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SUBSTITUENT INTERACTION EFFECTS IN AROMATIC MOLECULES IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

Retention volumes of monosubstituted benzenes, chlorobenzenes, benzoic acids, phenols, anilines and pyridines have been measured in the reversed-phase mode of a high-performance liquid chromatographic system. Buffered methanol–water and acetonitrile–water eluents were used with an octadecylsilylsilica adsorbent. From the net retention volumes a substituent interaction effect was derived, and described with the Taft equation. The resultant values of the ϱ-parameters were compared with values holding for gas–liquid chromatography and for normal-phase liquid chromatography, derived in previous investigations, and with values holding for batch partition coefficients. They were interpreted in terms of hydrogen bonding between the solutes and the eluent.

Batch partition experiments with monosubstituted benzenes, phenols and pyridines were carried out with an *n*-hexadecane/methanol–water system, in order to obtain more information concerning the retention mechanism. A general discussion is given on retention mechanisms in reversed-phase chromatography.

INTRODUCTION

The influence of substituents on the properties of the parent molecule has since long been recognized. An attempt to quantify these effects has been made by Hammett[1] who proposed a set of substituent parameters based on the dissociation constants of benzoic acids. These σ-parameters turned out to be useful for interpreting the influence of substituents on other chemical equilibrium constants and even on reaction rate constants.

Further developments in describing the influence of substituents were made in the field of adsorption and partition. Examples can be found in the well known compilation of Leo *et al.*[2] of partition data in octanol–water systems. Most of the literature[3–5] describes only the individual substituent effect of a single group. Mutual interactions in multisubstituted molecules were mostly neglected. However, simple addition of individual group contributions is incorrect for an accurate prediction of retention. Nieuwdorp *et al.*[6] demonstrated the presence of a substituent inter-

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action effect with gas–liquid chromatography (GLC). These workers investigated substituted phenols, anilines and pyridines and developed an extension of the Hammett and Taft[7] equations for a better description of those systems.

In view of the explosive growth of liquid chromatography an analogous approach in this field is a logical continuation. Hammett-like formula have been tested in normal-phase systems by Snyder[8] for silica and alumina as adsorbents and Hammers and co-workers[9–11] have investigated non-polar and polar bonded phases in normal-phase adsorption chromatography. Nowadays reversed-phase liquid chromatography is a popular method[12] and we have therefore set out to investigate substituent interaction effects in this chromatographic system, and to describe them with the equations developed by Hammett, Taft or Nieuwdorp. In the course of this work we became interested in the retention mechanism and so performed batch partition experiments, and compared the results with those from corresponding high-performance liquid chromatographic (HPLC) systems.

THEORETICAL

The series of solutes used in this study can be described schematically as $i\phi k$, a general formula for disubstituted benzenes. The variable substituent is denoted by i, while within a certain series k is the fixed polar group. The substituents i were fluoro, chloro, bromo, iodo, methyl, nitro, cyano, methoxy, acetyl and carboxymethyl ester. Phenols, anilines, pyridines and benzoic acids formed series of test solutes.

In this article $i\phi$OH, $i\phi NH_2$, $i\phi$N and $i\phi$COOH will be used as notations for the investigated series. (For the sake of simplicity pyridines are also denoted by $i\phi k$ and $i\phi N$, although the nitrogen atom is a part of the ring.)

For chemical equilibrium constants K the Hammett equation has the following form:

$$\log K^{i\phi k} - \log K^{H\phi k} = \varrho_k \, \sigma_i \tag{1}$$

The reaction constant ϱ_k reflects the sensitivity of the fixed group k towards electronic shifts in the benzene nucleus caused by substituent i. The substituent parameter σ_i indicates the influence of a varying substituent i on the fixed functional group. Hammett developed a σ_i scale by defining $\varrho_k \equiv 1$ for dissociation constants of benzoic acids in water at 25°C. This set of σ values can be used to determine ϱ values for other systems.

The σ values depend on the position of the substituent, which results in σ_m values for *meta* and σ_p values for *para* substituents. The reaction constant ϱ has the same value for *meta*- and *para*-substituted solutes. To increase the somewhat limited applicability of the Hammett equation, an extension was formulated by Taft and Lewis[7] which reads as follows:

$$\log K^{i\phi k} - \log K^{H\phi k} = \varrho_I \, \sigma_I + \varrho_R \cdot \sigma_R \tag{2}$$

Taft and Lewis use σ_I and σ_R parameters, and so distinguish an inductive (I) and a resonance (R) effect for each substituent, which are equal for *meta* and *para* positions. *Meta*- and *para*-substituted solutes must be considered separately in this model, which results in different ϱ_I and ϱ_R-values for *meta* and *para* series.

In *ortho*-substituted compounds steric and other short-range intramolecular interactions interfere with a description of substituent effects in terms of σ parameters only[8]. For this reason we have not carried out measurements on *ortho*-substituted solutes.

Because in this work retention volumes are measured we have to convert from chemical equilibrium constants to retention volumes. The basic retention formula for a solute in liquid chromatography[13] is:

$$V_N = V_R - V_M = K\Phi_s \tag{3}$$

where V_N is the net retention volume, V_M is the mobile phase volume and the instrumental dead volume, Φ_s is either the volume of the stationary phase in partition chromatography or the adsorbent surface area in adsorption chromatography, and K is the distribution coefficient of an eluted compound.

