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RETENTION RELATIONSHIPS FOR AROMATIC HYDROCARBONS ELUTED FROM CAPPED AND UNCAPPED OCTADECYL SILICA GELS

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SUMMARY

Retention characteristics of 54 aromatic hydrocarbons have been measured on three reversed-phase packing materials, ODS Hypersil (a fully capped octadecyl bonded reversed-phase silica gel), uncapped ODS Hypersil (an intermediate in the production of ODS Hypersil), and LiChrosorb RP-18, using three different eluent compositions, 90:10, 80:20, and 70:30 methanol–water (by weight).

For all nine combinations of packing material and eluent log k' shows a linear dependence on carbon number for the *n*-alkylbenzenes and for those polymethylbenzenes in which all methyl groups are *ortho* to each other. The gradients of the plots are essentially identical for ODS Hypersil and LiChrosorb RP-18, but are slightly lower for uncapped ODS Hypersil. For compounds with the same carbon number, those with higher hydrogen numbers are more retained. Examination of the correlation of log k' for all 54 solutes with carbon number, hydrogen number and connectivity index shows that none of these parameters provides a satisfactory universal correlation.

For isomeric alkylbenzenes, retention is significantly affected by the number of alkyl groups, by the structure of the alkyl groups and by the arrangement of the groups on the benzene ring. It is noted specifically that *ortho*-substituted alkylbenzenes have lower retention than isomers where the groups are not adjacent. This is termed the "reverse *ortho* effect". Branching of alkyl substituents reduces retention.

While the chromatographic behaviour of the 54 solutes on capped and uncapped ODS Hypersil is broadly similar, the retention of the alkylbenzenes is slightly increased by capping while the retention of polynuclear aromatics is slightly reduced by capping. LiChrosorb behaves similarly to uncapped ODS Hypersil except that its retention is much greater.

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INTRODUCTION

A detailed understanding of the relationship between retention and molecular structure in liquid chromatography (LC) enables us to interpolate the chromatographic behaviour of homologues and related solutes, to estimate the chromatographic behavour of structurally similar compounds, and to optimise the chromatographic conditions for the separation of complex mixtures in analytical and preparative separations.

In the case of aromatic hydrocarbons, relationships between retention and molecular structure have been extensively studied for both adsorption (normal-phase LC) and reversed-phase LC.

Relationships for alumina were early established by Snyder[1,2] and later in more detail by Popl and co-workers[3–5]. More precise data was obtained by Kříž *et al.* in their studies of alkylbenzenes[6] and alkylbiphenyls[7], using a moisture control system (MCS) which provided a constant water content on the adsorbent surface.

Relationships for silica gel were established by Popl *et al.*[5,8,9] for mono- and diaromatic hydrocarbons, and by Kříž and co-workers for alkylbenzenes[10], alkylnaphthalenes[11] and alkylbiphenyls[12], again using MCS.

Retention measurements for *n*-alkylbenzenes as a function of mobile phase composition and temperature for reversed-phase systems have been made by Melander and co-workers[13–15], by Grushka *et al.*[16], by Colin and co-workers[17–20] and by Berendsen and De Galan[21]. The relation of retention to molecular structure has been extensively considered[16,17,22–26].

Jandera[27] used *n*-alkylbenzenes as reference compounds to predict retention on the basis of interaction indices. Sleight[28] studied alkylbenzenes with different substitution patterns, and correlated log k' with the total number of carbon atoms. He also established the strictly linear relationship between log k' and carbon number for the *n*-alkylbenzenes.

Shabron *et al.*[29] compared the retentive behaviour on μBondapak C_{18} of alkyl substituted aromatic and hydroaromatic compounds having different parent groups, and obtained empirical correlation factors for different structural features He pointed out, for example, that when the parent compound contained a non-aromatic double bond, as with indene and acenaphthalene, the two double bonded C-atoms had the same effect on retention as a single aliphatic C-atom. Koopmans and Rekker[30] studied the relationship of retention to hydrophobicity and connectivity indices for polymethylbenzenes and basic polyaromatics using methanol–water (70:30, v/v) as eluent and μBondapak C_{18} as stationary phase. Karger *et al.*[31] studied hydrophobic effects using basic phases. Kiselev *et al.*[25] compared retention of polymethylbenzenes and of *n*-alkylbenzenes on silanized silica gel using 2-propanol–water (40:60, v/v) as eluent with their retention on underivatized silica with hexane as eluent. In the latter system the polymethylbenzenes were less retained than the *n*-alkylbenzenes of the same carbon number.

Smith[32] carried out a comprehensive study of the retentive behaviour of C_1–C_4 substituted alkylbenzenes on three different reversed-phase silica gels using methanol–water (70:30, v/v) as eluent. He confirmed the well established linear relationship between log k' and carbon number for the *n*-alkylbenzenes and established a similar relationship for polymethylbenzenes with adjacent methyl groups.

The lower slope of the latter was explained on the basis of the increased electron density on the benzene ring caused by the increased methyl substitution. Smith found that the two bicyclic aromatics, biphenyl and naphthalene, eluted much earlier than would be expected from their carbon numbers. Log k' correlated well with the connectivity index for the *n*-alkylbenzenes and polymethylbenzenes but poorly for other isomeric alkylbenzenes.

The present study aimes at finding relationships between molecular structure and retention for an extended series of 54 aromatic hydrocarbons including, alkylaromatics, cycloaromatics, hydroaromatics, and polynuclear aromatics, using three different ODS bonded silica gels, and three different methanol–water eluents. The three bonded silica gels were: ODS Hypersil, which is a fully capped octadecyl bonded silica gel manufactured by Shandon Southern Products (Runcorn, U.K.), uncapped ODS Hypersil, which is the intermediate in the production of ODS Hypersil just prior to capping, and LiChrosorb RP-18 manufactured by Merck (Darmstadt, F.R.G.). Comparison of the results from capped and uncapped ODS Hypersil was expected to reveal the effects of underivatized silanol groups, while comparison of ODS Hypersil and LiChrosorb RP-18 was expected to indicate any significant differences between two materials both claiming the same bonding functionality.

