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RETENTION BEHAVIOR OF LARGE POLYCYCLIC AROMATICS IN BONDED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The retention behavior of 31 large polycyclic aromatic hydrocarbons was studied on polymeric octadecyl-bonded columns, with methanol–dichloromethane mobile phases. Three types of elution behavior were observed, dependent on the degree of intramolecular strain in the polycyclic aromatic hydrocarbons.

INTRODUCTION

High-performance liquid chromatography (HPLC) has become one of the preferred methods for the separation of polycyclic aromatic hydrocarbons (PAHs) because it does not suffer many of the limitations that have plagued other techniques. Because HPLC is not limited, for example, by the low volatility of PAHs like gas chromatography is, compounds with more than six rings can be analyzed. When the appropriate columns and detectors are used, HPLC can also differentiate the large number of isomers possible for the same molecular formulae, which mass spectrometry cannot do. Even with these advantages, the extreme complexity of many sample types has limited their complete characterization by HPLC. Recent advances, including higher-resolution columns and more specific detectors, have partially overcome this limitation. The full-spectrum ultraviolet–visible absorbance or fluorescence detectors, such as those utilizing a photodiode array, are ideal for PAH detection since the spectrum of each PAH isomer has enough characteristic maxima and minima to form a unique pattern, allowing identification and differentiation from the other possible isomers[1].

However, these instrumental advances have still not permitted the complete characterization of the most complex samples. There are so many possible structures for PAHs of a particular carbon number that the lack of standard compounds has also been a severe limitation. Even though many more standards have recently become available, there are still not enough to identify every peak in many chromatograms or to confirm or exclude the occurrence of every possible PAH isomer. The retention times of known PAHs could be used as an additional characterization parameter in the identification of unknown peaks, since the behavior of known compounds that are eluted in a similar retention time range could be a clue to the structure of unknown peaks. This approach has been suggested by several research groups

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who have proposed relationships between the retention times and the number of rings or formal double bonds or the planar shape of the PAHs[2–4]. Those studies dealt only with the smaller PAHs of six rings or less, since they were the only ones readily available from commercial sources. However, recent work in our laboratory has shown that these types of relationships are not valid for one class of larger PAHs, *i.e.*, the peropyrene-type PAHs, dibenzo[*cd,lm*]perylene, and its benzologues of seven to nine rings[5,6].

To determine whether this anomalous elution behavior also occurred in other classes of PAHs, a series of experiments was performed to examine the retention behavior of a more varied group of PAHs. An earlier communication covered the results found for several normal bonded-phase columns with solvent mixtures of *n*-hexane and dichloromethane[7]. The predominant factor in retention strength was found to be the amount of intramolecular strain and the resulting degree of non-planarity of each PAH structure in the various solvent mixtures. It was found that the UV–visible absorbance spectra of the PAHs had progressively shallower minima between absorbance maxima as the steric strain and, thus, the non-planarity increased. Increased non-planarity also resulted in earlier-than-expected chromatographic elution when compared to other planar PAHs of the same carbon number. This report deals with the bonded-phase HPLC of the same set of 26 PAHs as well as 5 additional isomeric PAHs which had been recently synthesized or acquired.

The bonded-phase columns chosen for this study contained polymeric-bonded stationary phases since these have been shown to have the greatest resolving power for PAH isomers[8,9].

EXPERIMENTAL

As in the previous studies[5–7], this work was performed with a Du Pont Instruments Model 8800 quaternary solvent HPLC system. The unit was operated in the isocratic mode using only one solvent inlet. A Hewlett-Packard 1040A photodiode-array detector was used, and data were evaluated on a Hewlett-Packard HP 85 personal computer using the MCR-2 expanded version of the original Hewlett-Packard software. This upgraded software was purchased from Infometrix (Seattle, WA, U.S.A.). Data were stored for postrun evaluation on flexible disks using a Hewlett-Packard 82901M disk drive. The eight chromatographic signals that the detector monitored and stored were from 305 to 480 nm, in 25-nm increments, and spectra of the peaks were collected from 250 to 600 nm. A Valco C6U injector with a 10-μl loop was used for sample introduction. All mobile phase mixtures (consisting of methanol and dichloromethane) were premixed using Burdick & Jackson HPLC-grade solvents.