An important detail of this equation is the fact that the symbol K is not restricted to either an adsorption or a partition process taking place in the column. This is important because there exists a lack of full understanding of the retention mechanism in reversed-phase HPLC[14–18]. For the members of a series with a fixed centre k we can write

$$\log K^{i\phi k} = \log V_N^{i\phi k} - \log \Phi_s \tag{4}$$

and

$$\log K^{H\phi k} = \log V_N^{H\phi k} - \log \Phi_s \tag{5}$$

from which it follows that

$$\log \frac{K^{i\phi k}}{K^{H\phi k}} = \log \frac{V_N^{i\phi k}}{V_N^{H\phi k}} \tag{6}$$

Before eqns. 1 (or 2) and 6 can be combined, a correction must be made for the contribution of group i to log K. This contribution, the "primary substituent effect", can be calculated from data on substituted benzenes:

$$\log \frac{K^{i\phi H}}{K^{H\phi H}} = \log \frac{V_N^{i\phi H}}{V_N^{H\phi H}} \tag{7}$$

Combination of eqns. 2, 6 and 7 results in the final formula:

$$\log \frac{V_N^{i\phi k}}{V_N^{H\phi k}} - \log \frac{V_N^{i\phi H}}{V_N^{H\phi H}} = \varrho_I\,\sigma_I + \varrho_R \cdot \sigma_R \tag{8}$$

In this equation the left hand side represents the substituent interaction effect. Eqn. 8 will further be abbreviated to

$$\Delta \log V_N^{i\phi k} - \Delta \log V_N^{i\phi H} = \Delta\Delta \log V_N^{i\phi k} = \varrho_I\,\sigma_I + \varrho_R \cdot \sigma_R \tag{9}$$

In the batch partition experiments the two phases consisted of *n*-hexadecane and the eluent used in the HPLC experiments, mutually saturated. Partition coefficients, P, can be calculated from the UV absorbances of the eluent by the formula[19]

$$P = \frac{C_{\text{hexadecane}}}{C_{\text{eluent}}} = \frac{A_{\text{before equilibration}} - A_{\text{after equilibration}}}{A_{\text{after equilibration}}} \cdot \frac{V_{\text{eluent}}}{V_{\text{hexadecane}}} \tag{10}$$

where C represents the concentration of the solute, A its absorbance in the eluent and V the volume of the equilibrated quantities of both phases.

The Taft equation for partition coefficients is analogous to eqn. 8:

$$\log \frac{P^{i\phi k}}{P^{H\phi k}} - \log \frac{P^{i\phi H}}{P^{H\phi H}} = \varrho_I\, \sigma_I + \varrho_R \cdot \sigma_R \tag{11}$$

In further dealing with this equation it will be abbreviated in the same way as eqn. 8.

EXPERIMENTAL

Chemicals

The solutes were from Fluka (Buchs, Switzerland) (grade purissimus), Baker (Deventer, The Netherlands) (analysed-reagent grade), Aldrich Europe (Beerse, Belgium) (laboratory-use quality) and ICN Pharmaceuticals (Plainview, NY, U.S.A.) (qualified by "for investigational use").

The eluent components methanol, acetonitrile and phosphoric acid were from Baker (analysed-reagent grade). Disodium hydrogen phosphate and sodium dihydrogen phosphate were from E. Merck (Darmstadt, G.F.R.) (pro analysi). Water was distilled twice from an alkaline potassium permangate solution.

LiChrosorb 10 RP-18 (Merck) was used as adsorbent. It has a reported[20] carbon content of 0.286 g per g bare LiChrosorb Si-100. This corresponds with a surface concentration of 4.4 μmole octadecyl groups per square meter.

The *n*-hexadecane was a product of Aldrich Europe, with a purity of 99%.

Apparatus

The liquid chromatograph was a Packard-Becker Model 8200 (Delft, The Netherlands), equipped with a UV detector with a fixed wavelength of 254 nm. The eluent flow-rate was continuously monitored with a siphon counter (Waters Assoc., Milford, MA, U.S.A.) which had been calibrated with a type ABU 12 autoburette (Radiometer, Copenhagen, Denmark). The column and eluent vessel were kept at (25.0 ± 0.1)°C with a Haake circulating-water thermostat Model E52 (Karlsruhe, Germany). The column (precision-bore stainless steel, 25 cm × 2.1 mm I.D.) was packed by forcing an ultrasonically degassed and homogenized slurry of the adsorbent in carbon tetrachloride (*ca.* 10%, w/v) into it with *n*-hexane at 350 atm. The packing was settled by flushing 300 ml of *n*-hexane and 300 ml of methanol through the column[21]. The column was weighed when filled with *n*-hexane and with methanol, respectively, to obtain the void volume of the column[22]. This volume, measured twice, was (622 ± 10) μl. A similar measurement with *n*-hexane and tetrachloromethane gave a slightly higher value. The weight of the packing material was 0.63 g. Dead volumes of connecting capillaries were measured by coupling column inlet and outlet directly together with a volumeless device and injecting a solute. Samples of 3 μl were injected on-stream with a high-pressure resistant syringe (SGE, Melbourne, Australia).

Procedure

Chromatographic experiments. Eluent modifier concentrations were chosen to obtain capacity ratios (k') between 1 and 10. The eluent flow was *ca.* 1 ml min^{-1}, which required pressure drops of 100 and 150 atm with acetonitrile–water and methanol–water, respectively. Triplicate measurements were made with reproducibilities of *ca.* 20 μl or 4% for the most strongly retained solutes.

To take into account any possible slight differences in eluent compositions, the retention of the nitro-containing compounds in each series of solutes was measured daily, and if necessary, minor corrections were made. These measurements served also as a control for column performance.

Special attention had to be paid to measurements in eluents with pH 2, since literature reports different opinions on adsorbent stability at very low pH values[23,24]. When measurements at pH 2 had been made, the column was filled with eluent of pH 3.5 at the end of the day, so as to avoid adsorbent deterioration overnight. A buffer concentration of 25 mM sufficed because of the very small sample concentration. This factor also enlarged column lifetime[24]. In the course of this study no sign of column deterioration has been observed.

Suppression of ionization of all compounds was accomplished by choosing appropriate pH values.

The pH of the aqueous phosphate buffer was adjusted while using a Methrohm E 350 B pH meter (Herisau, Switzerland). Methanol or acetonitrile was then added. Upon addition of an organic solvent to water, the pK of the phosphoric acid in the buffer solution increases, and hence its pH. In 50% (v/v) methanol we estimate the increase at *ca.* 0.5 unit. However, the pK values of the acidic solutes increase by about the same amount, whereas the pK values of the basic solutes decrease by about 0.5 unit[25]. This implies that all solutes are in the uncharged form when the pH before addition of the organic solvent is adjusted to 2.0 for benzoic acids, 3.5 for phenols and 7.1 for anilines and pyridines[26]. Special care was taken in degassing the eluents. In contrast with recent reports[27] we achieved excellent performance by ultrasonically degassing after gently heating the mixtures to 40°C.

