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LIQUID CHROMATOGRAPHIC RETENTION BEHAVIOR OF LARGE, FUSED POLYCYCLIC AROMATICS

NORMAL BONDED PHASES

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SUMMARY

The normal bonded-phase retention behavior of 26 large polycyclic aromatic hydrocarbons (PAHs) was investigated. The retention on aminopropyl-, nitropropyl-, and tetranitrofluorenoimidopropyl-derivatized columns were compared using *n*-hexane-methylene chloride mobile phases. The PAHs studied showed three distinct types of retention behavior. Changes in elution order are dependent on the degree of non-planarity of the compounds in the solvent systems used, as evidenced by the UV-VIS spectra.

INTRODUCTION

The analysis of crude oils or residua and their resulting products is a complex task. Complimentary methods are necessary for accurate characterization of a feed and the products of any process because many types of hydrocarbon- and heteroatom-containing molecules are present. One important class is the polycyclic aromatic hydrocarbons (PAHs). The separation of mixtures of PAHs by high-performance liquid chromatography (HPLC) has become a routine technique for identifying these components in many types of samples. Both normal- and reversed-phase separations have been used. A lack of standard compounds, for use in comparison of detector responses and retention times, has been one major limitation in development of PAH analyses. Another has been the complexity of most samples, making unequivocal identification of the chromatographic peaks difficult or impossible. These problems have been partially overcome by the synthesis and isolation of large PAHs, the production of columns with greater separating power, and the development of more selective detectors. The "full-spectrum" photodiode array detector is an ideal detector of PAHs. This detector collects the UV spectrum of an eluting peak by monitoring the UV intensity at all wavelengths in a spectral range. The UV spectra of the PAHs are very characteristic, containing several absorbance maxima and the corresponding minima. The location of these spectral features is determined by the size and shape of the PAH¹. Thus, very similar PAHs can be easily identified and quantified. These developments, however, do not totally solve the problems of separation and identification.

Other characteristics of the PAHs must be used to differentiate them. For example, the use of the retention times could be another identifying characteristic for those PAHs that cannot be unambiguously identified by their UV spectra. Retention time relationships have been proposed for both normal- and reversed-phase HPLC as a useful parameter for identification of the PAH²⁻⁵. These studies found correlations between the number of π electrons or rings and the expected retention times. Other parameters, such as the partition coefficients between aqueous and organic phases, solubilities in various solvents, and molecular shape, have all been used for predicting retention behavior (see ref. 6 and references contained therein, and ref. 7). All of these studies were of PAHs of six or less rings (the only ones readily available commercially). Recent work in our laboratory on the reversed-phase separation of several seven-, eight-, and nine-ring PAHs of the peropyrene class showed a much more complex behavior^{8,9}. The retention of these PAHs was based on solute-solvent interactions that were a function of the intramolecular steric strain of the PAHs. This strain would not be present in the smaller PAHs used in most studies, but would be more prevalent with an increase in the number of rings in the molecule¹⁰. Intramolecular steric strain produces anomalous retention behavior in the peropyrene-type PAHs because the effective surface area of the PAHs becomes smaller in certain solvents. The sterically strained PAHs could become non-planar in those solvents, reducing the strain but at the same time reducing the area of the PAH that interacted with the chromatographic stationary phase.

Since many PAHs appear to have structural features similar to those with anomalous retention behavior and also possess UV spectral features suggesting steric strain^{8,9}, a series of experiments was undertaken. While anomalous retention behavior had only been seen in reversed-phase separations, the proposed mechanism of a solvent-induced non-planarity should also occur in other types of chromatography, producing similar behavior for many PAHs besides those studied previously. The same "strong" solvents (ethyl acetate, tetrahydrofuran, chloroform, and methylene chloride) are used in both reversed- and normal-phase chromatography, so the solvent interactions with the PAHs should be similar in both types of chromatography. These were also the solvents which caused anomalous elution behavior and spectroscopic changes for the peropyrene-type PAHs. A number of 26 fused-ring PAHs (Fig. 1 and Table I) were chosen as standard compounds because of size and shape differences. These included various isomeric sets that differed in their UV spectral characteristics and possibilities for intramolecular strain. Three types of bonded normal-phase columns were studied: an aminopropyl-bonded phase, a nitropropyl-bonded phase and a tetranitrofluorenoimidopropyl (TENF)-bonded phase. These columns have greatly differing affinities and selectivities for PAHs, thereby allowing study of a wide range of mobile phases.