EXPERIMENTAL

Equipment and chemicals

The HPLC equipment comprised a Waters Model M6000 pump (Waters Assoc., Croydon, U.K.), a Rheodyne injection valve (Shandon Southern Products), a Cecil Model 212 UV spectrophotometer (Cecil Instruments, Cambridge, U.K.). Columns were of the Shandon design, 160 mm × 5 mm I.D. They were packed by the slurry method using 2-propanol as slurry medium with methanol and finally hexane as follower liquids. For chromatography a flow-rate of 1 ml/min was used throughout. ODS Hypersil and uncapped ODS Hypersil were kindly gifted by Shandon Southern Products. LiChrosorb RP-18 was obtained from BDH, Poole, U.K. Eluents were water–methanol mixtures. The methanol was HPLC grade from Rathburn Chemicals, Walkerburn, U.K.; water was doubly distilled and stored in glass. Methanol–water mixtures were made up by weight, not volume, to avoid uncertainties arising from volume changes on mixing. Most of the hydrocarbons were kindly provided by the Institute of Chemical Technology, Prague, Checkoslovakia; the remainder were purchased from U.K. chemical suppliers.

Retention data were analysed and processed on an HP-85 personal computer (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with an 82905B impact printer, an 8901 disk drive and a Servigor Model 281 plotter. Visicalc software was used to prepare, compute and print tables.

Procedure

On the basis of preliminary chromatographic runs, mixtures containing from two to five completely resolved hydrocarbons were prepared in methanol. Injections of between 0.5 and 5 μl of each such mixture were made in triplicate. Column capacity ratios, k', were calculated from the recorder traces using direct measurement of retention distances according to

$$k' = (t_R - t_0)/t_0 \tag{1}$$

TABLE I

RETENTION DATA

C = No. of carbon atoms in molecule, H = No. of hydrogen atoms in molecule, Con. = connectivity index. C_1 = column with LiChrosorb RP-18, C_2 = column with Hypersil ODS, C_3 = column with Hypersil ODS-uncapped.

Compound	*C*	*H*	*Con.*	*Capacity factor*								
				*70:30**			*80:20*			*90:10*		
				C_1	C_2	C_3	C_1	C_2	C_3	C_1	C_2	C_3
1 Benzene	6	6	2.0000	1.41	0.89	0.79	0.74	0.46	0.43	0.41	0.25	0.24
2 Toluene	7	8	2.4107	2.38	1.47	1.28	1.16	0.71	0.68	0.59	0.37	0.35
3 Ethylbenzene	8	10	2.9713	3.54	2.23	1.85	1.58	0.99	0.90	0.74	0.47	0.43
4 *n*-Propylbenzene	9	12	3.4713	5.66	3.61	2.81	2.30	1.44	1.24	0.98	0.62	0.55
5 *n*-Butylbenzene	10	14	3.9713	9.20	5.87	4.37	3.36	2.12	1.72	1.32	0.84	0.71
6 *n*-Amylbenzene	11	16	4.4713	15.35	9.75	6.85	4.99	3.09	2.41	1.75	1.12	0.92
7 *n*-Hexylbenzene	12	18	4.9713	25.41	16.03	10.79	7.40	4.55	3.39	2.37	1.49	1.18
8 *n*-Heptylbenzene	13	20	5.4713	42.85	26.67	17.22	11.14	6.73	4.83	3.23	1.98	1.54
9 *n*-Octylbenzene	14	22	5.9713	–	44.36	27.54	16.60	10.12	6.87	4.37	2.62	1.99
10 *n*-Nonylbenzene	15	24	6.4713	–	–	–	24.21	14.72	9.75	6.00	3.47	2.62
11 *n*-Decylbenzene	16	26	6.9713	–	–	–	36.22	21.28	13.96	8.18	4.61	3.40
12 *n*-Tridecylbenzene	19	32	8.4713	–	–	–	–	–	–	20.32	10.74	7.21
13 *o*-Xylene	8	10	2.8274	3.72	2.30	1.92	1.82	1,04	0.94	0.84	0.50	0.47
14 *m*-Xylene	8	10	2.8214	4.03	2.52	2.07	1.81	1.09	1.00	0.87	0.55	0.48
15 *p*-Xylene	8	10	2.8214	4.12	2.68	2.09	1.80	1.15	0.95	0.87	0.54	0.48
16 Isopropylbenzene	9	12	3.3541	5.89	3.35	2.92	2.16	1.36	1.17	0.88	0.57	0.51
17 *o*-Ethyltoluene	9	12	3.3880	5.46	3.43	2.57	2.28	1.36	1.16	1.04	0.64	0.57
18 *m*-Ethyltoluene	9	12	3.3820	5.70	3.77	2.86	2.34	1.50	1.28	1.05	0.67	0.58
19 *p*-Ethyltoluene	9	12	3.3820	6.01	3.86	2.99	2.43	1.53	1.32	1.08	0.68	0.59
20 1,2,3-Trimethylbenzene	9	12	3.2440	5.79	3.72	2.94	2.51	1.53	1.34	1.17	0.71	0.64
21 1,2,4-Trimethylbenzene	9	12	3.2380	6.37	4.47	3.18	2.67	1.62	1.40	1.21	0.72	0.66
22 1,3,5-Trimethylbenzene	9	12	3.2321	6.82	4.29	3.33	2.84	1.74	1.47	1.23	0.77	0.69

