CHROM. 6860

REVERSED-PHASE CHROMATOGRAPHY OF SOME AROMATIC HYDRO-CARBONS

STRUCTURE–RETENTION RELATIONSHIPS

R. B. SLEIGHT

The British Petroleum Company Limited, Group Research and Development Department, Great Burgh, Epsom, Surrey (Great Britain)

SUMMARY

The chromatographic behaviour of some aromatic hydrocarbons has been studied on columns of Zipax Permaphase ODS in water–methanol mixed solvents. The increase in retention with the number of aromatic rings in a molecule is noted. A simple relation between k' (measure of retention) and empirical formulae for unsubstituted polynuclear aromatic hydrocarbons is suggested. The substitution of alkyl side chains on to an aromatic nucleus causes a further increase in retention. Comparisons show that aliphatic carbon atoms are more effective at increasing retention than their aromatic counterparts. Chromatographic retention of alkylbenzenes can be related to the number of carbon atoms in the aliphatic chain. These relationships together with that linking mobile phase composition to retention might be used to give an approximate idea of the k' value of an aromatic hydrocarbon given its structure.

The carbon content of unsubstituted polynuclear aromatic hydrocarbons could be estimated from their positions in these reversed-phase chromatograms. Furthermore, given the identity of the aromatic nucleus of an aralkyl hydrocarbon, the extent of aliphatic substitution could also be estimated. Such structural assignments can be reinforced by spectroscopic examination of the separated fractions.

INTRODUCTION

Liquid chromatography (LC) has been widely used for the separation of aromatic hydrocarbon compounds. The liquid–solid mode has predominated using either alumina[1–5] or silica[5–7] for column packing materials, either on an analytical[2–4,6] or preparative[1,5,7] scale. However, useful results have also been obtained using Sephadex LH-20[8,9] or conventional normal phase liquid–liquid chromatographic systems[10,11]. In all of these cases the type of separation obtained is similar. Compounds are retained according to the extent of their aromatic nuclei. The presence of alkyl side chains seems to cause a greater deviation from the behaviour of the parent compounds on silica gel than on alumina for alkylbenzenes[4]. With both types of packing

the presence of alkyl side chains tends to cause a diminution in retention, although this is not universally so[12].

The LC of unsubstituted aromatic hydrocarbons in the reversed-phase mode gives a similar result to normal-phase work[13]. Compounds with larger numbers of aromatic rings elute later than those with fewer. However, in normal-phase LC retention depends largely on the interaction between the aromatic π-electrons and polar functional groups in the stationary medium. Reversed-phase chromatography of these compounds is more dependent on their limiting solubility in the polar mobile phase. Thus it might be anticipated that the addition of an aliphatic side chain to an aromatic hydrocarbon might result in a large reduction in its solubility in a polar carrier solvent, but only a small perturbation to the π-electron system. This should result in increasing retention with increasing alkylation of aromatic hydrocarbons in reversed-phase chromatography. This type of selectivity contrasts sharply with the insensitivity to alkyl substituents shown by normal-phase liquid chromatography of such compounds.

EXPERIMENTAL

The apparatus used in this work was a Varian V-4100 high-pressure liquid chromatograph with, in series, differential refractive index (RI) and ultraviolet (UV) absorption detectors. Columns were 150 cm lengths of 1.9 mm I.D. stainless-steel tubing. Prior to packing the columns, their interiors were cleaned by washing with a series of solvents: acetone, benzene, isopropanol, distilled water, nitric acid (50%), distilled water and acetone. The latter was removed by a stream of air and the columns dry-packed. During this procedure the packing material was added slowly and continuously while the column, in a vertical position, was rotated about its vertical axis. Vibrations were applied to the side of the column with a Burgess Vibro-tool. The packing material used was Zipax Permaphase ODS (DuPont Instruments). When packed the columns were bent into a square configuration so that they could be housed in the thermostatted water-bath as the column was maintained at 60°.

Stop flow injection of samples was facilitated by installing a three-way valve (Hoke Selectomite) between the injector and the pump. This enabled the column to be depressurised while full column pressure was maintained at the pump, which was stopped. On reconnecting the pump to the column, the former being simultaneously re-started, the pressure rose rapidly to its equilibrium value.