EXPERIMENTAL

All chromatograms were run on a DuPont Model 8800 liquid chromatograph in the isocratic mode at ambient temperature. A Valco C6U injection valve with a 25- μ l loop was used for sample introduction. A Hewlett-Packard 1040A photodiode array detector was used, and all data were evaluated with that unit's HP 85 computer. The software used was the original HP package expanded with Infometrix MCR 2

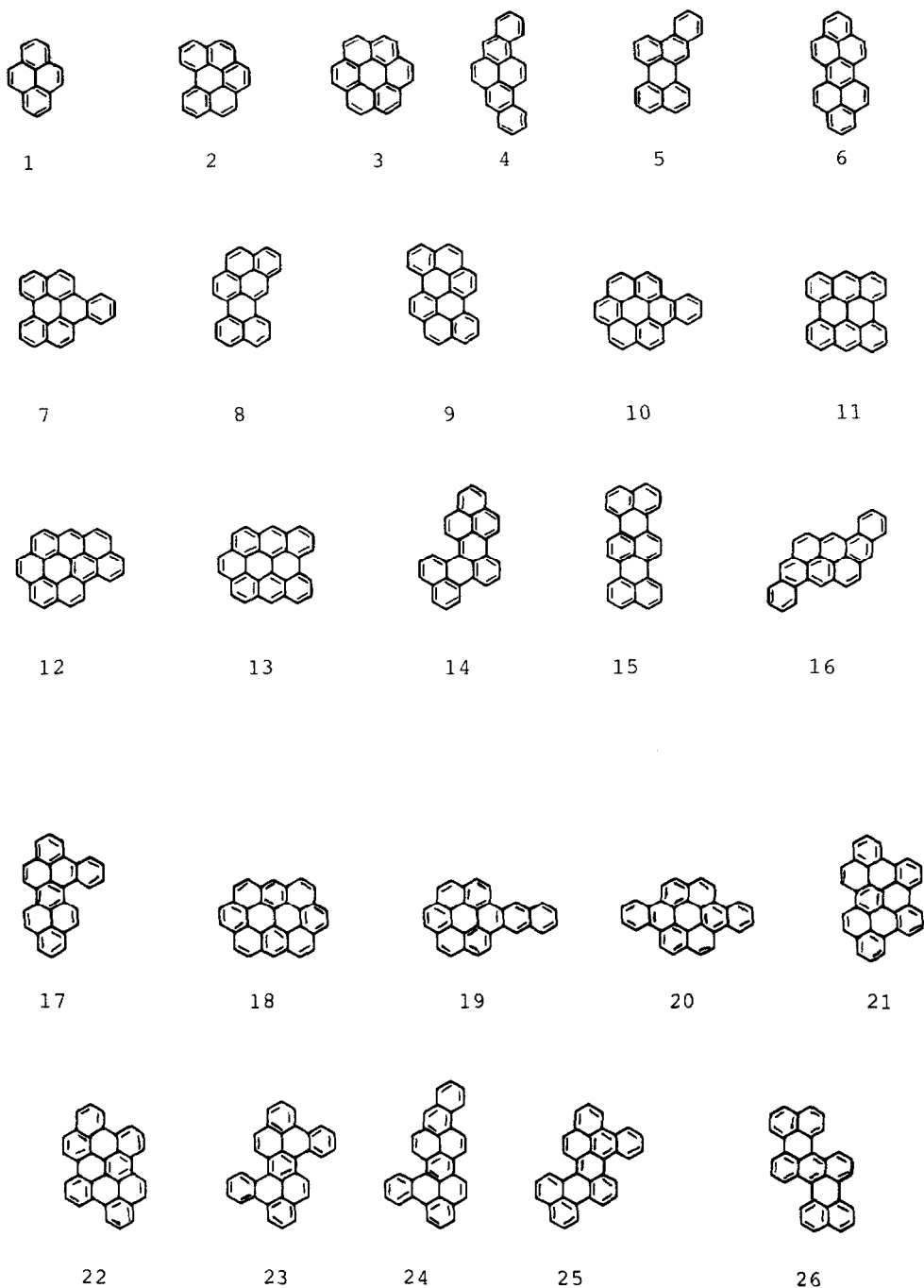


Fig. 1. PAHs studied (see Table I for names).