23	Isobutylbenzene	10	14	3.8272	8.20	5.56	3.95	3.01	1.98	1.59	1.22	0.79	0.67
24	*sec.*-Butylbenzene	10	14	3.8921	7.50	4.67	3.55	2.81	1.84	1.46	1.16	0.76	0.62
25	*tert.*-Butylbenzene	10	14	3.6607	6.62	3.84	3.13	2.45	1.50	1.28	1.02	0.69	0.58
26	*p*-Cymene	10	14	3.7647	8.87	5.55	4.00	3.13	2.02	1.64	1.28	0.82	0.69
27	*o*-Diethylbenzene	10	14	3.9487	7.62	5.12	3.71	2.92	1.91	1.55	1.24	0.82	0.68
28	*m*-Diethylbenzene	10	14	3.9427	8.43	5.21	3.96	3.08	1.99	1.61	1.26	0.82	0.69
29	*p*-Diethylbenzene	10	14	3.9427	8.95	5.60	4.15	3.30	2.10	1.70	1.34	0.85	0.72
30	1,2,3,4-Tetramethylbenzene	10	14	3.6607	9.33	5.97	4.55	3.78	2.26	1.92	1.66	0.99	0.86
31	1,2,3,5-Tetramethylbenzene	10	14	3.6547	10.07	6.22	4.75	3.99	2.39	2.00	1.67	1.02	0.89
32	1,2,4,5-Tetramethylbenzene	10	14	3.6547	10.19	6.59	4.82	3.96	2.40	1.99	1.69	1.00	0.89
33	Pentamethylbenzene	11	16	4.0774	15.35	9.66	7.10	5.62	3.37	2.71	2.32	1.36	1.17
34	4-*tert.*-Butyltoluene	11	16	4.0714	12.16	7.55	5.40	3.94	2.57	1.98	1.52	0.99	0.80
35	*m*-Diisopropylbenzene	12	18	4.7081	14.76	10.23	6.70	4.60	3.18	2.32	1.64	1.11	0.90
36	*p*-Diisopropylbenzene	12	18	4.7081	16.71	11.35	7.59	5.32	3.53	2.55	1.80	1.22	0.94
37	Hexamethylbenzene	12	18	4.5000	23.01	14.29	10.33	8.07	4.76	3.69	3.21	1.86	1.56
38	Cyclohexylbenzene	12	16	5.0159	16.22	10.23	7.29	5.30	3.24	2.57	2.00	1.21	1.02
39	*p*-Di-*tert.*-butylbenzene	14	22	5.3214	31.62	21.93	13.06	8.20	5.71	3.81	2.53	1.74	1.25
40	1,2,3-Triisopropylbenzene	15	24	5.9848	38.90	28.18	15.85	9.33	6.79	4.26	2.65	1.91	1.31
41	*p*-Dicyclohexylbenzene	18	26	8.0317	–	–	–	37.50	21.93	12.88	8.36	5.09	3.63
42	Hexaethylbenzene	18	30	7.8640	–	–	–	19.72	12.02	6.55	4.11	2.99	1.91
43	Indane	9	10	3.5345	5.07	2.89	2.39	2.14	1.29	1.15	1.02	0.62	0.57
44	Indene	9	8	3.2112	2.96	1.69	1.60	1.42	0.83	0.81	0.71	0.42	0.43
45	Tetrahydronaphthalene	10	12	4.0345	7.38	4.40	3.61	3.06	1.82	1.58	1.38	0.82	0.73
46	Naphthalene	10	8	3.4047	3.65	1.97	1.88	1.64	0.90	0.92	0.79	0.44	0.46
47	Phenanthrene	14	10	4.8154	12.30	5.22	5.71	4.51	1.95	2.25	1.87	0.83	0.98
48	Anthracene	14	10	4.8094	12.02	5.31	5.71	4.51	1.95	2.25	1.85	0.84	0.98
49	Biphenyl	12	10	4.0714	5.97	3.21	2.90	2.35	1.29	1.25	1.01	0.57	0.57
50	Fluorene	13	10	4.6118	8.75	4.49	4.22	3.49	1.70	1.79	1.50	0.74	0.81
51	Acenaphthene	12	10	4.4451	8.09	4.33	3.90	3.38	1.77	1.74	1.51	0.82	0.81
52	*o*-Terphenyl	18	14	6.1487	13.49	8.43	6.00	4.30	2.59	2.12	1.45	0.90	0.80
53	*m*-Terphenyl	18	14	6.1427	24.04	12.79	10.26	7.13	3.69	3.35	2.28	1.22	1.21
54	*p*-Terphenyl	18	14	6.1427	33.19	13.96	13.06	9.53	3.97	3.98	2.92	1.28	1.39

* Composition of methanol–water (w/w) mobile phase.

where t_R is the retention time (or distance on chart) for a solute peak, and t_0 is the retention time (or distance on chart) of the unretained solute peak. The retention time of the unretained solute peak was taken as the time interval from the moment of injection to the time when the trace for the solvent disturbance crossed the baseline. The solvent disturbance peak was generated by the methanol in which the samples were dissolved. Retention data were reproducible to better than 2% from run to run provided that an entire set of data were obtained over a period of 10–18 h. Column activity was checked several times during any such experiment by injecting a standard mixture of n-alkylbenzenes.

RESULTS AND DISCUSSION

Table I lists the values of the capacity factors for the 54 hydrocarbons on the three stationary phases and with the three eluent compositions. Each value represents the average of at least three measurements. The majority of compounds examined were alkylbenzenes and had the empirical formula C_nH_{2n-6}, but compounds with the following empirical formulae were also characterised: C_nH_{2n-8} (indane, tetralin, cyclohexylbenzene), C_nH_{2n-10} (indene), C_nH_{2n-12} (naphthalene), C_nH_{2n-14} (biphenyl and acenaphthene) and so on up to C_nH_{2n-22} (terphenyls).

