	−0.045	−0.070
IIb	0.325	0.283	0.228	0.178	0.139
IIc	0.463	0.427	0.382	0.345	0.304
IId	0.408	0.364	0.308	0.256	0.219
IIe	0.630	0.591	0.541	0.487	0.460
IIf	0.248	0.210	0.159	0.112	0.089
IIg*	0.422	0.382	0.325	0.272	0.248
IIh	0.954	0.915	0.863	0.806	0.770
IIIa	−0.185	−0.206	−0.241	−0.268	−0.308
IIIb	−0.172	−0.195	−0.229	−0.268	−0.294
IIIc	0.589	0.563	0.523	0.475	0.443
IIId	0.828	0.794	0.749	0.695	0.667
IVa	0.541	0.487	0.422	0.345	0.304
IVb	0.737	0.676	0.602	0.525	0.487
IVc	0.788	0.728	0.654	0.577	0.539

* IIg belongs to 5-(3′-chloro-4′-methoxyphenyl)-5-oxopentanoic acid (X = 3-Cl-4-CH_3O, Y = $CH_2CH_2CH_2$) with log P (measured experimentally) 2.17.

which is a measure of the solvent system selectivity to changes in solute lipophilicity, rises with temperature. These linear relationships can be used for calculation of log P for the acids I–III from the corresponding experimental values of log k' (Table V). However, this is not true in the case of acids IV (Table VI), the lipophilicity of which is influenced by intramolecular hydrophobic interaction of both aromatic nuclei. The lipophilicity, expressed by the log P_{exp} values, is lower in comparison with the log P_{calc} values calculated by the fragmental method. The effect of this hydrophobic interaction on the retention behaviour in HPLC is exhibited to a lesser extent when methanol or acetonitrile is used as a modifier. The corresponding values, log P_{chrom}, lie between the values of log P_{exp} and log P_{calc}. Such an interaction is almost absent in the case of tetrahydrofuran.

From the experimental log k' values summarized in Tables I–III, the dependencies of log k' on temperature were calculated (*cf.*, Table VII). They show a regular course over the whole range of temperature used, provided methanol or acetonitrile is

TABLE III

LOGARITHMS OF CAPACITY FACTORS (log k') OF ACIDS I–IV AT VARIOUS TEMPERATURES

40% Tetrahydrofuran was used as the mobile phase.

No.	*Temperature (°C)*				
	20	*30*	*40*	*50*	*55*
	1/T ($K^{-1} \cdot 10^3$)				
	3.413	*3.300*	*3.195*	*3.096*	*3.049*
Ia	0.380	0.338	0.314	0.305	0.371
Ib	0.675	0.612	0.556	0.524	0.571
Ic	0.731	0.671	0.617	0.587	0.634
Id	0.897	0.818	0.752	0.711	0.749
Ie	1.030	0.947	0.851	0.809	0.841
If	1.169	1.069	0.978	0.915	0.939
IIa	0.290	0.259	0.230	0.225	0.305
IIb	0.498	0.433	0.383	0.362	0.421
IIc	0.590	0.537	0.497	0.477	0.533
IId	0.686	0.615	0.556	0.522	0.573
IIe	0.792	0.725	0.663	0.626	0.668
IIf	0.450	0.416	0.386	0.376	0.440
IIg	0.590	0.523	0.466	0.436	0.490
IIh	1.030	0.947	0.861	0.824	0.858
IIIa	0.243	0.211	0.188	0.189	0.262
IIIb	0.174	0.151	0.131	0.130	0.211
IIIc	0.955	0.868	0.793	0.745	0.780
IIId	1.208	1.112	1.022	0.960	0.983
IVa	0.981	0.897	0.826	0.780	0.785
IVb	1.083	0.990	0.907	0.852	0.881
IVc	1.268	1.157	1.055	0.983	1.001

TABLE IV

REGRESSION EQUATIONS: $\log P = a \log k' + b$

The log P values summarized in Table I were used for calculation of regression eqns. 3–17.