The solvent system comprised various mixtures of methanol and water, both of which were distilled before use. Degassing was accomplished by boiling before introduction to the pump.

RESULTS AND DISCUSSION

To compare the retention of the compounds studied, k' values (chromatographic capacity factors) are quoted, such that:

$$V_e = V_0 (1 + k') \tag{1}$$

where V_e and V_0 are the elution volumes of the compound concerned and that of an

TABLE I

k' VALUES FOR SOME AROMATIC HYDROCARBONS ON ZIPAX ODS

Solvent, methanol–water (7:3); temperature, 60°.

Compound	No. of carbon atoms	k'	Log k'
Benzene	6	0.09	−1.05
Naphthalene	10	0.23	−0.64
Diphenyl	12	0.34	−0.47
Phenanthrene	14	0.55	−0.26
Anthracene	14	0.67	−0.17
Fluoranthene	16	0.89	−0.05
Pyrene	16	0.94	−0.03
Chrysene	18	1.73	0.24
Naphthacene	18	1.71	0.23
Perylene	20	2.75	0.44
3,4-Benzopyrene	20	3.10	0.49

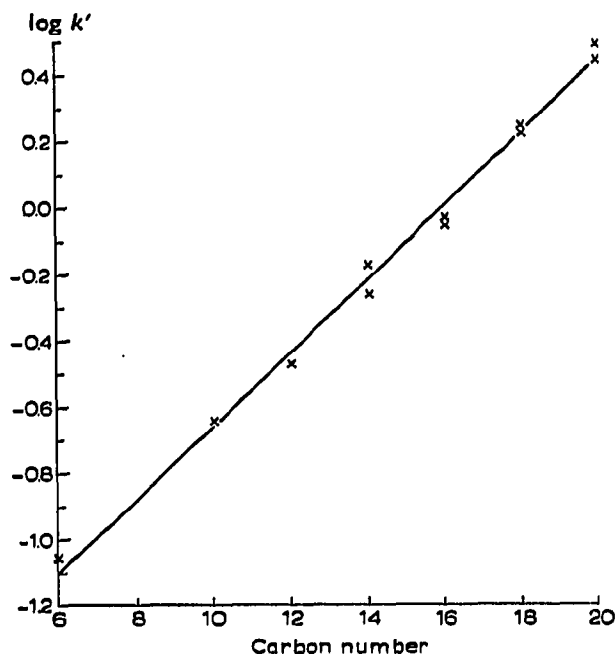
Fig. 1. Plot of log k' against carbon number for unsubstituted aromatic hydrocarbons chromatographed on Zipax ODS. Solvent, methanol–water (7:3); temperature, 60°.

unretained component, respectively. The latter was taken to be the elution volume of methanol. The k' values of some unsubstituted aromatic hydrocarbons are shown in Table I. It was thought that an attempt should be made to relate the structure of these compounds to their chromatographic behaviour. The simple solution of plotting log k' against carbon number (Fig. 1) gave a surprisingly good straight-line fit with the experimental data such that

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

where C is the number of carbon atoms in an aromatic hydrocarbon and k' is its chromatographic capacity factor, $a = -1.74 \pm 0.04$ and $b = 0.109 \pm 0.003$.

Prediction of the k' value of an aromatic hydrocarbon using this column/solvent system, Zipax ODS/methanol–water (7:3), given the carbon number of the compound, seems possible.

Schmit *et al.*[13] have suggested that under the conditions described in this work a linear relation exists between log k' and the percentage of organic modifier in the carrier solvent. Combination of this finding with a linear log k'–carbon number relationship in a fixed solvent suggests that an exponentially increasing solvent gradient should give rise to an elution pattern for unsubstituted aromatic hydrocarbons which is linear in carbon number. Furthermore the prediction of the liquid chromatographic properties, on Zipax ODS, of a whole range of aromatic hydrocarbons on the basis of a minimum of experimental work should be possible. The testing of these assertions for a range of solvent systems is necessary to show how widely these ideas are applicable. It is of interest to note that linear plots of log k' against solvent composition have also been found on Zipax ODS for chlorinated benzenes, alkyl anthraquinones and phthalate esters[13], and to speculate whether structure–retention relationships can be framed for these compounds also.