Batch experiments. Before performing the actual partition experiments both the eluent and the *n*-hexadecane were saturated with each other. Both phases were equilibrated during a week by permanent shaking, the eluent being refreshed four times during this period. Constancy of composition was checked by gas chromatographic analysis. Afterwards the layers were roughly separated by a separatory funnel and thoroughly centrifuged. Solutes were dissolved in the eluent up to concentrations giving absorbances between 0.4 and 0.6. Since phenols and pyridines have log ε values of 3 and higher[28] solute concentrations were low enough to be sure of the absence of dimerization in the *n*-hexadecane layer[29,30]. Aliquots (each 1.5 ml) of the sample solutions were equilibrated with 7.5 ml of *n*-hexadecane for 30 min by means of a testtube rotator. To ensure the absence of emulsions the tubes were centrifuged at 1300 *g* for 30 min. After these manipulations a constant blank was measured spectrophotometrically.

Before drawing a sample of the aqueous bottom layer, the upper layer and the upper part of the bottom layer were carefully removed by means of a water suction pump. Absorbances of 1-ml aliquots of the eluent phase only (not only the organic phase[31], nor both phases[32]), were measured spectrophotometrically at 254 nm in the quartz cell of a Vitatron MPS type 940-320 spectrophotometer (Dieren, The Netherlands). Solutes were used as received because the prior HPLC experiments had not shown any spurs due to impurities.

RESULTS

The results of the measurements in methanol–water are shown in Tables I and II, while those in acetonitrile–water are in Table III. The precision of the log V_N values can be estimated by comparing data for the series of substituted benzenes at pH 2.0, 3.5 and 7.1 and of substituted chlorobenzenes at pH 3.5 and 7.1. Doing so (for substituents 0–10) we find a standard deviation of 0.007 in log V_N, corresponding with an experimental uncertainty of 1.6% in the means of the triplicate V_N determinations. This precision can be expected to hold for all measurements, except those on bromo- and iodobenzene. The mean V_N values of these solutes have relative standard deviations of *ca.* 3% due to tailing. Before testing the two-parameter eqn. 8, graphs of $\Delta\Delta$ log V_N *versus* $-\ \Delta$pK (where K is the ionization constant of the disubstituted

TABLE I

LOG V_N VALUES FOR MONOSUBSTITUTED BENZENES AND MONOSUBSTITUTED CHLOROBENZENES AT SEVERAL pH VALUES IN METHANOL–WATER (50:50, v/v)

Substituent, i	*Substituent No.*	*Log V_N*						
		Series (pH)						
		iϕH (2.0)	*iϕH (3.5)*	*iϕH (7.1)*	*iϕCl (3.5)*		*iϕCl 7.1*	
					m	*p*	*m*	*p*
H	0	0.64	0.64	0.64	–	–	–	–
F	1	0.62	0.71	0.73	1.06	0.98	1.06	0.98
Cl	2	0.96	0.99	1.00	1.41	1.33	1.41	1.34
Br	3	1.11	1.14	1.10	1.51	1.43	1.53	1.45
I	4	1.27	1.26	1.30	–	–	–	–
CH_3	5	0.97	1.02	1.01	1.38	1.38	1.38	1.37
NO_2	6	0.40	0.50	0.49	0.85	0.74	0.86	0.75
CN	7	0.23	0.25	0.23	0.58	0.52	0.59	0.52
OCH_3	8	0.59	0.63	0.68	1.11	1.07	1.13	1.08
$COCH_3$	9	0.30	0.34	0.34	0.71	0.71	0.73	0.72
$COOCH_3$	10	–	0.59	0.60	–	–	–	–
OH	11	−0.07	0.02	0.01	0.53	0.50	–	–
NH_2	12	0.12	0.17	−0.12	–	–	0.36	0.32
=N–	13	–	–	−0.07	–	–	–	–
COOH	14	0.20	–	–	–	–	–	–