We have examined the following aspects of the structure–retention relationship for the 54 solutes. (i) Correlations between log k' and (a) the number of carbon atoms in each molecule, (b) the number of hydrogen atoms in each molecule, and (c) the connectivity index of each molecule[33]. (ii) The effects of the following structural features: (a) the length of the alkyl chain, (b) the number of alkyl groups, (c) the arrangement of the alkyl groups (the *ortho* effect), and (d) the shape of the alkyl groups. (iii) The effect of capping an ODS silica gel.

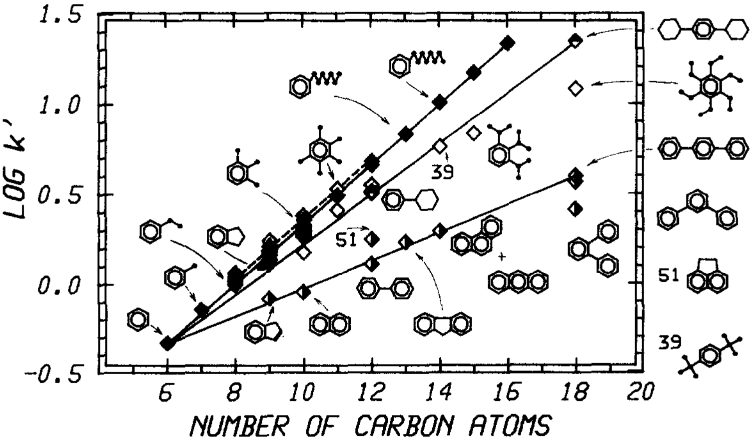

Fig. 1. Dependence of log k' for 54 aromatic hydrocarbons on carbon number. Packing material, ODS Hypersil; eluent, methanol–water (80:20, w/w). Solutes identified by number are listed in Table I. Symbol representation: (◆) *n*-alkylbenzenes, (◇) polymethylbenzenes and other alkylbenzenes, (⬘) cyclohexyl benzenes, (⬖) polycyclic aromatics. Broken line is drawn through points for the all-*ortho* polymethylbenzenes; full lines are drawn (in order from the top) through points for *n*-alkylbenzenes, cyclohexylbenzenes, and polycyclic aromatics.

Correlations of log k′ with structural features

Correlation with carbon number. In reversed-phase LC, k' invariably increases with carbon number for any series of compounds, although with adsorbents in normal-phase LC[6,7] k' may hardly change (alumina) or may even decrease (silica gel) with increase in n..

Fig. 1 shows the dependence of log k' upon carbon number with ODS Hypersil as packing and methanol–water (80:20) as eluent. Similar results were obtained for the other packing materials and other mobile phases. More or less linear dependences were observed for *n*-alkylbenzenes, polymethylbenzenes, cyclohexylbenzenes, and polynuclear aromatics. The dependence for *n*-alkylbenzenes is strictly linear for all members except benzene (slighly low k') and toluene (slightly high k'), and is shown by the uppermost full line. The dependence for the polymethylbenzenes is also linear when all the methyl groups are adjacent (broken line above the full line for the *n*-alkylbenzenes) as found by Smith[32]. The other polymethylbenzenes show slightly higher retention than the all-*ortho* isomers. By contrast, the points for branched chain alkylbenzenes and cyclohexylbenzenes are significantly below those for the *n*-alkylbenzenes. The points for the cyclohexylbenzenes mark more or less the lower limit of this group (hexaethylbenzene being the exception). In general the points for all the alkyl- and cycloalkylbenzenes fall within a relatively narrow band marked out by the two full lines in Fig. 1. Log k' values for the polynuclear aromatics (PNAs) fall well below those for the other aromatics and show considerable variance from a straight line. *o*-Terphenyl shows particularly low retention, and acenaphthene (compound 51) particularly high retention relative to the general body of the PNAs. In addition the general gradient of the log k' *versus* n plot for the PNAs is about half that for the *n*-alkylbenzenes, confirming the earlier results of Shabron *et al.*[29].

Correlations with hydrogen number. The H-atoms in the sample molecules had a remarkably large effect on their retention in the reversed-phase systems studied. This is seen clearly by comparing Figs. 2 and 3. Fig.. 2 shows chromatograms where the number of C-atoms was kept constant while the number of hydrogen atoms was changed. Fig. 3 shows chromatograms where the number of hydrogen atoms was kept constant while the number of carbon atoms was changed. The effect of adding

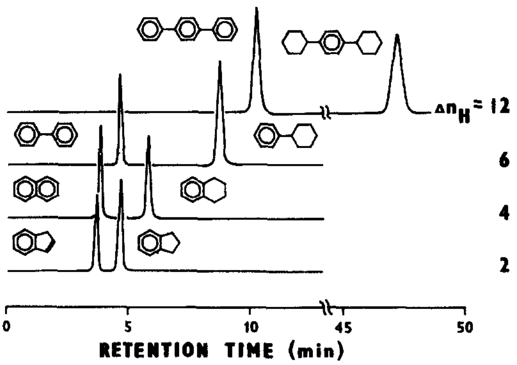

Fig. 2. Chromatogram of selected mixtures showing the effect of addition of hydrogen atoms when the carbon number is fixed. Column packing, ODS Hypersil; eluent, methanol–water (80:20). Δn_H gives the difference in the hydrogen number for the solutes in each chromatogram.