Fig. 2 shows the chromatogram obtained from a mixture of aromatic hydrocarbons. Peak A corresponded to the elution volume of phenanthrene. It was necessary to discover the identities of B, C and D. Larger-scale separations were carried out and the individual fractions were collected. Concentration of these separated components was greatly facilitated by solvent extraction from the aqueous methanolic phase into a relatively small volume of isooctane. After UV spectra had been obtained, these solutions were evaporated down in "Reactivials" (Pierce Chemical Co.) prior to mass spectroscopic examination. This suggested that A, B, C and D were, respectively, phenanthrene (or anthracene) and mono-, di- and trimethyl-substituted phenanthrenes (or anthracenes). The UV spectra ruled out the possibility of anthracenes in favour of phenanthrenes.
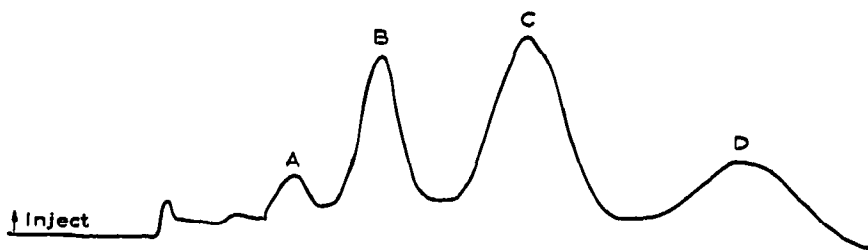

Fig. 2. Separation of phenanthrene (A) and methyl- (B), dimethyl- (C) and trimethylphenanthrenes (D) on Zipax ODS. Solvent, methanol–water (6:4); temperature, 60°.

The point of chromatographic interest in this analysis was that the addition of alkyl substituents to an aromatic hydrocarbon did cause increased retention in reversed-phase liquid chromatography. This was presumably because of increased insolubility in the mobile phase. To test this finding further it was decided to examine some other alkylated aromatic hydrocarbons. Table II shows the measurements which were made. In every case it is seen that increased alkylation leads to increased reten-

TABLE II

k' VALUES OF SOME ALKYL-SUBSTITUTED AROMATIC HYDROCARBONS ON ZIPAX ODS

Solvent, methanol–water (7:3); temperature, 60°.

Compound	No. of aliphatic carbons	k'	Log k'
Toluene	1	0.17	−0.77
Ethylbenzene	2	0.23	−0.64
Isopropylbenzene	3	0.24	−0.62
n-Butylbenzene	4	0.41	−0.39
Durene	4	0.44	−0.36
1,3-Diisopropylbenzene	6	0.54	−0.27
Diisopropyltoluene	7	0.76	−0.12
1,4-Di-tert.-butylbenzene	8	0.98	−0.01
1,3,5-Triisopropylbenzene	9	1.19	0.17
1,2,4,5-Tetraisopropylbenzene	12	2.03	0.31
Hexaethylbenzene	12	2.06	0.31
2-Methylnaphthalene	1	0.32	−0.49
2-Isopropylnaphthalene	3	0.57	−0.24
2,6-Di-tert.-butylnaphthalene	8	2.52	0.40
Fluorene	1	0.44	−0.36
2-Methylfluorene	2	0.71	−0.15
Methylphenanthrene	1	0.81	−0.09
Dimethylphenanthrene	2	1.22	0.09
Trimethylphenanthrene	3	1.74	0.24
1-Methyl-7-isopropylphenanthrene	4	2.41	0.38
1,3-Dimethylpyrene	2	2.52	0.40

tion. Some examples of separations which illustrate this point are shown in Figs. 3 and 4.

Attempts to quantify this increase in retention with increasing proportions of alkyl side chains resulted in plotting log k' against the number of carbon atoms in alkyl substituents for the benzene, naphthalene and phenanthrene derivatives studied (Fig. 5). It is to be noted that mono- and poly-substituted compounds were included. The straight-line plots of the form

$$\log k' = A + BC'$$ (3)

where C' is the total number of alkyl carbons, showed increasing values of B as the aromatic nucleus increased in size (see Table III).