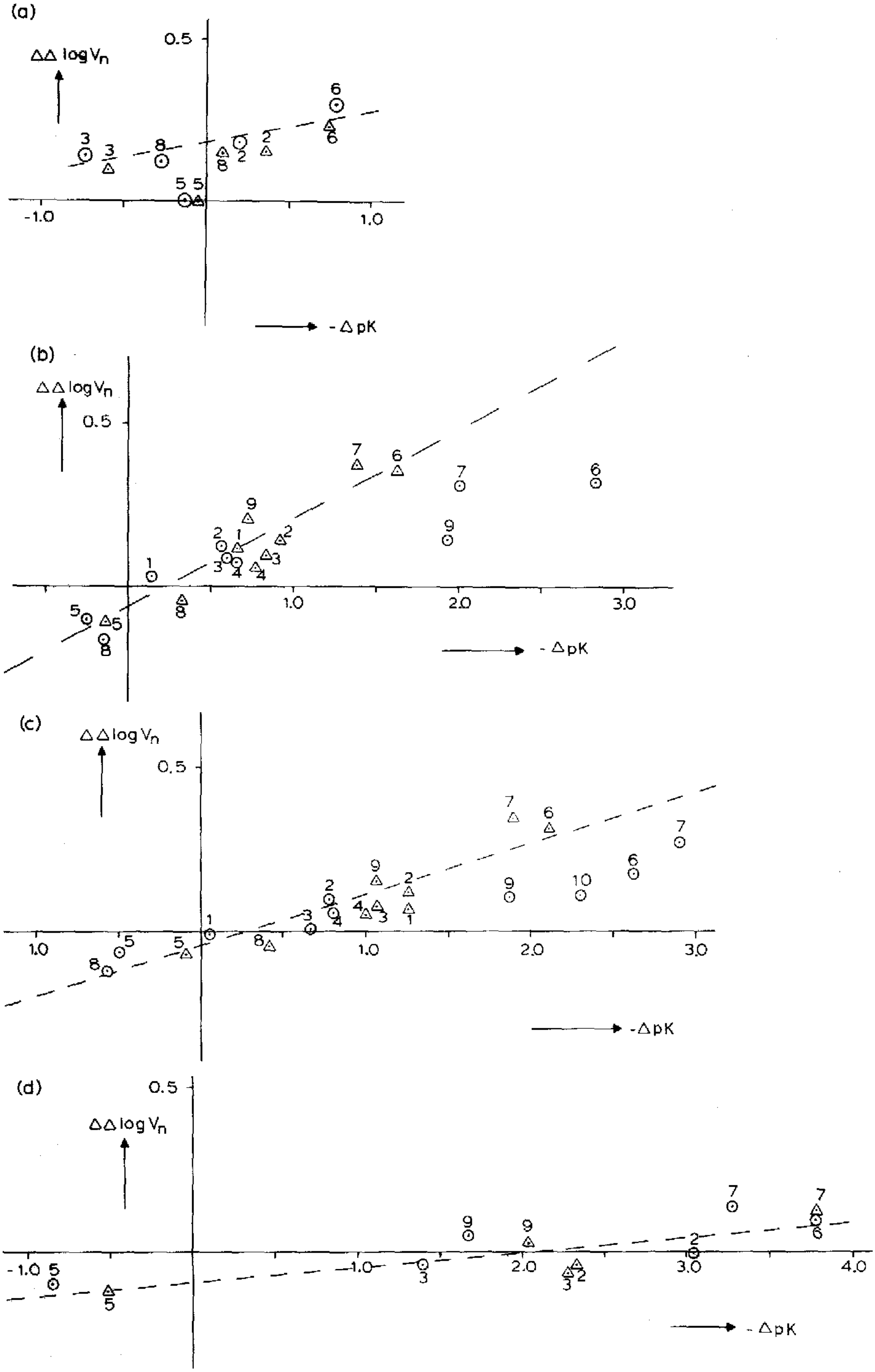

Fig. 1. ΔΔ log V_N^{ijk} values in 50% methanol–water at appropriate pH, as a function of − ΔpK values taken from the literature. Substituent numbers and pH according to Table I. The symbols ◬ and ⊙ refer to the *meta* and *para* positions, respectively. (a), *k* = COOH, p*K* from refs. 28 and 33; (b), *k* = OH, p*K* from refs. 33–40; (c), *k* = NH_2, p*K* from refs. 34 and 35; (d), *k* = =N–, p*K* from refs. 35 and 41.

TABLE II

LOG V_N VALUES IN METHANOL–WATER (50:50, v/v)

i	*Log V_N*			
	Series (pH)			
	iϕCOOH (2.0)	*iϕOH* (3.5)	*iϕNH$_2$* (7.1)	*iϕN* (7.1)
m-F	0.34	0.21	0.04	–
p-F	0.32	0.12	−0.03	–
m-Cl	0.68	0.53	0.36	0.26
p-Cl	0.70	0.50	0.32	0.28
m-Br	0.77	0.62	0.43	0.34
p-Br	0.80	0.61	0.42	0.36
m-I	0.86	0.72	0.59	0.41
p-I	0.98	0.72	0.60	–
m-CH_3	0.51	0.29	0.18	0.19
p-CH_3	0.50	0.30	0.19	0.20
m-NO_2	0.19	0.25	0.05	–
p-NO_2	0.26	0.20	−0.10	−0.13
m-CN	0.05	0.02	−0.18	−0.36
p-CN	0.01	−0.05	−0.25	−0.34
m-OCH_3	0.30	−0.03	−0.13	–
p-OCH_3	0.27	−0.15	−0.21	–
m-$COCH_3$	−0.04	−0.06	−0.25	−0.34
p-$COCH_3$	0.01	−0.13	−0.31	−0.31
m-$COOCH_3$	–	0.15	–	–
p-$COOCH_3$	–	0.16	−0.05	–

benzene) were drawn to visualize the presence of a substituent interaction effect. Since acid–base equilibration can be described with σ values, a correlation of $\Delta\Delta$ log V_N with ΔpK is an indication for a correlation of $\Delta\Delta$ log V_N with σ constants. These graphs are shown in Fig. 1 for all solute series and confirm the existence of a substituent interaction effect that is related to σ constants. Any missing point in these figures is due to a lack of available pK values. Deviations from straight lines in these plots can be regarded as limitations of the two-dimensional presentation. An effect that must be described with a two-parameter equation should be graphically illustrated with a three-dimensional figure. Testing of the two-parameter equation by regression analysis seemed to be justified by these plots.

The $\Delta\Delta$ log V_N values for all series were correlated with the substituent constants σ_I and σ_R given by Nieuwdorp *et al.*[42]. (Nieuwdorp's values of σ_I and σ_R have been estimated by an advanced statistical procedure, taking into account the whole body of relevant data). This treatment generates regression coefficients identical to the ϱ_I and ϱ_R parameters in eqn. 8. The results are given in Table IV for both methanol–water and acetonitrile–water eluents. The s_y values in this table correspond with the standard deviation of an individual $\Delta\Delta$ log V_N value from the calculated value. An extraordinary group of substances consists of solutes with both *i* and *k* being OH, NH_2, =N– or COOH. The log V_N values of these compounds are collected in Table V. The results of the batch partition experiments are given in Table VI and

TABLE III

LOG V_N VALUES IN ACETONITRILE–WATER (30:70, v/v)

i	*Log* V_N				
	Series (pH)				
	$i\phi H$ (2.0)	$i\phi H$ (3.5)		$i\phi COOH$ (2.0)	$i\phi OH$ (3.5)
H	0.77	0.77		–	–
F	0.88	0.88	*m*	0.41	0.35
			p	0.43	0.23
Cl	1.22	1.20	*m*	0.75	0.57
			p	0.78	0.55
Br	1.32	1.29	*m*	0.85	0.70
			p	0.88	0.68
I	1.49	1.47	*m*	1.01	0.84
			p	1.03	0.83
CH_3	1.19	1.17	*m*	0.59	0.39
			p	0.59	0.40
NO_2	0.73	0.71	*m*	0.31	0.33
			p	0.37	0.28
CN	0.50	0.52	*m*	0.06	0.18
			p	0.11	0.10
OCH_3	0.84	0.82	*m*	0.32	0.15
			p	0.33	0.05
$COCH_3$	0.49	0.46	*m*	−0.03	−0.01
			p	−0.01	−0.11
$COOCH_3$		0.78	*m*	–	0.24
			p	–	0.20
OH	–	0.06			
COOH	0.17	–			

the calculated ϱ_I, ϱ_R and s_y values are collected in Table IV. The precision of the log *P* values can, at first instance, be estimated by comparing data for the series of substituted benzenes at pH 3.5 and 7.1, respectively. Doing so we find a standard deviation of 0.024 in log *P*, corresponding with an experimental uncertainty of 5.5% in the means of the duplicate *P* determinations.