Approximately 30 types of commercial octadecyl-bonded stationary phases were evaluated for retention capability. In agreement with earlier work[8,9], the polymeric phases were superior to the monomeric ones. Using a definition of the "best" columns as those which had the ability to separate selected isomeric pairs of similar structure, such as dibenzo[*cd,lm*]perylene and naphtho[8,1,2*bcd*]perylene, it was found that the best were those packed with Vydac 218TP5 (Separations Group, Hesperia, CA, U.S.A.). Column dimensions were 25 cm × 0.46 cm I.D. This type of column was used for the remainder of the study.

The structures of the 31 PAHs used are shown in Fig. 1, and the appropriate IUPAC names are given in Table I. All were either synthesized in our laboratory[5–7] or purchased from a variety of suppliers (primarily from Dr. Werner Schmidt, Ahrensberg, F.R.G.). Two PAHs benzo[*lm*]phenanthro[4,5,6*abcd*]perylene and tetrabenzo[*a,cd,f,lm*]perylene (compounds 21 and 31), were the products of new syntheses,

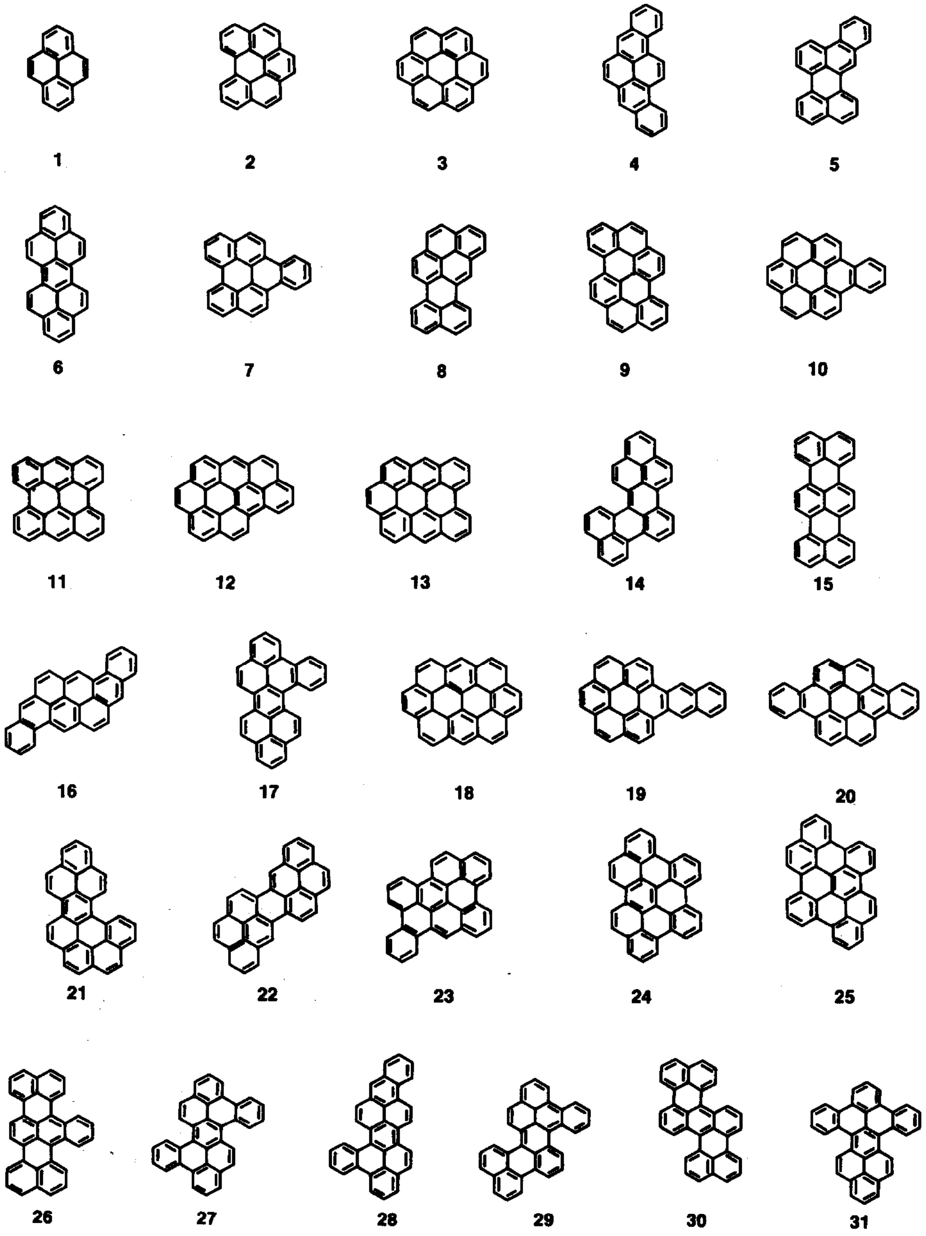


Fig. 1. The structures of the polycyclic aromatic hydrocarbon standards used.

TABLE I

IUPAC NAMES FOR THE 31 POLYCYCLIC AROMATIC HYDROCARBONS STUDIED

Compound	*IUPAC name*
1	Pyrene
2	Benzo[*ghi*]perylene
3	Coronene
4	Benzo[*rst*]pentaphene
5	Benzo[*b*]perylene
6	Dibenzo[*cd,lm*]perylene
7	Naphtho[1,2,3,4*ghi*]perylene
8	Naphtho[8,1,2*bcd*]perylene
9	Benzo[*pqr*]naphtho[8,1,2*bcd*]perylene
10	Benzo[*a*]coronene
11	Phenanthro[1,9,10,8*fghij*]perylene
12	Naphtho[8,1,2*abc*]coronene
13	Dibenzo[*bc,ef*]coronene
14	Phenaleno[1,9*ab*]perylene
15	Tribenzo[*de,kl,rst*]pentaphene