Eqn.	*Temperature (°C)*	*a*	*b*	*n*	*r*	*s*	*F*
3	22	1.657	0.949	17	0.992	0.114	903.9
4	30	1.708	1.054	17	0.992	0.111	961.5
5	40	1.738	1.245	17	0.993	0.105	1072.8
6	50	1.852	1.307	17	0.993	0.108	1011.3
7	57	1.892	1.418	17	0.992	0.111	962.5
8	22	2.431	1.175	18	0.991	0.114	911.5
9	30	2.459	1.253	18	0.992	0.111	966.5
10	40	2.481	1.367	18	0.992	0.108	1005.0
11	50	2.531	1.474	18	0.991	0.113	925.1
12	55	2.533	1.555	18	0.992	0.113	931.7
13	20	2.602	0.478	18	0.982	0.162	435.9
14	30	2.796	0.521	18	0.981	0.166	413.8
15	40	3.032	0.541	18	0.980	0.172	383.0
16	50	3.261	0.507	18	0.980	0.173	380.6
17	55	3.506	0.198	18	0.979	0.174	373.7

TABLE V

EXPERIMENTAL AND CALCULATED VALUES OF log P FOR ACIDS I–III

Compound			*log* P_{exp}*	*log* k'**			*log* P_{chrom}***		
No.	*X*	*Y*		*A*	*B*	*C*	*A*	*B*	*C*
Ig	3-Cl-4-$CH_2=CHCH_2O$		2.75	1.082	0.632	0.865	2.74	2.71	2.73
IIi	4-CH_3O	CH_2CH_2	1.38	0.195	0.098	0.360	1.27	1.41	1.42
IIk	3-Cl-4-CH_3O	$CH_2CH_2CH_2$	2.17	0.803	0.438	0.623	2.28	2.24	2.10
IIl	3-Cl-4-CH_3O	$CH_2CH(CH_3)CH_2$	2.60	1.052	0.585	0.812	2.69	2.60	2.59
IIIe	4-C_6H_5	CH_3	2.71	1.187	0.698	0.945	2.91	2.87	2.94

* Values were determined in octanol–buffer (pH 3.5) by the shake-flask method.

** For mobile phases with 50% methanol (A), 40% acetonitrile (B) and 40% tetrahydrofuran (C).

*** Calculated from eqn. 3 (A), eqn. 8 (B) and eqn. 13 (C).

TABLE VI

EXPERIMENTAL AND CALCULATED VALUES OF log P FOR ACIDS IV

No.	log P_{exp}*	log P_{calc}**	log P_{chrom}***		
			A	B	C
IVa	2.00	2.71	2.40	2.49	3.03
IVb	2.45	3.32	2.87	2.97	3.30
IVc	2.60	3.57	3.04	3.09	3.78

* Values were determined in octanol–buffer (pH 3.5) by the shake-flask method.
** See Experimental.
*** Calculated from eqn. 3 (A), eqn. 8 (B) and eqn. 13 (C).

used in the mobile phase. Anomalous behaviour was observed for tetrahydrofuran. The dependence log k' *vs.* $1/T$ shows curvature at 50–55°C. In this case, ΔH^0 values for the distribution process were calculated from log k' values at 20, 30, 40 and 50°C. The departure from linearity is the more striking the lower is the log k' of the compound. For example, the log k' value of compounds IIa, IIIa, IIIb at 55°C is even higher than that at 20°C. The slope of the dependence log k' *vs.* $1/T$ is positive at temperatures lower than 50°C and the corresponding ΔH^0 is negative, while it becomes positive at temperatures over 50°C. An influence of the anomalous temperature dependencies is evident also in the relationships between log P and log k'. The lines corresponding to eqns. 13–17 intersect.

The values of ΔH^0 were calculated from the temperature dependencies according to eqn. 2 and are summarized in Table VII. Relationships between log k' and ΔH^0 are expressed by eqns. 18–20 for the system in which 50% methanol is used as the mobile phase:

	n	r	s	F	
$\log k'_{313} = 0.128\,(-\Delta H^0) - 1.627$	6	0.983	0.092	116.8	(18)
$\log k'_{313} = 0.128\,(-\Delta H^0) - 1.842$	11	0.969	0.136	138.3	(19)
$\log k'_{313} = 0.128\,(-\Delta H^0) - 1.765$	17	0.949	0.141	136.4	(20)

The values of k'_{313} were measured at 313 K, close to the harmonic mean temperature. Eqn. 18 is valid for the arylacetic acids I, eqn. 19 for the acids II and III, the lipophilicity of which is influenced by an interaction of the hydrophilic fragments. The isokinetic relationship in both groups of compounds is characterized by the same value of the slope and by very similar values of the intercept. The combination of all three series of acids I–III gives rise to eqn. 20. The acids IV, where the lipophilicity and retention behaviour are affected by the intramolecular hydrophobic interaction of the aromatic nuclei, cannot be included in eqn. 20. The deviation of these acids from eqn. 20 is apparent from Fig. 1. For clarity, the line corresponding to eqn. 21, fitted only through three points, is also shown:

	n	r	s	F	
$\log k'_{313} = 0.094\,(-\Delta H^0) - 1.394$	3	0.968	0.063	15.1	(21)

TABLE VII

THERMODYNAMIC QUANTITIES OF RETENTION FOR ACIDS I IV

Values correspond to the general linear relationship $\log k' = a(1/T) + b$; values of the correlation coefficient for all equations are higher than 0.990.