DISCUSSIONS

The primary substituent effect

Before exploring the substituent interaction effect, which is a secondary substituent effect, we must justify the correction for the primary substituent effect in eqn. 8. Therefore we examined following equations:

$$\log V_N^{i\phi H} - \log V_N^{H\phi H} = \varrho_I \sigma_I + \varrho_R \cdot \sigma_R \tag{12}$$

$$\log V_N^{i\phi OH} - \log V_N^{H\phi OH} = \varrho_I \sigma_I + \varrho_R \cdot \sigma_R \tag{13}$$

$$\log V_N^{i\phi OH} - \log V_N^{H\phi OH} = \varrho_I \sigma_I + \varrho_R \cdot \sigma_R + q(\log V_N^{i\phi H} - \log V_N^{H\phi H}) \tag{14}$$

TABLE IV

CALCULATED ϱ_I AND ϱ_R PARAMETERS FOR $\Delta\Delta$ LOG V_N DATA IN METHANOL–WATER (50:50, v/v) (A) AND ACETONITRILE–WATER (30:70, v/v) (B), AND FOR $\Delta\Delta$ LOG P DATA IN n-HEXADECANE/METHANOL–WATER (50:50, v/v) (C)

	i	*k*		ϱ_I	ϱ_R	s_y
A	1–9	COOH	*m*	0.34 ± 0.04	0.03 ± 0.08	0.05
	1–9		*p*	0.41 ± 0.02	0.08 ± 0.04	0.03
	1–10	OH	*m*	0.49 ± 0.03	0.42 ± 0.05	0.04
	1–10		*p*	0.40 ± 0.03	0.50 ± 0.05	0.03
	1–9	NH_2	*m*	0.42 ± 0.03	0.41 ± 0.05	0.03
	1–10		*p*	0.28 ± 0.03	0.37 ± 0.06	0.04
	2–5,7,9	=N–	3	0.07 ± 0.06	0.38 ± 0.15	0.06
	2,3,5,6,7,9		4	0.10 ± 0.04	0.34 ± 0.11	0.04
B	1–9	COOH	*m*	0.28 ± 0.01	0.01 ± 0.01	0.01
	1–9		*p*	0.36 ± 0.01	0.03 ± 0.01	0.01
	1–10	OH	*m*	0.46 ± 0.04	0.33 ± 0.07	0.05
	1–10		*p*	0.35 ± 0.02	0.37 ± 0.03	0.02
C	1–10	OH	*m*	1.99 ± 0.03	0.59 ± 0.06	0.10
	1–10		*p*	1.65 ± 0.04	0.70 ± 0.07	0.08
	2,3,5–8	=N–,	3	0.20 ± 0.09	1.00 ± 0.08	0.07
	2,3,5–8		4	0.26 ± 0.08	0.89 ± 0.07	0.08

Calculated values of the regression coefficients ϱ_I, ϱ_R and q are shown in Table VII. If we survey the column of s_y values in this table we see that the primary substituent effect can by no means be described by a linear combination of σ_I and σ_R constants, neither with substituted benzenes nor with substituted phenols. On the other hand, with eqn. 14 we obtained a standard error of fit which is similar to the experimental

TABLE V

LOG V_N VALUES FOR $i\phi k$ COMPOUNDS IN WHICH BOTH i AND k ARE POLAR SUBSTITUENTS

In the case of the pyridines, 3 and 4 have to be read instead of *m* and *p*, respectively.

i	*Log V_N*					
	Eluent					
	Methanol–water (50:50, v/v)				*Acetonitrile–water (30:70, v/v)*	
	k(pH)					
	COOH (2.0)	*OH (3.5)*	*NH_2 (7.1)*	*=N– (7.1)*	*COOH (2.0)*	*OH (3.5)*
m-OH	−0.30	−0.59	−0.66	−0.50	−0.28	−0.46
p-OH	−0.39	−0.80	−0.77	−0.94	−0.42	−0.62
m-NH_2	−0.54	−0.48	−0.74	−0.49	−0.72	−0.53
p-NH_2	−0.63	−0.53	−0.90	−0.55	−0.63	−0.71
m-COOH	−0.48	–	–	–	−0.68	–
p-COOH	−0.65	–	–	–	−0.74	–

TABLE VI

LOG P VALUES IN n-HEXADECANE/METHANOL–WATER (50:50, v/v)

i	*Log P*					
	Series (pH)					
	iφH *(3.5)*	*iφH* *(7.1)*		*iφOH* *(3.5)*		*iφN* *(7.1)*
H	0.02	0.02		–		–
F	0.04	0.05	*m*	−0.39	3	–
			p	−0.60	4	–
Cl	0.37	0.39	*m*	−0.02	3	−1.10
			p	−0.17	4	−1.07
Br	0.51	0.47	*m*	0.14	3	−1.00
			p	−0.04	4	−1.01
I	0.62	0.66	*m*	0.20	3	−0.80
			p	0.05	4	–
CH_3	0.34	0.36	*m*	−0.90	3	−1.12
			p	−0.92	4	−1.13
NO_2	−0.44	−0.41	*m*	−0.27	3	–
			p	−0.50	4	−1.43
CN	−0.53	−0.52	*m*	−0.48	3	−1.64
			p	−0.65	4	−1.66
OCH_3	−0.11	−0.09	*m*	−1.02	3	–
			p	−1.17	4	–
$COCH_3$	−0.62	−0.58	*m*	−1.13	3	−1.72
			p	−1.19	4	−1.74
$COOCH_3$	−0.72	−0.69	*m*	−1.20	3	–
			p	−1.28	4	–
OH	−1.15	–				
=N	–	−1.34				

error (see below, in *Statistics* section). In eqn. 14 the primary substituent effect is accounted for in the last term. Furthermore, the least-squares estimates of q are about unity. This points towards the correctness of the use of eqn. 8 for further calculations on the substituent interaction effect as eqn. 8 is identical with eqn. 14 for $q = 1$.