TABLE I
MODEL COMPOUNDS USED IN THIS STUDY

Compound number	Name
1	Pyrene
2	Benzo[ghi]perylene
3	Coronene
4	Benzo[rsi]pentaphene
5	Benzo[b]perylene
6	Dibenzo[cd,lm]perylene
7	Naphtho[1,2,3,4ghi]perylene
8	Naphtho[8,1,2bcd]perylene
9	Benzo[pqr]naphtho[8,1,2bcd]perylene
10	Benzo[a]coronene
11	Phenanthro[1,9,10,8fghi]perylene
12	Naphtho[8,1,2abc]coronene
13	Dibenzo[bc,ef]coronene
14	Phenaleno[1,9ab]perylene
15	Tribenzo[de,kl,rst]pentaphene
16	Pyranthrene
17	Tribenzo[a,cd,lm]perylene
18	Ovalene
19	Naphtho[2,3a]coronene
20	Dibenzo[a,j]coronene
21	Dibenzo[fg,ij]phenanthro[2,1,10,9,8,7pqrstuv]pentaphene
22	Benzo[cd]chryseno[4,5,6,7fghijk]perylene
23	Tetrabenzo[a,cd,j,lm]perylene
24	Benzo[rsi]phenanthro[1,10,9,cde]pentaphene
25	Benzo[cd]naphtho[1,8jk]perylene
26	Tetrabenzo[de,hi,op,si]pentacene

(Infometrix, Seattle, WA, U.S.A.). The solvent-effect comparison UV spectra were run on a Perkin-Elmer Lambda 3 spectrophotometer as in the previous work⁹.

The bonded-phase columns were a 25 × 0.46 cm I.D. DuPont aminopropyl-bonded phase, a 20 × 0.46 cm I.D. Macherey-Nagel nitropropyl-bonded phase, and a 30 × 0.46 cm I.D. ES Industries TENF-bonded phase. All packages were derivatized 5- μ m spherical silicas. All mobile phases were premixed using Burdick & Jackson solvents. The PAHs were either synthesized in our laboratory^{8,9} or purchased from various commercial sources. Three mixtures of eight or nine PAHs were dissolved in a minimum amount of methylene chloride and diluted with *n*-hexane, to ensure solubilization of the PAHs and to minimize adverse chromatographic effects. Relative retention times were measured as the average of the values found for each PAH at each set of chromatographic conditions, two or three replicate values being the norm. In the spectral comparisons, the PAHs were dissolved in methylene chloride and either diluted with nine parts *n*-hexane or methylene chloride.

RESULTS AND DISCUSSION

The use of plots of the logarithm of relative retention times *versus* percentage of strong solvent in the mobile phase can indicate changes in the various factors that

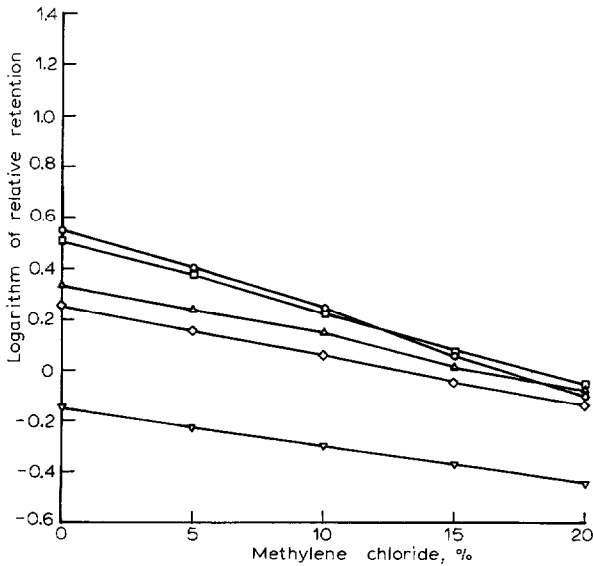


Fig. 2. Relative retention plots of the PAHs of molecular weights 202-302. ▽ = Pyrene; ◇ = benzo[ghi]perylene; △ = coronene; □ = benzo[rsi]pentaphene; ○ = benzo[b]perylene.

