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INFLUENCE OF INTRAMOLECULAR INTERACTIONS ON CHROMATOGRAPHIC BEHAVIOUR OF ARYLALIPHATIC ACIDS

II. ENTHALPY-ENTROPY COMPENSATION IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The capacity factors of various arylaliphatic acids have been determined at various temperatures on a μ Bondapak C₁₈ reversed-phase system with buffered aqueous methanol as eluent (pH 3.0). Two groups of derivatives of arylaliphatic acids were evaluated, the lipophilicity of which is affected by intramolecular interactions. A series of arylacetic acids without intramolecular interactions was used for the sake of comparison. Analysis of the data obtained showed a linear correlation between the logarithms of the capacity factors and the corresponding changes in enthalpy. Enthalpy-entropy compensation is operating in this system, with differences in the slopes between the particular groups of acids. It may be concluded that the decrease in lipophilicity of some derivatized acids is probably due to the differences in the mechanism of the solute-stationary phase interactions. The linear relationship between $\log k'$ and ΔH^0 values implies that a decrease in lipophilicity of the *ortho*-dialkoxy derivatives is connected with a decrease in changes in entropy on passing from a mobile to a stationary phase. Such a conclusion is in agreement with a hypothesis of steric hindrance of solvation.

INTRODUCTION

Much attention has been recently given¹⁻³ to the role of temperature in reversed-phase high-performance liquid chromatography (HPLC). For a mechanistic interpretation of this type of chromatography, an extrathermodynamic approach based on enthalpy-entropy compensation⁴⁻⁷ has been used. Applying this principle, Melander *et al.*⁴ derived eqn. 1

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

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.

When enthalpy-entropy compensation is indeed operative in a family of compounds, a linear relationship for eqn. 1 is observed between the corresponding $\log k'$ values determined at the harmonic mean temperature T_{harm} (or close to this temperature, T_{eval}) and the ΔH^0 values. The compensation temperature, β , can be evaluated from the slope of eqn. 1. The change in the standard enthalpy, ΔH^0 , of the transfer of a solute from the mobile to the stationary phase can be calculated from the slope of the linear relationship between $\log k'$ and $1/T$ (van 't Hoff plot), expressed by eqn. 2, where ΔS^0 is the change in standard entropy. The mechanism of the process is assumed to be invariant over the temperature range investigated.

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

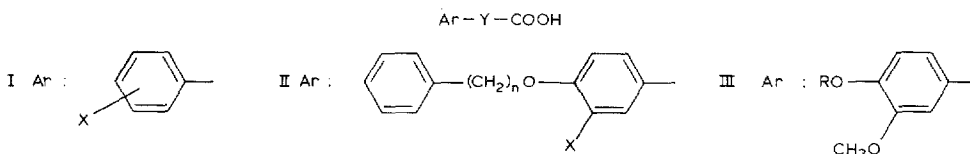
A number of authors⁵⁻⁸ have shown that the reversible binding of various solutes to hydrocarbon stationary phases manifests enthalpy-entropy compensation under a wide range of conditions. An identical intrinsic mechanism of the interactions of solutes with a bonded stationary phase may be concluded from the closeness of the compensation temperatures, β , calculated for various sets of compounds.

In an earlier paper⁹ we found that the intramolecular interactions studied affect the lipophilicity of arylaliphatic acids in a similar way both in HPLC and thin-layer chromatography (TLC) and in the reference system *n*-octanol-water. In the series of arylacetic acids, where the derivatives with intramolecular interactions were involved, the relationships between $\log P$ [*n*-octanol buffer (pH 3.4)] and $\log k'$ is expressed by eqn. 3.

$$\log P = 1.761 \log k' + 1.954 \quad \begin{array}{cccc} n & r & s & F \\ 21 & 0.991 & 0.098 & 1075 \end{array} \quad (3)$$

The capacity factors k' were determined on μ Bondapak C₁₈ using 60% methanol-buffer (pH 3.0) as a mobile phase.

We have now applied the enthalpy-entropy compensation concept to the retention behaviour of the arylaliphatic acids I-III in HPLC. The influence of the type of substituent on the linearity of a $\log k'_T - \Delta H^0$ dependence and the compensation temperature, β , has been evaluated.