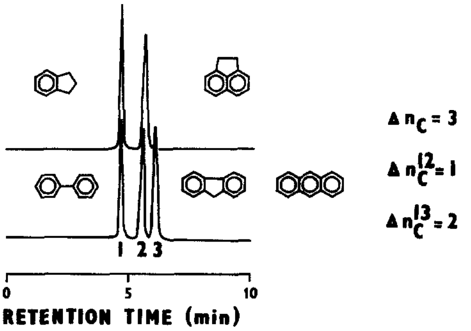

Fig. 3. Chromatograms of selected mixtures showing the effect of addition of carbon atoms when the hydrogen number is fixed. Conditions as for Fig. 2. Δn_c gives the difference in the carbon number for the solutes in each chromatogram.

another hydrogen atom is only slightly less than that of adding another carbon atom. Addition of hydrogen atoms invariably increases retention: thus indane is more retained than indene, cyclohexylbenzene is more retained than biphenyl, and tetrahydronaphthalene is more retained than naphthalene.

Fig. 4 plots the dependence of log k' upon hydrogen number using the same data as for Fig. 1. Necessarily a strict linear dependence is still observed for the *n*-alkylbenzenes and for the all-*ortho* polymethylbenzenes. However the cyclohexylbenzenes now fall on the same line as the *n*-alkylbenzenes, while the points for the PNAs now lie above those for the *n*-alkylbenzenes, and the best straight line through them has a much higher slope.

Correlations with connectivity indices. The connectivity index has been found to give a useful correlation with retention in reversed-phase systems[34]. For example

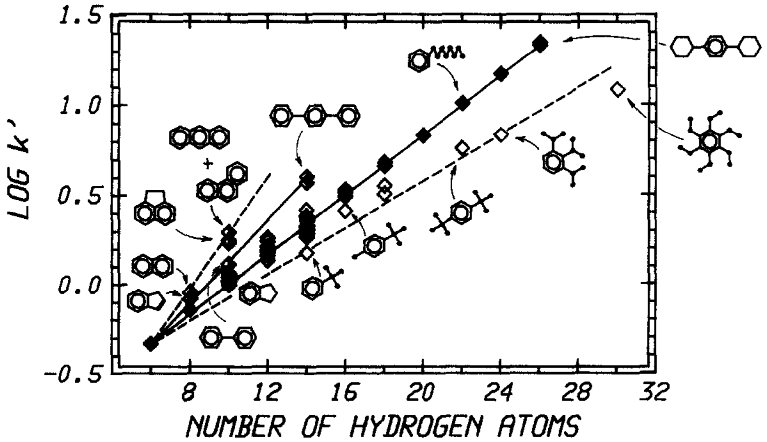

Fig. 4. Dependence of log k' upon hydrogen number. Conditions and data as for Fig. 1. Symbol identification: (◆) *n*-alkylbenzenes, (◇) polymethylbenzenes and other alkylbenzenes, (◈) polycyclic aromatics. Lines in order from the top are drawn through points for: condensed ring polynuclear aromatics (broken line), polyphenyls (full line), *n*-alkylbenzenes (full line), and propylbenzenes (broken line).

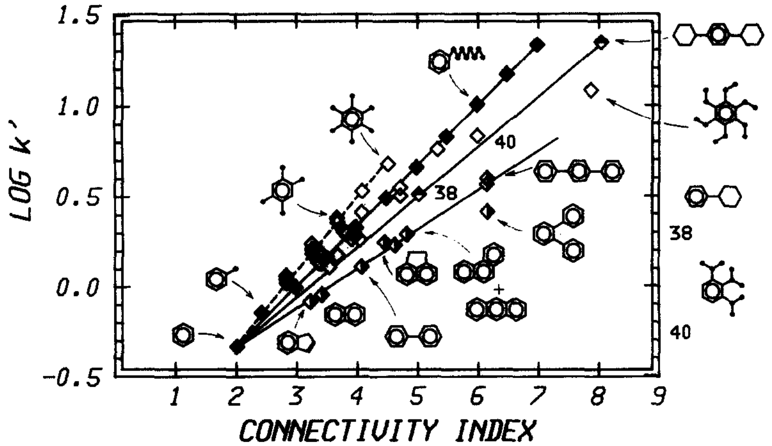

Fig. 5. Dependence of log k' upon connectivity index. Conditions, data, symbols and lines as for Fig. 1.

the log k' values of a large set of C_5–C_{11} alkanes with different degrees of branching were found to lie within a much narrower band when plotted against the connectivity index than when plotted against the carbon number.

Fig. 5, again using the same data as Fig. 1, plots log k' against the connectivity index, and is rather similar to Fig. 1. Evidently the connectivity index mainly reflects the number of carbon atoms in the group under study. The four more or less linear dependences are again shown. The polymethylbenzenes now show a considerably higher slope than the *n*-alkylbenzenes, while the slope for the PNAs is only a little less than that for the cyclohexylbenzenes. Amongst the PNAs only *o*-terphenyl is now exceptional; the point for acenaphthene falls on the line of the other PNAs, and the spread of points about the line is generally less than in Fig. 1 and 4. While the

TABLE II

PARAMETERS OF LINEAR REGRESSION FOR ALKYLBENZENES

log $k' = bn + a$; where k' = capacity factor, n = carbon number, a and b are regression coefficients, r^2 = coefficient of determination.

Column	*Mobile phase ratio*	*b*	*a*	r^2
LiChrosorb RP-18	70:30	0.210	−1.118	0.99894
	80:20	0.168	−1.142	0.99955
	90:10	0.129	−1.166	0.99931
Hypersil ODS	70:30	0.212	−1.335	0.99947
	80:20	0.166	−1.328	0.99976
	90:10	0.124	−1.321	0.99970
Hypersil uncapped	70:30	0.191	−1.259	0.99921
	80:20	0.148	−1.237	0.99921
	90:10	0.112	−1.268	0.99947

correlation in specific areas may be better than when using carbon and hydrogen numbers it is not significantly better overall.