No.	*50% Methanol*			*40% Acetonitrile*			*40% Tetrahydrofuran*		
	Slope, a	*Intercept, b*	$-\Delta H^0$ (*kJ mol*$^{-1}$)	*Slope, a*	*Intercept, b*	$-\Delta H^0$ (*kJ mol*$^{-1}$)	*Slope, a*	*Intercept, b*	$-\Delta H^0$ (*kJ mol*$^{-1}$)
Ia	699.0	−2.152	13.46	493.6	−1.567	9.51	303.4	−0.658	5.84
Ib	864.6	−2.345	16.65	523.3	−1.427	10.08	545.8	−1.188	10.51
Ic	873.2	−2.141	16.81	489.0	−1.175	9.42	522.9	−1.054	10.07
Id	998.5	−2.302	19.23	526.0	−1.123	10.13	665.3	−1.375	12.81
Ie	1075.5	−2.352	20.71	539.0	−1.027	10.38	774.5	−1.612	14.91
If	1211.8	−2.569	23.34	561.8	−0.928	10.82	876.0	−1.821	16.87
IIa	682.2	−2.121	13.14	456.6	−1.459	8.79	275.1	−0.649	5.30
IIb	997.6	−2.779	19.21	534.1	−1.482	10.28	455.2	−1.306	8.77
IIc	1016.2	−2.627	19.57	445.6	−1.044	8.58	427.0	−0.869	8.22
IId	907.1	−2.404	17.47	545.0	−1.436	10.49	596.5	−1.351	11.49
IIe	1101.5	−2.690	21.21	486.3	−1.018	9.36	591.6	−1.227	11.39
IIf	725.4	−2.029	15.86	457.0	−1.303	8.80	293.6	−0.552	5.65
IIg				503.4	−1.285	9.69	568.9	−1.353	10.95
IIh	1270.2	−2.777	24.46	534.2	−0.851	10.29	774.5	−1.612	14.91
IIIa	729.0	−2.502	14.04	344.5	−1.337	6.63	197.3	−0.500	3.80
IIIb	700.7	−2.420	13.49	340.4	−1.333	6.55	252.6	−0.620	4.86
IIIe	1110.3	−2.614	21.38	424.1	−0.841	8.17	743.2	−1.583	14.31
IIId	1186.6	−2.593	22.85	459.6	−0.728	8.85	852.9	−1.703	16.42
IVa	1124.4	−2.940	21.65	691.3	−1.797	13.31	711.1	−1.447	13.69
IVb	1234.0	−3.370	24.69	714.0	−1.685	13.75	807.3	−1.673	15.54
IVc	1296.9	−3.136	24.97	711.7	−1.626	13.71	976.8	−2.066	18.81

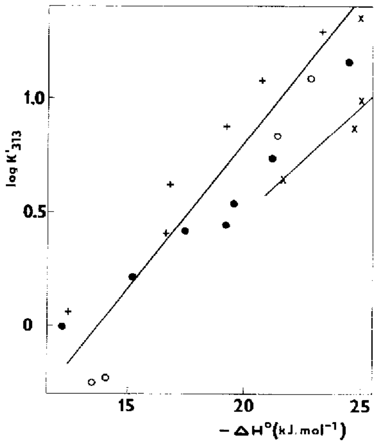


Fig. 1. Compensation plot, log k'_{313} *vs.* $-\Delta H^0$ for the acids I–IV. The log k'_{313} and $-\Delta H^0$ were taken from Tables I and VII, respectively. Mobile phase: 50% methanol. Solutes: +, arylacetic acids (I); ○, aryloxoalkanoic acids (II); ●, arylhydroxyalkanoic acids (III); ×, substituted benzyloxyphenylacetic acids (IV).

Deviations in the slope and intercept in comparison with eqn. 20 are similar to those ascertained for the arylalkoxyarylacetic acids[7,9], where the retention behaviour and lipophilicity are influenced by the same intramolecular hydrophobic interactions.