EXPERIMENTAL

Experiments have been carried out using a liquid chromatograph assembled from a Model 6000A pump, a U6K injector, a 440 fixed-wavelength detector and an

M 730 data module (Waters Assoc., Milford, MA, U.S.A.). To maintain the temperature ($\pm 0.1^\circ\text{C}$), a water-jacketed column connected to a circulating water-bath was used. A $\mu\text{Bondapak C}_{18}$ column (300×3.9 mm) was obtained from Waters Assoc. A mixture of methanol and 0.0025 *M* aqueous phosphate buffer (pH 3.0; ratio 3:2) was used as mobile phase. Double-distilled water, filtered through 0.45- μm Millipore filters, was used throughout, and methanol was of Lichrosolv quality (E. Merck, Darmstadt, F.R.G.). The eluent flow-rate was 1 ml/min. Detection was performed by UV absorption at 280 nm, 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 by the relationship $k' = (t_R - t_0)/t_0$.

The syntheses of the compounds I–III are described elsewhere^{10–14}.

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 coefficient of multiple correlation (r), the standard deviation (s) and the Fischer-Snedecor criterion, (F).

RESULTS AND DISCUSSION

The experimental values for the capacity factors of the series of acids I–III determined in the temperature range 20–60°C are given in Table I. The arylacetic acids (I) (where intramolecular interactions do not take place) were used as a standard series and series II and III were compared with this. The fall in lipophilicity for the arylalkoxy derivatives (II) is probably due to hydrophobic interactions of both aromatic nuclei¹⁵. Any actual approach of the aromatic nuclei has not been proved experimentally but indirect support for this hypothesis stems from the decrease in lipophilicity which occurs on lengthening the connecting chain between the aromatic nuclei. A decrease in lipophilicity for 3-methoxy-4-alkoxy derivatives has been tentatively ascribed to steric hindrance to solvation^{16,17}. The latter can negatively affect a gain in entropy during passage from an aqueous to an organic phase.

Enthalpy changes were calculated from regression analysis of the relationship between $\log k'_T$ and $1/T$ (Table I) according to eqn. 2 and are summarized in Table II. The relationship between $\log k'_T$ and ΔH^0 for the acids I–III is expressed by eqn. 4. The $\log k'_T$ values were calculated from experimental data obtained at 313°K, which was close to the harmonic mean of the temperature range used ($T_{\text{harm}} = 309.8^\circ\text{K}$). If the group of acids is divided into the individual series I–III (Fig. 1), the statistical significances of eqns. 5–7, respectively, are considerably better than that of eqn. 4.

$$\log k'_T = -0.089\Delta H^0 - 0.901 \quad \begin{matrix} n \\ 20 \end{matrix} \quad \begin{matrix} r \\ 0.947 \end{matrix} \quad \begin{matrix} s \\ 0.121 \end{matrix} \quad \begin{matrix} F \\ 157.7 \end{matrix} \quad (4)$$

$$\log k'_T = -0.096\Delta H^0 - 0.994 \quad \begin{matrix} n \\ 6 \end{matrix} \quad \begin{matrix} r \\ 0.996 \end{matrix} \quad \begin{matrix} s \\ 0.035 \end{matrix} \quad \begin{matrix} F \\ 505.8 \end{matrix} \quad (5)$$

$$\log k'_T = -0.086\Delta H^0 - 0.945 \quad \begin{matrix} n \\ 7 \end{matrix} \quad \begin{matrix} r \\ 0.987 \end{matrix} \quad \begin{matrix} s \\ 0.038 \end{matrix} \quad \begin{matrix} F \\ 191.2 \end{matrix} \quad (6)$$

$$\log k'_T = -0.107\Delta H^0 - 1.118 \quad \begin{matrix} n \\ 7 \end{matrix} \quad \begin{matrix} r \\ 0.997 \end{matrix} \quad \begin{matrix} s \\ 0.047 \end{matrix} \quad \begin{matrix} F \\ 726.7 \end{matrix} \quad (7)$$