control retention^{11,12}. If the various factors that effect retention had the same mechanisms in each solvent mixture, then a linear plot would result. If there was a drastic change in the controlling mechanisms or in the relative influence of the mechanism, the plot would curve. The logarithm of the relative retention values found with *n*-hexane-methylene chloride mixtures on the aminopropyl-bonded phase column are

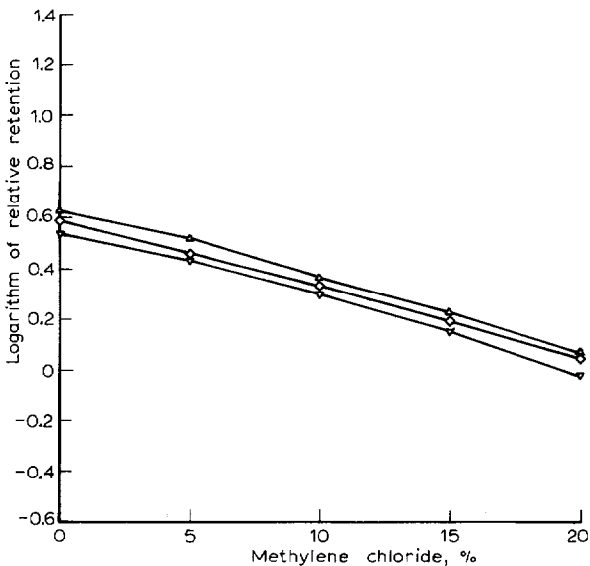


Fig. 3. Relative retention plots of the PAHs of molecular weight 326. ▽ = Dibenzo[cd,lm]perylene; ◇ = naphtho[1,2,3,4ghi]perylene; △ = naphtho[8,1,2bcd]perylene.

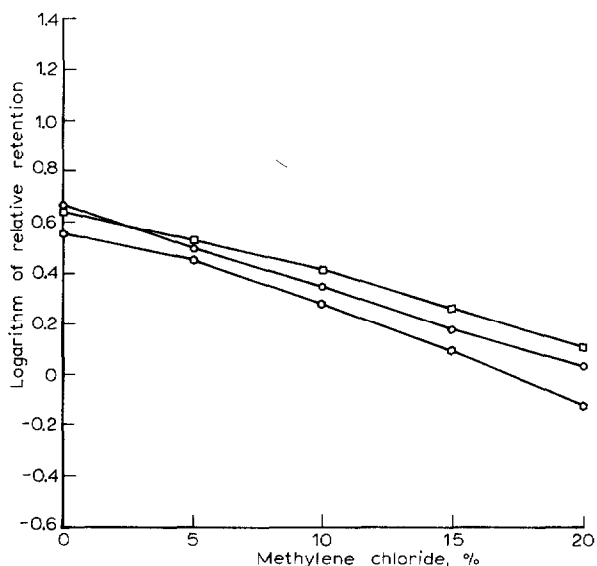


Fig. 4. Relative retention plots of the PAHs of molecular weight 350. □ = Benzo[*pqr*]naphtho[8,1,2*bcd*]perylene; ○ = benzo[*a*]coronene; ○ = phenanthro[1,9,10,8*fghij*]perylene.

shown in Figs. 2–8. The seven sets of data are divided into groups according to molecular weight to facilitate comparison of isomers or other PAHs of similar carbon number. From the prior work it was known that three types of behavior were possible: PAHs could always be either planar or non-planar or only become non-planar at higher concentrations of the strong solvent. All three were seen in this work.

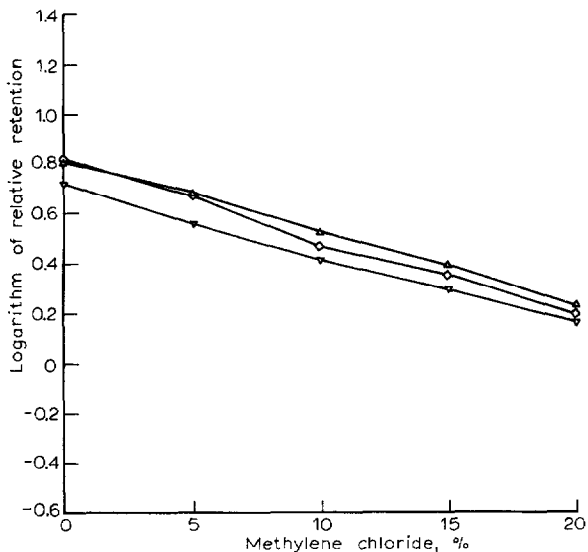


Fig. 5. Relative retention plots of the PAHs of molecular weight 374–376. ▽ = Naphtho[8,1,2*abc*]coronene; ◇ = dibenzo[*bc,ef*]coronene; △ = phenaleno[1,9*ab*]perylene.