Fig. 3. Separation of pyrene from 1,3-dimethylpyrene on Zipax ODS. Solvent, methanol–water (7:3); temperature, 60°. 1 = Pyrene; 2 = 1,3-dimethylpyrene.
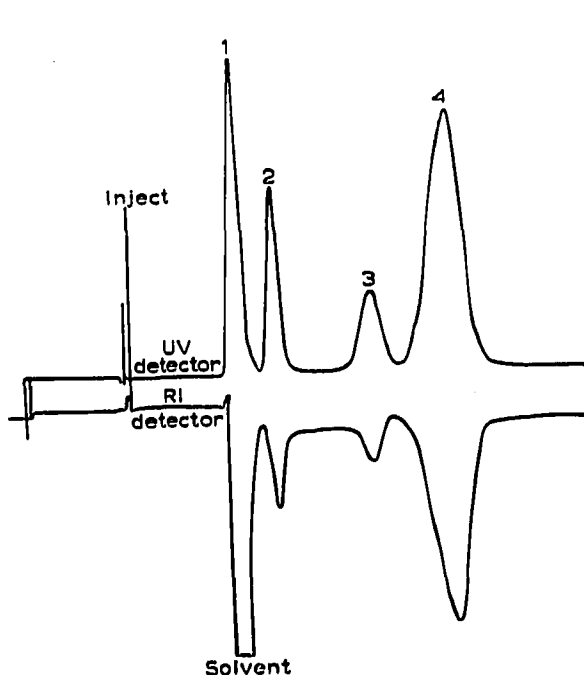
Fig. 4. Separation of benzene, cumene, diisopropylbenzene and diisopropyltoluene on Zipax ODS Solvent, methanol–water (65:35); temperature, 60°. 1 = Benzene; 2 = cumene; 3 = diisopropylbenzene; 4 = diisopropyltoluene.
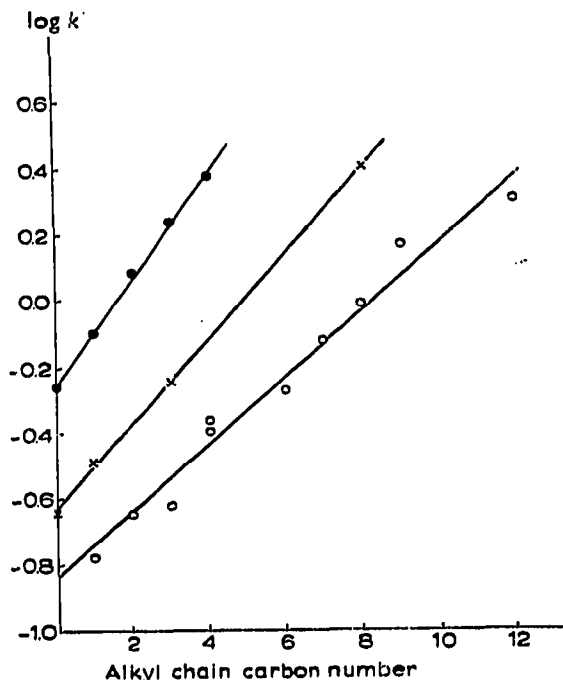
Fig. 5. Plot of log k' against alkyl side chain carbon number for alkylbenzenes (O), naphthalenes (×) and phenanthrenes (●) chromatographed on Zipax ODS. Solvent, methanol–water (7:3); temperature, 60°.

TABLE III

GRADIENT AND INTERCEPT VALUES OBTAINED FROM LEAST SQUARES FITS OF LOG k' AGAINST ALKYL CARBON NUMBER FOR ARALKYL HYDROCARBONS SUCH THAT LOG $k' = A + BC'$

C' is the alkyl side chain carbon number.