TABLE I
LOGARITHMS OF CAPACITY FACTORS ($\log k'$) OF ARYLALIPHATIC ACIDS I-III AT VARIOUS TEMPERATURES

Compound No.	Y	Substituent	Temperature ($^{\circ}\text{C}$)					Slope* (a)	Intercept* (b)	
			20	30	40	50	55			60
			$1/T$ ($^{\circ}\text{K} \cdot 10^3$)							
			3.413	3.300	3.195	3.096	3.049	3.003		
Ia	CH_2	4- <i>iso</i> - $\text{C}_3\text{H}_7\text{O}$	0.449	0.375	0.301	0.228	0.200	0.160	702.1	-1.944
Ib	CH_2	4- C_2H_5	0.578	0.489	0.408	0.330	0.296	0.258	776.5	-2.073
Ic	CH_2	4- <i>tert</i> - C_4H_9	0.975	0.862	0.762	0.666	0.622	0.576	970.6	-2.339
Id	CH_2	4- <i>iso</i> - C_3H_7	0.800	0.705	0.615	0.529	0.492	0.454	846.1	-2.088
Ie	CH_2	4- <i>n</i> - $\text{C}_6\text{H}_{13}\text{O}$	1.509	1.374	1.245	1.125	1.069	1.010	1213.5	-2.632
If	CH_2	4- <i>n</i> - $\text{C}_5\text{H}_{11}\text{O}$	1.195	1.077	0.965	0.859	0.812	0.762	1051.1	-2.393
Ila	CH_2	4- $\text{C}_6\text{H}_5\text{CH}_2\text{O}$	0.870	0.764	0.659	0.556	0.509	0.457	1007.2	-2.563
Ilb	CH_2	3-Cl-4- $\text{C}_6\text{H}_5\text{CH}_2\text{O}$	1.089	0.972	0.853	0.741	0.687	0.630	1120.2	-2.729
Ilc	CH_2	3-Cl-4- $\text{C}_6\text{H}_5(\text{CH}_2)_2\text{O}$	1.304	1.175	1.050	0.925	0.863	0.807	1218.1	-2.848
Ild	CH_2	3- CH_3O -4- $\text{C}_6\text{H}_5\text{CH}_2\text{O}$	0.656	0.564	0.470	0.391	0.349	0.310	845.1	-2.227
Ile	CH_2CH_2	4- $\text{C}_6\text{H}_5\text{CH}_2\text{O}$	1.091	0.973	0.858	0.745	0.690	0.638	1109.0	-2.690
Ilf	CH_2CH_2	3-Cl-4- $\text{C}_6\text{H}_5\text{CH}_2\text{O}$	1.299	1.172	1.046	0.924	0.867	0.807	1201.5	-2.797
Ilg	$\text{CH}=\text{C}(\text{CH}_3)$	3- CH_3O -4- $\text{C}_6\text{H}_5\text{CH}_2\text{O}$	1.222	1.089	0.981	0.864	0.805	0.753	1149.1	-2.695
IIla	CH_2	3- CH_3O -4- <i>iso</i> - $\text{C}_3\text{H}_7\text{O}$	0.171	0.106	0.045	-0.012	-0.041	-0.064	575.0	-1.792
IIlb	CH_2	3- CH_3O -4- <i>n</i> - $\text{C}_6\text{H}_{13}\text{O}$	1.268	1.157	1.050	0.940	0.885	0.837	1057.7	-2.336
IIlc	CH_2	3- CH_3O -4-cyclo(C_6H_4) CH_2O	1.299	1.189	1.080	0.973	0.922	0.869	1049.7	-2.279
IIld	CH_2CH_2	3- CH_3O -4- <i>iso</i> - $\text{C}_3\text{H}_7\text{O}$	0.474	0.405	0.334	0.265	0.236	0.200	668.5	-1.804
IIle	CH_2CH_2	3- CH_3O -4- <i>n</i> - $\text{C}_6\text{H}_{13}\text{O}$	1.502	1.373	1.252	1.140	1.085	1.030	1151.1	-2.426
IIlf	$\text{CH}=\text{CH}$	3- CH_3O -4- <i>iso</i> - $\text{C}_3\text{H}_7\text{O}$	0.564	0.479	0.392	0.323	0.288	0.252	759.7	-2.030
IIlg	$\text{CH}_2\text{CH}(\text{CH}_3)$	3- CH_3O -4- <i>n</i> - $\text{C}_6\text{H}_{13}\text{O}$	1.619	1.484	1.358	1.236	1.182	1.130	1193.9	-2.456

* Values correspond to the general linear relationship $\log k' = a(1/T) + b$; for all regression equations the values of r^2 were higher than 0.998.