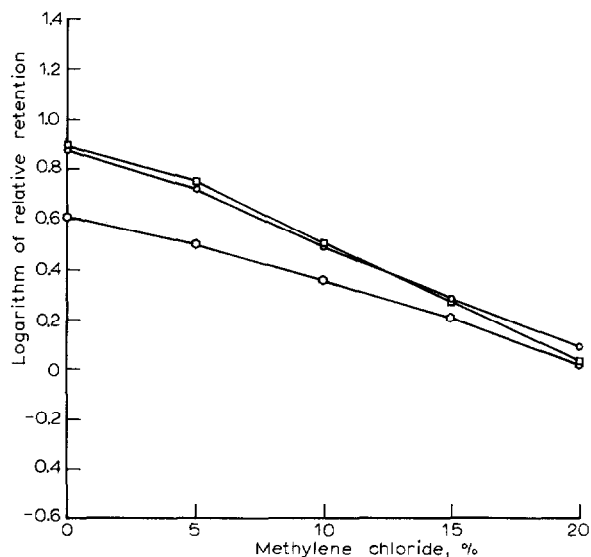


Fig. 6. Relative retention plots of the PAHs at molecular weight 376. □ = Tribenzo[de,kl,rst]pentaphene; ○ = pyranthrene; ◇ = tribenzo[a,cd,lm]perylene.

Most of the molecules appeared to remain in a planar conformation in all the solvent mixtures, as evidenced by their approximately linear plots. Their UV spectra had shifts in the band positions, but no changes in band shape or the number of bands. Coronene and ovalene (compounds 3 and 18) behaved this way. In contrast, the plots for some of the PAHs showed some curvature. Benzo[rs]t]pentaphene and

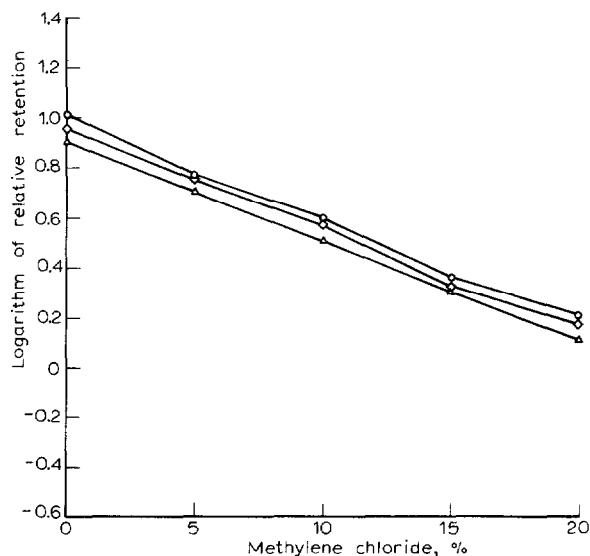


Fig. 7. Relative retention plots of the PAHs of molecular weights 398-400. △ = Ovalene; ◇ = naphtho[2,3a]coronene; ○ = dinbenzo[a,j]coronene.