TABLE VII

CALCULATED ϱ_I, ϱ_R AND q VALUES FOR LOG V_N DATA OF SUBSTITUTED BENZENES AND PHENOLS IN METHANOL–WATER (50:50, v/v) AT pH 3.5 WITH SUBSTITUENTS 1–10

Equation		ϱ_I	ϱ_R	q	s_y
12		−0.01 ± 0.24	−0.87 ± 0.44	–	0.31
13	*m*	0.49 ± 0.22	−0.45 ± 0.40	–	0.28
	p	0.40 ± 0.25	−0.37 ± 0.46	–	0.32
14	*m*	0.49 ± 0.02	0.33 ± 0.04	0.90 ± 0.03	0.02
	p	0.40 ± 0.03	0.53 ± 0.06	1.03 ± 0.04	0.03

Substituent interaction effects

The main goal of this work is to determine whether eqn. 8 is a valuable tool for describing solute retention. A survey of Table IV for the standard deviations s_y of the regressions on chromatographic experiments shows that they are not much larger than the experimental error in $\Delta\Delta \log V_N$ (0.014). Therefore eqn. 8 can be concluded to hold well (see *Statistics* section).

As can be expected, s_y values from calculations on batch partition experiments, shown in the same table, are worse. This is mainly due to the rather large number of manipulations involved in measuring a single P value, each one of which introduces a small error[2]. As already mentioned in the Results section, the experimental error for these experiments is more than three times greater than the corresponding error for chromatographic experiments.

We now turn to the evaluation of the calculated ϱ parameters. Starting with values calculated from chromatography (Table IV), the positive sign of the ϱ values of the acidic carboxylic acids and phenols as well as the basic anilines and pyridines is striking. This contrasts with ϱ values calculated from the GLC measurements mentioned previously[6] (Table VIII). The GLC results have been explained by the occurrence of hydrogen bonding, with the hydroxyl or amino group acting as proton donor and the stationary phase polyethylene glycol (PEG) as proton acceptor. The opposite sign of the ϱ values for the pyridines was explained in terms of a dipole–dipole interaction between the pyridines and the stationary phase (hydrogen bonding cannot occur in this case).

TABLE VIII

CALCULATED ϱ_I AND ϱ_R PARAMETERS FOR DATA GIVEN BY NIEUWDORP *et al.*[6] WITH SUBSTITUENTS 1–8

Stationary phase	*k*		ϱ_I	ϱ_R	s_y
PEG	OH	*m*	0.43 ± 0.02	0.22 ± 0.03	0.02
		p	0.62 ± 0.02	0.56 ± 0.04	0.02
	NH_2	*m*	0.38 ± 0.01	0.14 ± 0.03	0.02
		p	0.75 ± 0.05	0.80 ± 0.10	0.06
	=N–	3	−0.51 ± 0.03	−0.19 ± 0.08	0.03
		4	−0.40 ± 0.01	0.02 ± 0.03	0.01
Apiezon M	OH	*m*	0.16 ± 0.02	0.04 ± 0.03	0.02
		p	0.27 ± 0.03	0.23 ± 0.05	0.03
	NH_2	*m*	0.12 ± 0.01	0.10 ± 0.01	0.01
		p	0.30 ± 0.03	0.36 ± 0.06	0.03
	=N–	3	−0.16 ± 0.02	−0.10 ± 0.04	0.01
		4	−0.25 ± 0.01	−0.17 ± 0.02	0.01

Another difference is found when our results are compared with those on retention behaviour in normal-phase systems as investigated by Hammers and co-workers[9–11]. In this mode liquid–solid adsorption is the retention mechanism. The reported ϱ values (Table IX) were explained in terms of hydrogen bonding between the solutes and the adsorbent. The positive signs of the ϱ values for the phenols indicate the proton-donating properties of these solutes.

TABLE IX

ϱ PARAMETERS IN NORMAL-PHASE SYSTEMS AS CALCULATED BY HAMMERS *et al.*[9–11] ON OCTADECYLSILYLSILICA (ODS), N-2-CYANOETHYL-N-METHYLAMINOSILICA (CNA) AND AMINOBUTYLSILYLSILICA (ABS)

Bonded phase	k	i	ϱ	s_y
ODS	OH	1–3,5–8	1.04 ± 0.15	0.19
	NH_2	1–3,5–8	-2.60 ± 0.16	0.16
	=N–	2,3,5,7	-3.39 ± 0.21	0.12
CNA	OH	1–3,5–10	0.82 ± 0.16	0.21
	NH_2	1–3,5–9	-1.76 ± 0.16	0.16
	=N–	2,3,5,7	-3.03 ± 0.35	0.19
ABS	OH	1–3,5–10	2.56 ± 0.20	0.26
	NH_2	1–3,5–9	-0.46 ± 0.26	0.27
	=N–	2,3,5,7	-2.42 ± 0.45	0.28

Anilines and pyridines act as proton acceptors towards the silanol sites of the adsorbent, and have negative ϱ parameters. These results are analogous to earlier findings by Snyder[43] for phenols and pyridines on both bare silica and alumina.

The signs of the ϱ values in our reversed-phase system agree with those calculated from batch partition experiments (Table IV), and with the signs of ϱ values calculated from literature data of the octanol–water system (Table X). Thus the positive signs exhibited by all investigated series, whether acidic or basic, are not a peculiar feature of the complicated reversed-phase system. We believe that all solutes examined act as proton acceptors towards the aqueous phase. This is a necessity for the pyridines, and rather obvious for the anilines, but, at first sight, seems odd for the phenols and benzoic acids. However it must be realized that in the case of the phenols the aqueous phase was acidified to pH 3.5 and in the case of the benzoic acids to pH 2.0, in order to suppress the basic properties of the aqueous phase towards these substances. It is plausible that the so-generated H_3O^+ and $CH_3OH_2^+$ ions act as proton donors towards phenols and benzoic acids.