We conclude that none of the simple structural parameters examined can provide a unique correspondence with retention for the range of aromatic compounds covered.

Effect of structural changes on retention

Alkyl chain length. Numerous studies have established that the retention of *n*-alkylbenzenes increases exponentially with chain length according to

$$\log k' = a + bn \tag{2}$$

where n is the number of carbon atoms in the molecule. The data obtained in this work and listed in Table I, provide the regression coefficients for the three stationary phases and the three eluents listed in Table II. Only k' values of benzene and toluene show deviations from eqn. 2. If, for example, toluene and benzene are omitted from the data for ODS Hypersil with methanol–water (70:30) as eluent, the correlation coefficient is increased from 0.99947 to 0.99989.

The regression coefficients giving the values of b in eqn. 2 are essentially the same for ODS Hypersil and LiChrosorb RP-18, but are slightly lower for uncapped ODS Hypersil. This is what might be expected in view of the presence of accessible silanol groups in the uncapped material. Of course, in this work, the eluents contained a considerable proportion of water which would undoubtedly reduce the adsorptive effects of the silanol groups drastically. Nevertheless they still have some of the repulsive effect on alkyl chains which they show when the *n*-alkylbenzenes are chromatographed in non-polar eluents on bare silica gel[6,7].

Number of alkyl groups. Retention increases with increasing number of alkyl groups when the total carbon number is fixed, and of, course, whenever the carbon

TABLE III

PARAMETERS OF LINEAR REGRESSION FOR *ORTHO*-SUBSTITUTED METHYLBENZENES

Benzene, toluene, *o*-xylene, 1,2,3-trimethylbenzene, 1,2,3,4-tetramethylbenzene, pentamethylbenzene and hexamethylbenzene. $\log k' = bn + a$; where k' = capacity factor, n = carbon number, a and b are regression coefficients, r^2 = coefficient of determiniation.

Column	*Mobile phase*	b	a	r^2
LiChrosorb RP-18	70:30	0.202	−1.050	0.99937
	80:20	0.172	−1.140	0.99849
	90:10	0.149	−1.272	0.99967
Hypersil ODS	70:30	0.203	−1.256	0.99933
	80:20	0.168	−1.333	0.99956
	90:10	0.144	−1.453	0.99944
Hypersil uncapped	70:30	0.186	−1.210	0.99936
	80:20	0.154	−1.262	0.99787
	90:10	0.134	−1.404	0.99916

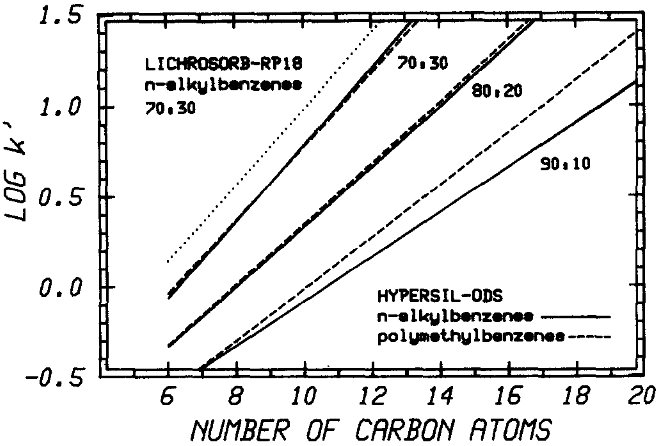

Fig. 6. Effect of eluent composition on selectivity of ODS Hypersil for *n*-alkylbenzenes (full lines) and all-*ortho* polymethylbenzenes (broken lines). The dotted line for LiChrosorb RP-18 is for *n*-alkylbenzenes using methanol–water (70:30) as eluent.

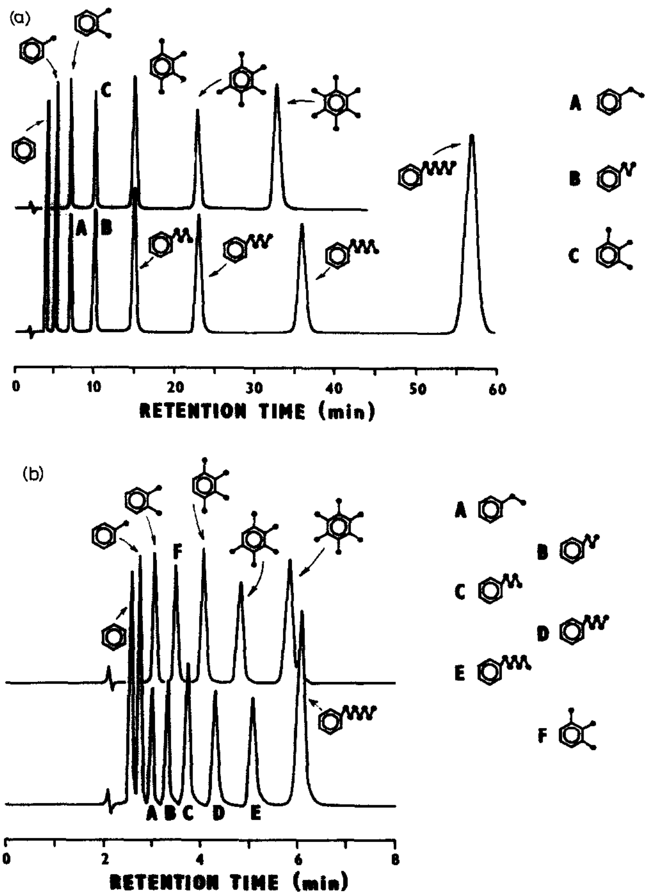

Fig. 7. (a) Chromatogram showing the close correspondance of retention times for the all-*ortho* polymethylbenzenes and *n*-alkylbenzenes having the same carbon numbers when using methanol–water (70:30) as eluent. (b) Chromatogram showing the selectivity for the same group when using methanol–water (90:10) as eluent. Packing material, ODS Hypersil.