The enthalpic changes, ΔH^0, connected with the distribution process where acetonitrile was used as a modifier altered only over a narrow range. Consequently, the isokinetic relationship is subject to large errors and is statistically not significant. The following equations were derived for the system with 40% tetrahydrofuran as a mobile phase, eqn. 22 for the acids I, eqn. 23 for the acids II and III and eqn. 24 for the acids I–III:

	n	*r*	*s*	*F*	
$\log k'_{313} = 0.060\,(-\Delta H^0) - 0.031$	6	0.993	0.032	271.2	(22)
$\log k'_{313} = 0.063\,(-\Delta H^0) - 0.094$	12	0.960	0.081	119.0	(23)
$\log k'_{313} = 0.063\,(-\Delta H^0) - 0.085$	18	0.970	0.068	252.0	(24)

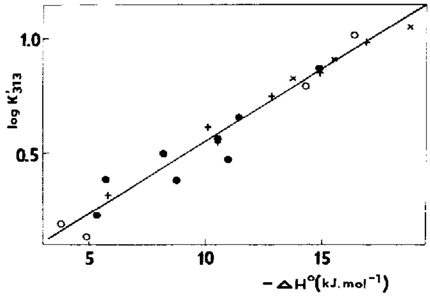


Fig. 2. Compensation plot, log k'_{313} *vs.* $-\Delta H^0$ for the acids I–IV. The log k'_{313} and $-\Delta H^0$ were taken from Tables III and VII, respectively. Mobile phase: 40% tetrahydrofuran. Symbols for solutes as in Fig. 1.

The values of ΔH^0 were calculated from the temperature dependencies in the range 20–50°C and so the isokinetic relationships are valid only in this range. The benzyloxy derivatives IV can also be introduced into the eqn. 24; no change in the slope and intercept resulted (*cf.*, eqn. 25):

	n	r	s	F	
$\log k'_{313} = 0.063\,(-\Delta H^0) - 0.080$	21	0.975	0.064	368.3	(25)

The validity of the isokinetic relationship for the acids I–IV is evident also from the plot in Fig. 2. It can be assumed that such an uniformity of the isokinetic relationship is due to the absence of intramolecular hydrophobic interactions in the distribution of these acids.

REFERENCES

1 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
2 M. Kuchař and V. Rejholec, *Česk. Farm.*, 28 (1979) 212.
3 M. Kuchař and V. Rejholec, *The Use of Quantitative Relationships between Structure and Biological Activity* (in Czech), Academia, Prague, 1988, p. 65
4 G. Cantelli-Forti, M. C. Guerra, A. M. Barbaro, P. Hrelia, G. L. Biagi and P. A. Borea, *J. Med. Chem.*, 29 (1986) 555.
5 J. M. McCall, *J. Med. Chem.*, 18 (1975) 549.
6 A. Kakoulidou, N. El Tayar, H. van der Waterbeemd and B. Testa, *J. Chromatogr.*, 389 (1987) 33.
7 M. Kuchař, V. Rejholec, E. Kraus, V. Miler and V. Rábek, *J. Chromatogr.*, 280 (1983) 279.
8 M. Kuchař, E. Kraus, M. Jelínková, V. Rejholec and V. Miller, *J. Chromatogr.*, 347 (1985) 335.
9 M. Kuchař, V. Rejholec and V. Miller, *QSAR in Toxicology and Xenobiochemistry*, Elsevier, Amsterdam, 1985, p. 321.
10 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.
11 W. R. Melander, D. E. Campbell and Cs. Horváth, *J. Chromatogr.*, 158 (1978) 215.
12 M. Kuchař, V. Rejholec, V. Miller and E. Kraus, *J. Chromatogr.*, 280 (1983) 289.
13 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525.
14 M. Kuchař, B. Brůnová, Z. Roubal, J. Schlanger and O. Němeček, *Collect. Czech. Chem. Commun.*, 45 (1980) 1401.
15 M. Kuchař, B. Brůnová, J. Grimová, V. Rejholec and V. Čepelák, *Collect. Czech. Chem. Commun.*, 51 (1986) 2617.
16 M. Kuchař, B. Brůnová, V. Rejholec, M. Jelínková, J. Holubek and O. Němeček, *Collect. Czech. Chem. Commun.*, 49 (1984) 122.
17 T. Fujita, J. Iwasa and C. Hansch, *J. Am. Chem. Soc.*, 8 (1964) 5175.
18 M. Kuchař, V. Rejholec, B. Brůnová and M. Jelínková, *J. Chromatogr.*, 195 (1980) 329.
19 A. Leo, P. Y. C. Jow, C. Silipo and G. Hansch, *J. Med. Chem.*, 18 (1975) 865.