TABLE II
THERMODYNAMIC QUANTITIES OF RETENTION FOR ARYLALIPHATIC ACIDS I-III

Compound No.	$\log k'_{313}$	$-\Delta H^0$ (kJ mol^{-1})	$-\Delta S^0$ ($\text{J}^\circ\text{K}^{-1} \text{mol}^{-1}$)
Ia	0.301	13.450	20.68
Ib	0.408	14.952	23.44
Ic	0.762	18.690	28.59
Id	0.615	16.292	23.73
Ie	1.245	23.366	34.28
If	0.965	20.239	29.64
IIa	0.659	19.293	32.48
IIb	0.853	21.457	35.71
IIc	1.050	23.333	37.88
IId	0.470	16.187	26.20
IIe	0.858	21.244	34.87
IIf	1.046	23.015	36.96
IIg	0.981	22.010	35.00
IIIa	0.045	11.072	17.96
IIIb	1.050	20.260	28.09
IIIc	1.080	20.110	26.98
IIId	0.334	12.805	18.00
IIIe	1.252	22.165	30.31
IIIf	0.392	14.555	22.48
IIIg	1.358	22.990	30.89

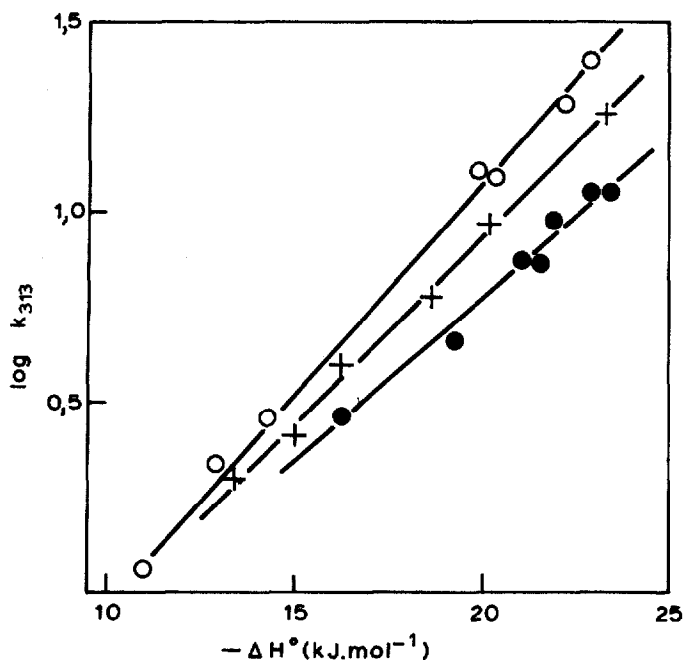


Fig. 1. Compensation plots, $\log k'_{313}$ vs. $-\Delta H^0$ for three groups of arylaliphatic acids. The $\log k'_{313}$ and $-\Delta H^0$ values are taken from Table II. Solutes: arylacetic acids I (+); phenylalkoxy derivatives II (●); 3-methoxy-4-alkoxy derivatives III (○).

The high values of the statistical criteria indicate that an enthalpy–entropy compensation mechanism is operative in each instance. At the same time, however, the different straight lines for the individual series of acids I–III are obvious. The corresponding compensation temperatures, β , of 735, 643 and 881°K, respectively, were calculated using eqns. 5–7. With the exception of the last value, these temperatures are comparable with those described (in the range 596–794°K) for various sets of compounds in other reversed-phase HPLC systems^{4–6}. These differences show a decrease in hydrophobic retention, especially for the series III, which is probably connected with the differences in the mechanisms of the solute–stationary phase interactions.

From the respective straight lines in Fig. 1 we may conclude that different changes in enthalpy correspond with the same value of $\log k'_T$. This implies that the same change in Gibbs free energy must be accompanied also by a different change in entropy. In the series of dialkoxy derivatives (III) (at least in the region of higher lipophilicity) the change in entropy corresponding to a given change in enthalpy is lower than that in the reference series of acids (I). It is probable that a decrease in lipophilicity of the derivatives III is connected with a decrease in the change in entropy during the transfer from the mobile to the hydrophobic stationary phases. Such a conclusion is in agreement with the hypothesis of steric hindrance of solvation^{16,17}. The decrease in lipophilicity of the acids II is probably not connected with a decrease in the change of entropy on passing from aqueous to organic phases.