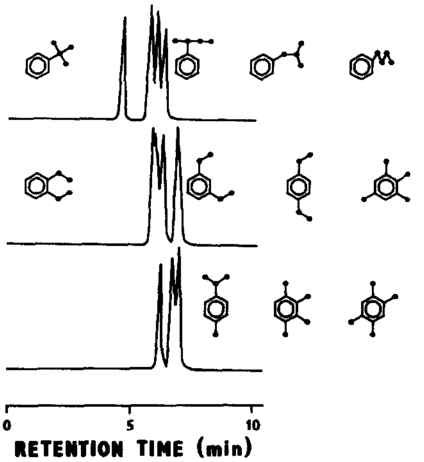

Fig. 8. Chromatograms of selected isomeric alkylbenzenes showing selectivity effects. Packing, ODS Hypersil; eluent, methanol–water (80:20).

number is increased. When all groups are *ortho* to each other there is a linear relationship between Log k' and n. The linear regression coefficients are given in Table III. Fig. 6 shows the plots with ODS Hypersil as packing for the three eluents used, and for both *n*-alkylbenzenes and polymethylbenzenes. The dotted line gives the plot for *n*-alkylbenzenes on LiChrosorb RP-18 for comparison using one of the eluents.

With the same eluent, the retention of the *n*-alkylbenzenes relative to that of the polymethylbenzenes having the same carbon number is roughly equivalent for the three stationary phases, but selectivity increases as the water content is lowered, being greatest for methanol–water (90:10). This is illustrated in Fig. 7a and b. Fig. 7a shows the near equivalence of the retention times of solutes of the same carbon number when using 70:30 eluent, while Fig. 7b shows the increased retention of the polymethylbenzenes over the *n*-alkylbenzenes in the 90:10 mixture.

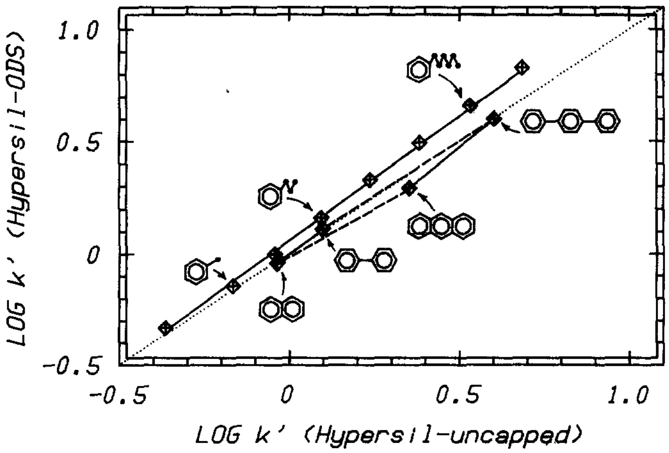

Fig. 9. Correlation of log k' on ODS Hypersil with log k' on uncapped ODS Hypersil. Eluent, methanol–water (80:20). Dotted line is locus for equal k' on the two packing materials.

The reversed ortho effect. The "*ortho* effect" is an effect observed with alumina and silica gel whereby the retention of substances with substituents in the *ortho* position is greater than that of isomers with substituents in other positions. With the ODS silica gels studied here the opposite effect is observed and the *ortho*-substituted isomers show the least retention. This is seen in the retention parameters for the xylenes, ethyltoluenes, diethylbenzenes, trimethylbenzenes and tetramethylbenzenes (see Table I). The inverse ortho-effect is particularly strong when large substituents are in ortho positions.

The inversion of the *ortho* effect on transferring from adsorption to reversed-phase chromatography is evidently just what one would expect.

Shape of alkyl groups. Branching of the alkyl group generally reduces retention as in other modes of chromatography. This is illustrated in Fig. 8 which shows chromatograms of the butylbenzenes and of other isomeric alkylbenzenes. The order of elution of the butylbenzenes, namely *tert.-*, *sec.-*, *iso-* and *n*-butylbenzene is in agreement with Smith[32] and is the same as found in gas chromatography. This order does not follow the connectivity index (see Table I).

Comparison of capped and uncapped ODS silica gels

Fig. 9 plots log k' on ODS Hypersil against log k' on uncapped ODS Hypersil. Points are shown for the *n*-alkylbenzenes, the polyphenyls, and the condensed ring aromatics. Points for the other alkylbenzenes lie on or close to the line for the *n*-alkylbenzenes. The dotted line (1:1 line) represents equal k' values on the two phases. For the alkylbenzenes the points lie above the 1:1 line so the process of capping ODS Hypersil slightly increases retention. This is no doubt due to a slight increase in carbon content produced by capping. Points for the polynuclear aromatics lie either on or below the 1:1 line. Accordingly capping either leaves their retention unchanged or decreases it. Apparently with the uncapped ODS Hypersil there still remains some small interaction between the residual silanol groups of the uncapped material and the aromatic rings. The effect of capping which removes these interactions is not

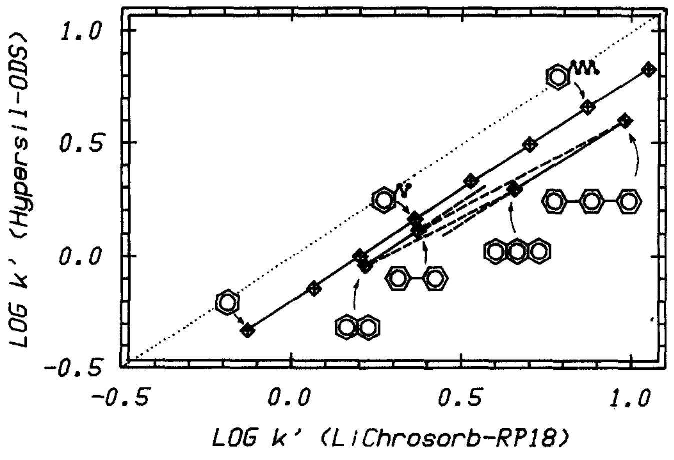

Fig. 10. Correlation of log k' on ODS Hypersil with log k' on LiChrosorb RP-18. Conditions as for Fig. 9.

quite compensated by the increased hydrophobic interaction resulting from the extra carbon content. Thus as shown earlier even with a high water content in the eluent the silanol groups still appear to have some adsorptive capacity.