Interaction between two polar substituents

A molecule such as aminophenol has *two* ways of hydrogen bonding with the

TABLE X

CALCULATED ϱ_I AND ϱ_R PARAMETERS FOR THE OCTANOL–WATER SYSTEM[2]

i	k		ϱ_I	ϱ_R	s_y
1–8	COOH	*m*	0.29 ± 0.05	-0.08 ± 0.09	0.06
1–8		*p*	0.37 ± 0.07	0.24 ± 0.13	0.08
1–10	OH	*m*	1.15 ± 0.06	0.62 ± 0.11	0.07
1–10		*p*	1.01 ± 0.04	0.89 ± 0.08	0.05
1,2,3,5,6,8	NH_2	*m*	0.96 ± 0.06	0.58 ± 0.10	0.06
1,2,3,5,6,8		*p*	0.97 ± 0.16	0.66 ± 0.27	0.15

eluent molecules: one by its hydroxyl and the other by its amino group. This means that the influence of both the amino group on the electron density in the hydroxyl group and of the hydroxyl group on the electron density in the amino group has to be taken into account. In other words, for the case of two polar substituents, eqn. 8 becomes

$$\Delta\Delta \log V_N^{i\phi k} = \{(\varrho^i\sigma^k)_I + (\varrho^i\sigma^k)_R\} + \{(\varrho^k\sigma^i)_I + (\varrho^k\sigma^i)_R\} \qquad (15)$$

Calculations using this formula can be carried out since ϱ parameters can be taken from Table IV. Calculations cannot be performed for benzoic acids and pyridines because no σ values for the carboxyl and pyridyl groups are available. This limits discussion to the aminophenols, dihydroxybenzenes and diaminobenzenes in methanol–water (50:50, v/v). The results (Table XI) show poor agreement between measured and calculated values. The aminophenols may have been partly ionized, at both pH 3.5 and 7.1, but this should make the experimental $\Delta\Delta \log V_N$ values *smaller* than the calculated ones.

TABLE XI

VALUES OF $\Delta\Delta$ LOG $V_N^{i\phi k}$ IN METHANOL–WATER (50:50, v/v) OF COMPOUNDS IN WHICH *i* AND *k* ARE HYDROXYL AND AMINO GROUPS

k		*ΔΔ log* $V_N^{i\phi k}$					
		i = OH (pH 3.5)			*i =* NH_2 *(pH 7.1)*		
		Meas.	*Calc.*	*Diff.*	*Meas.*	*Calc.*	*Diff.*
OH	*m*	0.01	−0.13	0.14	0.09	−0.23	0.32
	p	−0.20	−0.25	0.05	−0.02	−0.31	0.29
NH_2	*m*	−0.03	−0.23	0.20	0.13	−0.32	0.19
	p	−0.08	−0.31	0.23	−0.02	−0.32	0.30

Retention mechanisms in reversed-phase chromatography with alkyl-silica sorbents

A discussion of this subject is appropriate for two reasons. First, it is important to clarify the retention mechanism in the chromatographic systems that we have investigated. Second, our data from the batch partition experiments can shed some light on this mechanism.

Several possible retention mechanisms can be envisaged: (1) partition between the eluent and the bound organic layer, which may have taken up preferentially one of the components from the eluent[14]; (2) partition between the bulk eluent and a thick layer of adsorbed eluent, the composition of which is different from that of the bulk[15]; (3) interaction with residual silanol groups[16]; (4) adsorption on a monomolecular layer of adsorbed eluent[17]; (5) adsorption on the bound organic layer[18].

Mechanism 1. If retention is governed by partition between the eluent and the bound organic layer, the chromatographic distribution coefficient, *K*, is equal to the partition coefficient, *P*, determined by batch experiments. In other words,

$$\log V_N = \log K\Phi_s = \log P + \log \Phi_s \qquad (16)$$

Our data on log P (Table VI) enable us to investigate the validity of this relationship for the methanol–water (50:50, v/v)/octadecyl silica system, and a series of solutes ranging in polarity from benzene to substituted phenols. Fig. 2 shows data on log V_N as a function of log P. Inspection of this figure reveals that eqn. 16 must be rejected on the following arguments: (i) the data points are severely scattered; (ii) they do not lie on a straight line with a slope equal to unity; (iii) although the data points for the apolar solutes do lie on such a line, its intercept is much too large. From the intercept it would follow that V_s is equal to 5.06 ml, whereas the actual volume of the bound alkyl layer in the column is *ca.* 260 μl. Analogous conclusions were drawn by Hammers *et al.*[44] for the water/octadecyl silica system with a series of 27 solutes comprising methylbenzenes, fused arenes, halogenated benzenes, chloroanilines, chlorophenols and polar monosubstituted benzenes.

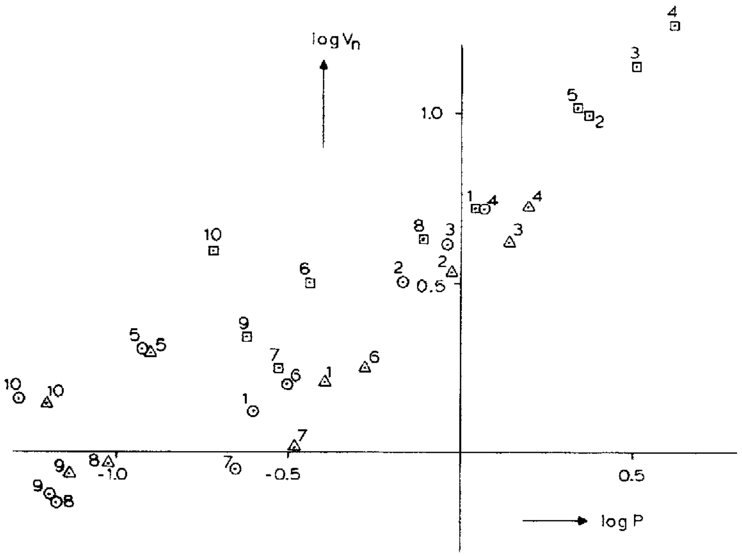

Fig. 2. Log V_N values of substituted benzenes (⊡) and *meta*- (△) and *para*- (⊙) substituted phenols, measured in methanol–water (50:50, v/v) at pH 3.5, as a function of the corresponding log P values for batch partition in the n-hexadecane/methanol–water (50:50, v/v) system.