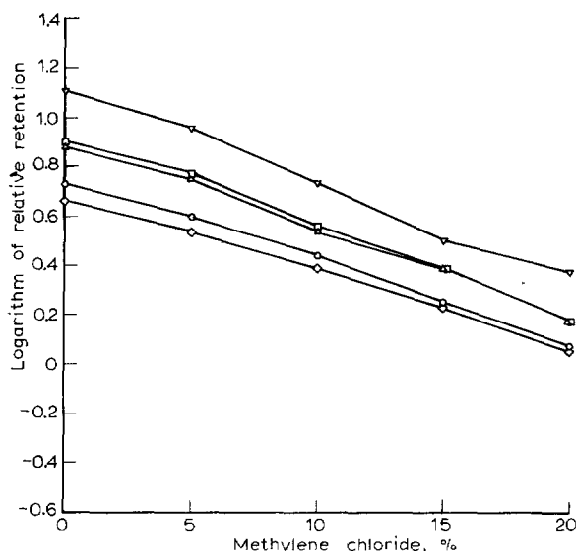


Fig. 8. Relative retention plots of the PAHs of molecular weights 424-426. ∇ = Benzo[*cd*]chryseno[4,5,6,7*fghijk*]perylene; \diamond = tetrabenzo[*a,cd,j,lm*]perylene; \triangle = benzo[*rsf*]phenanthro[1,10,9,*cde*]pentaphene; \square = dibenzo[*a,cd*]naphtho[1,8,*jk*]perylene; \circ = tetrabenzo[*de,hi,op,st*]pentacene.

benzo[*b*]perylene (compounds 4 and 5, Fig. 2), at a molecular weight of 302, are an example of the different types of behavior seen for an isomeric pair. Benzo[*b*]perylene had a significantly curved plot, while benzo[*rsf*]pentaphene gave an almost linear one. A curve plot was also observed for several of the other PAHs.

Another important trend was observed with tetrabenzo[*a,cd,j,lm*]perylene and tetrabenzo[*de,hi,op,st*]pentacene (compounds 23 and 26, Fig. 8). These molecules appeared to be so sterically strained that even in *n*-hexane they were non-planar. Their retention times were drastically smaller than other PAHs of similar molecular weight, but their unchanging non-planar conformation in all the solvent mixtures produced an approximately linear plot. The only way of identifying this type of behavior was comparison to other PAHs of the same carbon number. The non-planar PAHs' retentions were much less than the planar PAHs of the same carbon number. By contrast to those two strained, non-planar PAHs, compound 22 (with the same number of carbons) eluted much later. The two isomers of molecular weight 424, compounds 21 and 22, had identical retention behavior within experimental error; so only the line for compound 22 is shown in Fig. 8 for the two compounds.

In general, those PAHs whose UV spectra had shallow valleys between their highest wavelength absorbance bands appeared to elute earlier than expected. A correlation between these properties can be made. A parameter called the "shallowness factor" for a PAH was defined as the ratio of the average of the intensities of the two highest wavelength absorbance maxima and the intensity of the corresponding absorbance minimum. As the value of shallowness factor approached one, the spectral valley became smaller. The values for a particular set of PAHs in a particular solvent mixture can be compared with their relative retentions from an isocratic chromatographic run using the same solvent mixture. This experiment was performed

TABLE II
CORRELATION OF RETENTION TIME AND SHALLOWSNESS FACTOR

Compound number	Relative retention	Shalowness factor
22	2.399	3.27
25	1.510	2.21
24	1.484	2.10
26	1.192	1.42
23	1.148	1.35

using spectra collected both on-the-fly, using the photodiode array detector during the separations, and statically with solutions of the individual PAHs in the different solvent mixtures. The two experimental approaches yielded similar values, but the values from the static measurements were much more precise. Table II is a comparison between the relative retention times in *n*-hexane–methylene chloride (90:10) for the 34-carbon PAHs and the shallowness factor values found statically in the same solvent mixture. The shallowness factor values are precise to 0.02 units. It was seen that the shallower PAHs elute the earliest. To a lesser degree, this trend was also seen for the other sets of PAHs.

In our earlier reversed-phase work, a PAH was found that had spectral differences in weak and strong solvents⁹. The absorbance band for benzo[*rst*]phenanthro[1,10,9*cde*]pentaphene (compound 24), around 475 nm, underwent a band-shape change so that rather than the one large absorbance band seen in methylene chloride, the compound in *n*-hexane or methanol showed two smaller ones. In the current work, similar comparison spectra were obtained for another five of the PAHs. These were chosen because they had the greatest anomalous elution behaviors for PAHs that had not been studied in our earlier work. The two solvent mixtures used for the spectral comparisons were *n*-hexane–methylene chloride (90:10) and 100% methylene chloride. These PAHs showed two types of differences when the spectra in the two solvent mixtures were compared. First, the depth of the valley