Fig. 10 shows a similar plot comparing LiChrosorb RP-18 with ODS Hypersil. The retention on LiChrosorb RP-18 is substantially greater than that on ODS Hypersil as seen by the position of the points relative to the 1:1 line. This is no doubt due to the greater carbon content of LiChrosorb RP-18. Otherwise the relative positions of the points are very similar in Figs. 9 and 10, indicating that LiChrosorb RP-18 is closer to uncapped ODS Hypersil than to the standard capped ODS Hy-

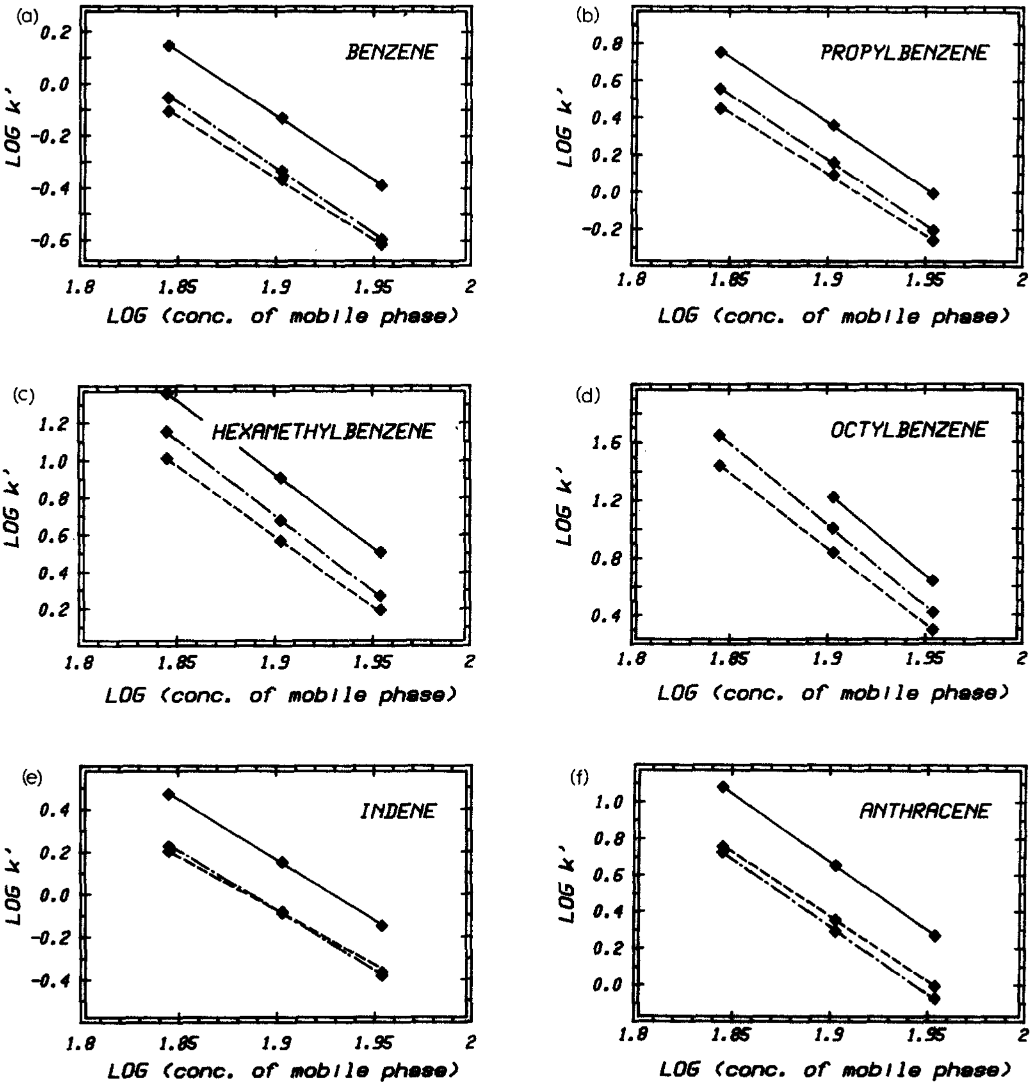

Fig. 11. Dependence of log k' upon eluent composition for the three packing materials. (———) LiChrosorb RP-18; (– · –) ODS Hypersil; (– – –) uncapped ODS Hypersil.

persil in its selectivity for different types of aromatic hydrocarbon. However the gradient of the line through the points for the *n*-alkylbenzenes is very close to unity in Fig. 10 whereas it is significantly above unity in Fig. 9. This similarity between LiChrosorb RP-18 and ODS Hypersil is also shown in Fig. 11a–f which give examples of the linear relationships between log k' and log (% methanol). The gradients for ODS Hypersil and LiChrosorb RP-18 are virtually identical while those for uncapped ODS Hypersil are significantly lower.

We note the danger in making judgements as to whether a material such as LiChrosorb RP-18 is capped or uncapped on the basis of retention measurements made only with hydrocarbons.

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