Mechanism 2. With methanol–water mixtures any contribution to retention from this mechanism can be ruled out, as adsorption of methanol to the alkyl silica does not proceed beyond a monolayer[44]. However, alkyl silica does adsorb large amounts of less polar organic co-solvents from their mixtures with water, and in these cases a partition mechanism may contribute to retention.

According to Slaats *et al.*[45] the volume of the solvent layer that is adsorbed on to alkyl silica is, in acetonitrile–water mixtures, about four times as large as in methanol–water mixtures. Organic co-solvents that are less polar than acetonitrile are even more strongly adsorbed. For instance, the adsorbed amount of tetrahydrofuran is about twice as large as that of acetonitrile[46]. The behaviour of a more exotic co-solvent, 1-pentanol, is enlightening in this respect[47]. This compound is only slightly soluble in water with a maximum concentration of 2.30% (v/v). When the concentration is 30–70% of this value, a monomolecular layer is adsorbed onto the surface of octyl silica. When the concentration of 1-pentanol in the solution is increased from 70 to 100% saturation, the adsorbed amount increases by a factor of 5. At the saturation point almost the complete pore volume is filled with 1-pentanol. The authors estimate that the contribution of partition to the retention volume varies from about 30% at 85% saturation to 95% at 100% saturation.

Mechanism 3. Interaction of the solutes with residual silanol groups is not likely to occur[48]. These groups are shielded by the octadecyl chains[49] and, besides, the eluent offers a vast number of hydroxyl groups[50]. Furthermore, it appears that log V_N data for acidic, neutral and basic solutes correlate well with the corresponding log P data for the octanol–water system[20,44].

Mechanism 4. In our opinion, adsorption on a monomolecular layer of adsorbed eluent rarely occurs. There are no arguments[46,51] for the assumption that the exchange of adsorbed eluent molecules such as methanol or acetonitrile for sample molecules does not occur. (It is, formally, the mechanism prevailing in ion-pair chromatography, if the counter ion that is added to the eluent is strongly adsorbed by the alkyl silica).

Mechanism 5. Adsorption on the bound organic layer is probably the retention mechanism in methanol–water eluents. Retention data are in good agreement with Locke's model for competitive adsorption in a monolayer[44,52]. Retention data in 1-pentanol–water eluents can also be described by assuming competitive adsorption in a monolayer, up to 70% of the saturation concentration of 1-pentanol.

Summarizing, we conclude that in our experiments with 50% (v/v) methanol and probably also with 30% (v/v) acetonitrile, retention is governed by adsorption on the bound alkyl layer.

Statistics

As has been mentioned above, the standard deviation of the $\Delta\Delta$ log V_N values is 0.014. This is much better than the standard errors of fit of the two-parameter regression analyses. These latter values vary from 0.01 to 0.06, as can be seen from Table IV. This can be understood if the exact expression for $\Delta\Delta$ log V_N is considered. If we overlook, for the sake of simplicity, the splitting into inductive and resonance parts we can write the exact eqn. 8 as

$$\Delta\Delta \log V_N^{i\phi k} = \varrho_i\sigma_k + \varrho_k\sigma_i \qquad (17)$$

Within a certain solute series the term $\varrho_i\sigma_k$ varies because the value of ϱ_i for the variable substituent *i* varies. The $\varrho_k\sigma_i$ term is the part we have been dealing with when calculating the ϱ parameters found in Table IV. The $\varrho_i\sigma_k$ term accounts for the influence of the fixed group *k* on the variable group *i*. It is small compared to the $\varrho_k\sigma_i$ term, but not negligible; its presence implies that the s_v values of the regressions are composed of the experimental error (0.014) and the neglected $\varrho_i\sigma_k$ terms:

$$(s_y)^2 = (0.014)^2 + (s')^2 \qquad (18)$$

where s' accounts for the neglected $\varrho_i\sigma_k$ terms. It turns out that s' varies from zero to 0.06. That this hypothesis is correct can be investigated as follows. Eqn. 17 should also hold for the substituted chlorobenzenes. However, in this case the fixed substituent k (Cl) and the variable substituents i are of comparable polarity. Hence, the terms $\varrho_i\sigma_k$ and $\varrho_k\sigma_i$ will be of comparable magnitude. This means that the variance within the $\Delta\Delta$ log V_N values of the substituted chlorobenzenes is equal to

$$s^2 = (0.014)^2 + 2(s')^2 \tag{19}$$

With eqn. 19 we find, from the data on *meta-* and *para*-substituted chlorobenzenes at pH 3.5 and 7.1, an estimate of 0.04 for s'. This is in good agreement with the estimate of 0.06 given above. Therefore we can conclude that the proposed two-parameter equation is valid as a description of the substituent interaction effect in all the solute series. Addition of a $\varrho_E\sigma_E$ term[6] does not give any improvement in the standard error of fit for our regressions. The single-term Hammett equation, on the other hand, does not hold at all.

CONCLUSIONS

The influence of substituents on the retention behaviour of benzoic acids, phenols, anilines and pyridines in reversed-phase liquid chromatography can be described using the Taft equation. For molecules with more than one strongly polar substituent the description fails. The signs of the values of the ϱ parameters in the Taft equation correspond with the signs of the ϱ parameters holding for batch partition coefficients, but not with the signs of the ϱ parameters holding for normal-phase liquid chromatography or for GLC. The signs can be explained by assuming that all the solutes (pyridines, anilines, phenols and benzoic acids) act as proton acceptors in hydrogen bonding with the (acidified) mixed aqueous–organic eluents.

Adsorption on the bound layer is the prevailing retention mechanism in our experiments.

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