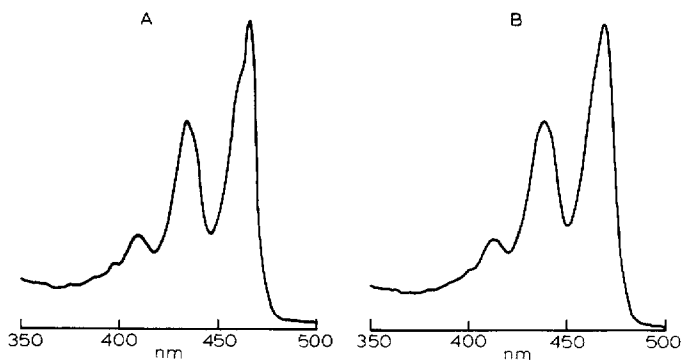


Fig. 9. Comparison spectra of naphtho[8,1,2*bcd*]perylene showing the changes in band-shape and shallowness with higher methylene chloride concentrations: (A) *n*-hexane–methylene chloride (90:10) and (B) 100% methylene chloride.

between the two highest wavelength absorbance bands was less in methylene chloride than in *n*-hexane. Secondly, some of these PAHs showed varying degrees of band shape changes similar to those seen previously with compound 24. The spectra of naphtho[8,1,2*bcd*]perylene, compound 8, are shown in Fig. 9. The change in the shallowness of the minimum, as well as the band shape changes, is seen. The shallowness factors for these six PAHs in the two solvents are shown in Table III. The values seen in methylene chloride were consistently lower than the values found in the predominantly *n*-hexane solution. This was only seen for the PAHs that had anomalous elution behavior. The values for the PAHs that had sharper absorbance bands and "normal" elution behavior, such as coronene or ovalene, differed by 0.008 units or less for the two solvents, with the greater value not always being the *n*-hexane one.

In addition to these spectral and chromatographic traits, these PAHs were also more soluble in *n*-hexane than isomers that had sharper spectral bands and longer retention times. This would also support the premise that these molecules are non-planar because they would also be less ordered crystallographically than the planar PAHs, if the solid state structure is similar to the solvated structure.

For the aminopropyl column, the three types of solution behavior of these PAHs caused any single isomeric set to have a wide range of retention times. Large overlaps with the other isomeric sets resulted. Thus any prediction of retention times by the number of rings or π electrons or by molecular shape were not possible.

This division of PAHs into classes based on the degree of planarity in solution is similar to the divisions made by Boschi *et al.*¹³ in their photoelectron spectroscopic study of the gas phase behavior of PAHs. The width of the photoelectron transitions were broader in sterically strained PAHs than those in the spectra of unstrained PAHs.

The nitropropyl-bonded packing column was more retentive than the aminopropyl-bonded phase; so slightly higher concentrations of methylene chloride could be used. The nitropropyl phase had an almost identical selectivity for separating isomers compared to the aminopropyl phase. Small differences in retention order, however, existed. This was seen especially at the higher methylene chloride concentrations. With the nitropropyl phase and its higher concentrations of methylene chloride, two pairs of closely eluting compounds reversed elution order compared to that seen with the aminopropyl phase. The ratios of the relative retention values in the various solvent mixtures for these pairs on the nitropropyl column are shown in

TABLE III
COMPARISON OF SHALLOWSNESS FACTOR IN DIFFERENT SOLVENTS

Compound number	<i>n</i> -Hexane	Methylene chloride
5	2.12	1.91
8	2.85	2.56
11	1.64	1.32
15	2.73	2.69
24	2.13	2.02
26	1.44	1.32

TABLE IV
RELATIVE RETENTION RATIOS INDICATING ELUTION ORDER CHANGES

Methylene chloride (%)	Compounds 7 and 8	Compounds 12 and 14	Compounds 15 and 16
5	0.943	0.959	0.889
10	0.971	1.016	0.936
15	1.002	1.052	0.953
20	1.066	1.087	0.987
25	1.093	1.115	1.010

Table IV. Values are also given for a third pair (compounds 7 and 8) which showed an elution order change on both columns. With lower methylene chloride concentrations, the elution order with the nitropropyl phase for all three pairs was the same as those seen with the aminopropyl phase. Since the ratios were less than one at lower concentrations and then became greater at higher concentrations, a change in elution order occurred that was caused by the use of greater proportions of methylene chloride possible with the nitropropyl column. Each of the PAHs that had been the later eluting compound at lower methylene chloride concentrations had a shallower spectrum than the earlier eluting one, indicating a greater susceptibility towards a non-planar structure. At higher concentrations of strong solvent, they went non-planar and eluted earlier than the other member of the pair.