16	Pyranthrene
17	Tribenzo[*a,cd,lm*]perylene
18	Ovalene
19	Naphtho[2,3*a*]coronene
20	Dibenzo[*a,j*]coronene
21	Benzo[*lm*]phenanthro[4,5,6*abcd*]perylene
22	Dinaphtho[2,1,7,8*defg*,2',1',7',8'*opqr*]pentacene
23	Dibenzo[*cd,k*]naphtho[8,1,2,3*fghi*]perylene
24	Dibenzo[*fg,ij*]phenanthro[2,1,10,9,8,7*pqrstuv*]pentaphene
25	Benzo[*cd*]chryseno[4,5,6,7*fghijk*]perylene
26	Dibenzo[*de,kl*]naphtho[1,2,3,4*rst*]pentaphene
27	Tetrabenzo[*a,cd,j,lm*]perylene
28	Dibenzo[*a,rst*]naphtho[8,1,2*cde*]pentaphene
29	Dibenzo[*j,lm*]naphtho[1,8*ab*]perylene
30	Tetrabenzo[*de,hi,op,st*]pentacene
31	Tetrabenzo[*a,cd,f,lm*]perylene

analogous to those previously employed in the production of the peropyrene-type PAHs. The five-ring ketones, naphthanthrone and dibenzoperinaphthenone (acquired from Dr. Schmidt), replaced the four-ring benzathrone in the condensation reaction with perinaphthenone. The isolation and characterization of these new PAHs was identical with that used in our earlier syntheses. Benzo[*lm*]phenanthro[4,5,6*abcd*]perylene is a nine-ring compound (molecular weight 400), forming yellowish-orange platelets when recrystallized from dichloromethane and acetonitrile. Its spectral characteristics are UV–visible absorbance maxima at 346, 385, 410, 434, and 478 nm in dichloromethane, with an intense, green fluorescence. Tetrabenzo[*a,cd,f,lm*]perylene is also a nine-ring molecule of molecular weight 426. It forms yellow platelets when recrystallized from dichloromethane and acetonitrile, has absorbance maxima in dichloromethane at 307, 333, 346, 395, 420, and 444 nm, and has a pale-blue fluorescence[10].