The use of linear ΔS^0 vs. ΔH^0 plots for the testing of compensation behaviour has often been criticized^{4,6} since this can lead to artifacts caused not by true compensation between these quantities but by statistical effects. However, these relation-

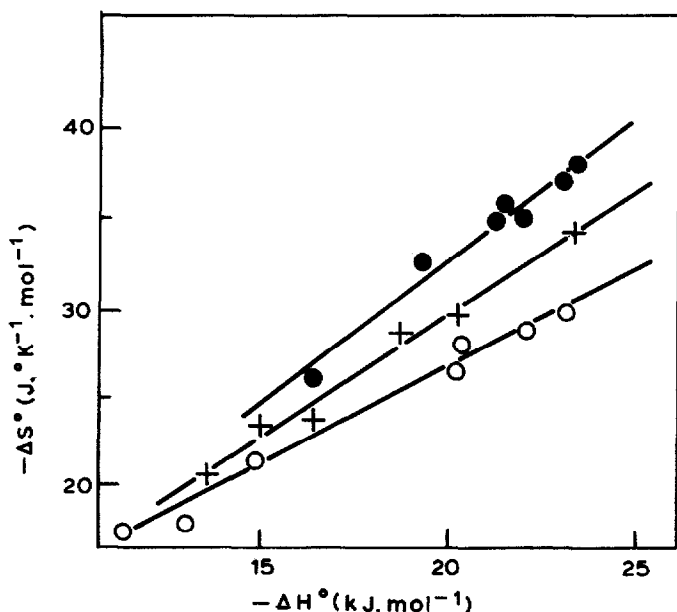


Fig. 2. Compensation plots, $-\Delta S^0$ vs. $-\Delta H^0$, for three groups of arylaliphatic acids. The $-\Delta S^0$ and $-\Delta H^0$ values are taken from Table II. Symbols for solutes as in Fig. 1.

ships provide a better view of the mutual differences in entropy changes for the same changes in enthalpy. The standard entropy changes, ΔS^0 , were calculated according to eqn. 2 and are summarized in Table II. It is necessary to know the ratio φ of the phases in the column. The value $\varphi = 0.137$ corresponds to 10% content of carbon in a stationary phase of weight 2.5 g with a retention volume of 2.7 ml of methanol. The value of φ is only approximate; however it is sufficient for a mutual comparison of the calculated changes in entropy. The linear ΔS^0 vs. ΔH^0 relationships for the whole group, as well as for the individual series of acids I–III, are expressed by eqns. 8–11. The plot in Fig. 2 clearly demonstrates the fall in entropy changes for the series of dialkoxy derivatives III compared with the reference series I, in agreement with above conclusions.

$$-\Delta S^0 = -1.482\Delta H^0 + 0.746 \quad \begin{matrix} n \\ 20 \end{matrix} \quad \begin{matrix} r \\ 0.931 \end{matrix} \quad \begin{matrix} s \\ 2.315 \end{matrix} \quad \begin{matrix} F \\ 117.8 \end{matrix} \quad (8)$$

$$-\Delta S^0 = -1.358\Delta H^0 + 2.519 \quad \begin{matrix} n \\ 6 \end{matrix} \quad \begin{matrix} r \\ 0.993 \end{matrix} \quad \begin{matrix} s \\ 0.677 \end{matrix} \quad \begin{matrix} F \\ 269.4 \end{matrix} \quad (9)$$

$$-\Delta S^0 = -1.553\Delta H^0 + 1.637 \quad \begin{matrix} n \\ 7 \end{matrix} \quad \begin{matrix} r \\ 0.986 \end{matrix} \quad \begin{matrix} s \\ 0.717 \end{matrix} \quad \begin{matrix} F \\ 172.7 \end{matrix} \quad (10)$$

$$-\Delta S^0 = -1.133\Delta H^0 + 4.898 \quad \begin{matrix} n \\ 7 \end{matrix} \quad \begin{matrix} r \\ 0.989 \end{matrix} \quad \begin{matrix} s \\ 0.904 \end{matrix} \quad \begin{matrix} F \\ 217.2 \end{matrix} \quad (11)$$

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