The TENF column was extremely retaining, allowing runs at 100% methylene chloride. Only about half of the compounds eluted, even under these conditions. Those that did elute showed an order that paralleled our earlier reversed-phase studies. The highly strained nine-ring PAHs tetrabenzo[*a,c,d,j,lm*]perylene and tetrabenzo[*de,hi,op,st*]pentacene (compounds 23 and 26) eluted before the much smaller benzo[*ghi*]perylene (compound 2). This was the most extreme example of the solvated structure effecting elution behavior, but compounds 5, 6, 8, 11, 14, 15, 17, 24, and 25 also showed earlier elution than expected when compared to isomers that are not sterically strained.

CONCLUSION

For each of the three columns examined, the solvent induced non-planarity of some PAHs caused the retention time range of an isomer set to be very large. The overlap between the isomer sets was then great enough to make any correlations between the number of π electrons or rings and the retention times impossible. Thus the use of parameters, such as the length-to-breadth ratio, appears to be valid only for the planar PAHs. The wide range of elution behavior was caused by three apparent types conformational behavior: (1) PAHs that were planar in all solvent mixtures and had predictable retention times that correlated with their ring number, (2) PAHs that were non-planar in all solvent mixtures and eluted much earlier than expected when compared to the planar PAHs, and (3) some PAHs which changed their degree of planarity with solvent composition so that their retention times could not be predicted by comparison to the planar PAHs. The degree of this non-planarity, and thus

the degree of earlier-than-expected chromatographic elution, was evidenced by the shallowness of the UV absorbance minima in the spectra of the PAHs. This amount of spectral shallowness changed with solvent in parallel fashion to the retention behavior changes. The degree of earlier-than-expected elution or the changes in elution order could be estimated by the UV spectra of the PAHs.

The use of gradient elution in PAH analyses must be carefully evaluated. The changing solvent composition can have a dramatic effect on retention order; so the identification of each peak must be verified. Single-wavelength detectors would not easily follow these changes. The separation of two or more closely eluting peaks could be accomplished by either a weaker or a stronger solvent mixture as well as the choice of different solvents, depending on which classes of PAHs are present.

The results from this new series of chromatographic experiments indicate that the use of retention time alone as an identifying parameter in HPLC for the larger PAHs is not valid, but its use for comparison to standard material would be appropriate if the reproducibility of the mobile phase composition is high enough to control the degree of non-planarity induced by the mobile phase. Primary identification of the PAHs must still be by comparison to standards and by spectral characterization.

ACKNOWLEDGEMENT

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REFERENCES

- 1 E. Clar, *The Aromatic Sextet*, Wiley-Interscience, New York, 1972.
- 2 R. B. Sleight, *J. Chromatogr.*, 83 (1973) 31.
- 3 S. A. Wise, W. J. Bennett and W. E. May, in A. Bjorsted and A. J. Dennis (Editors), *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*, Batelle Press, Columbus, OH, 1980, p. 791.
- 4 M. Novotny, M. Lee and K. Bartle, *J. Chromatogr. Sci.*, 12 (1974) 606.
- 5 M. Novotny, in E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci. Publ., Ann Arbor, MI, 1974, p. 199.
- 6 S. A. Wise, W. J. Bonnett, F. R. Guenther and W. E. May, *J. Chromatogr. Sci.*, 19 (1981) 457.
- 7 K. Jinno and M. Okamoto, *Chromatographia*, 18 (1984) 495.
- 8 J. C. Fetzer and W. R. Biggs, *J. Chromatogr.*, 295 (1984) 161.
- 9 J. C. Fetzer and W. R. Biggs, *J. Chromatogr.*, 322 (1985) 275.
- 10 E. Clar, *Polycyclic Hydrocarbons*, Vol. 1, Academic Press, New York, 1964, Ch. 16.
- 11 J. L. Glajch, E. I. du Pont de Nemours and Company, personal communication, 1985.
- 12 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd Edition, Wiley-Interscience, New York, 1979, pp. 587-588.
- 13 R. Boschi, E. Clar and W. Schmidt, *J. Chem. Phys.*, 60 (1974) 4406.