Aromatic nucleus	A	B
Benzene	−0.83	+0.100
Naphthalene	−0.63	+0.129
Phenanthrene	−0.25	+0.161

Decreasing the methanol content of the mobile phase increased retention. This was found to be more marked for compounds bearing alkyl substituents than for those which were unsubstituted (see Table IV). For example in methanol–water (7:3) unsubstituted anthracene elutes after 1,3-diisopropylbenzene. This order is reversed in methanol–water (6:4). An illustration of discrimination between varying degrees of unsaturation in hydrocarbons using reversed-phase chromatography has been made by Schmit[14].

TABLE IV

COMPARISON OF DEPENDENCE OF RETENTION ON SOLVENT COMPOSITION BE-
TWEEN ALKYLATED AND NON-ALKYLATED AROMATIC HYDROCARBONS ON
ZIPAX ODS

Temperature, 60°.

Compound	k' value	
	Methanol–water (7:3)	Methanol–water (6:4)
1,3-Diisopropylbenzene	0.54	3.63
Anthracene	0.67	2.82
2-Isopropylnaphthalene	0.57	3.03
Phenanthrene	0.55	2.42
2-Methylnaphthalene	0.32	1.45
Diphenyl	0.34	1.19

CONCLUSIONS

　　Reversed-phase LC of aromatic hydrocarbons on Zipax Permaphase ODS
with aqueous methanol mobile phases provides separations in which the position in
the chromatogram of a particular compound can be predicted from a knowledge of its
empirical formula. Retention of alkyl-substituted aromatic hydrocarbons is depen-
dent both on the aromatic and aliphatic carbon contents of the molecule. A route is
suggested to a more complete analysis of aromatic hydrocarbons in terms of aromatic
ring types and of alkyl substituents. Initially an aromatic class separation is required,
on a silica or alumina column. It has been suggested that the latter might be less
affected by the presence of alkyl substituents[4]. Rechromatographing these fractions
by reversed-phase chromatography should reveal the extent of alkylation within these
classes. Identification of the components eluting from the reversed-phase column can
be achieved up to a point using a combination of off-line mass and UV spectroscopy.
Problems remain, of deciding on response factors for quantitative work if UV de-
tectors are used. Clearly a more satisfactory detection system is desirable.

　　The behaviour of aromatic hydrocarbons with dissimilar ring structures but
the same carbon content, e.g. phenanthrene/anthracene, perylene/3,4-benzopyrene,
remains obscure. It would also be of interest, bearing in mind the limited number
of compounds examined to date, to know whether eqn. 3 (relating retention to the
extent of alkyl side chains) can be extended to aromatic systems other than those shown
in Table III (benzenes, naphthalenes and phenanthrenes).

ACKNOWLEDGEMENTS

REFERENCES

1 J. H. Weber and H. H. Oelert, Separ. Sci., 5 (1970) 669.
2 K. J. Bombaugh, R. F. Levangie, R. N. King and L. Abrahams, J. Chromatogr. Sci., 8 (1970) 657.

3 M. Popl, V. Dolansky and J. Mostecky, *J. Chromatogr.*, 59 (1971) 329.
4 M. Martin, J. Lohéac and G. Guiochon, *Chromatographia*, 5 (1972) 33.
5 D. E. Hirsch, R. L. Hopkins, H. J. Coleman, F. O. Cotton and C. J. Thompson, *Anal. Chem.*, 44 (1972) 915.
6 L. R. Snyder, *J. Chromatogr. Sci.*, 8 (1970) 692.
7 D. M. Jewell, J. H. Weber, J. W. Bunger and D. R. Latham, *Anal. Chem.*, 44 (1972) 1391.
8 M. Wilk, J. Rochlitz and H. Bende, *J. Chromatogr.*, 24 (1966) 414.
9 R. Gladen, *Chromatographia*, 5 (1972) 236.
10 R. E. Jentoft and T. H. Gouw, *Anal. Chem.*, 40 (1968) 923.
11 K. P. Hupe and H. Schrenker, *Chromatographia*, 5 (1972) 44.
12 D. M. Jewell, R. G. Ruberto and B. E. Davis, *Anal. Chem.*, 44 (1972) 2318.
13 J. A. Schmit, R. A. Henry, R. C. Williams and J. F. Dieckman, *J. Chromatogr. Sci.*, 9 (1971) 645.
14 J. A. Schmit, in J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley, New York, 1971, p. 394.