Four standard mixtures, each containing seven to nine PAHs, were used for the measurement of retention times. These mixtures were prepared by dissolving the PAHs in a minimum amount of dichloromethane and diluting with methanol. This procedure was necessary since many of the PAHs were not soluble in methanol alone, but using dichloromethane as the sample solvent produced unsymmetrical chromatographic peaks (this was especially true for the earliest eluted PAH). These non-Gaussian shapes made accurate measurement of the retention times difficult. The concentration of dichloromethane was estimated to be 20–40% in these solutions. The concentration of each PAH was 0.1–1.0 mg per 10 ml. The specific isomers of a particular carbon number were chosen by examination of their UV–visible absorbance spectra and structures so there would be a variety of both planar and non-planar PAHs in each solution. The relative retention times were calculated from the average of the values from three replicate runs. Relative retention time was defined as the corrected retention time (retention time minus void time) divided by the void time.

RESULTS AND DISCUSSION

The plots of the logarithms of the relative retention values *versus* dichloromethane concentration are shown as Figs. 2–7. The data were divided into these six

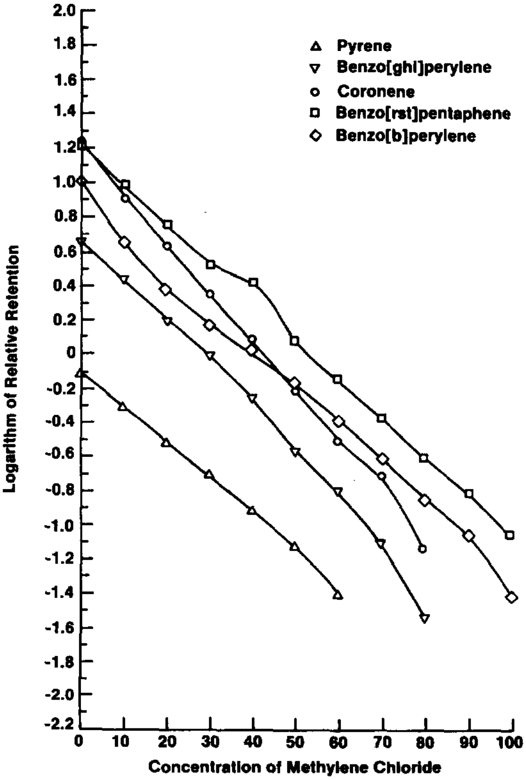
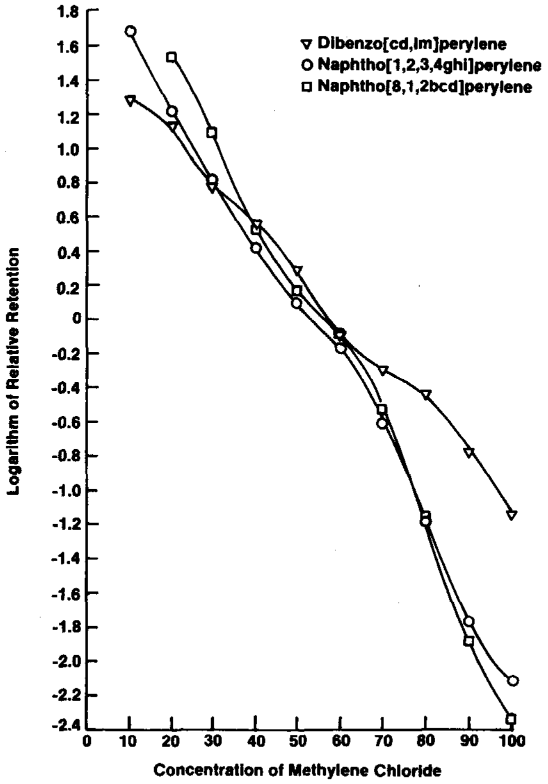


Fig. 2. Plots of the logarithm of the relative retention *versus* percentage dichloromethane for the hydrocarbons with 24 or fewer carbons.



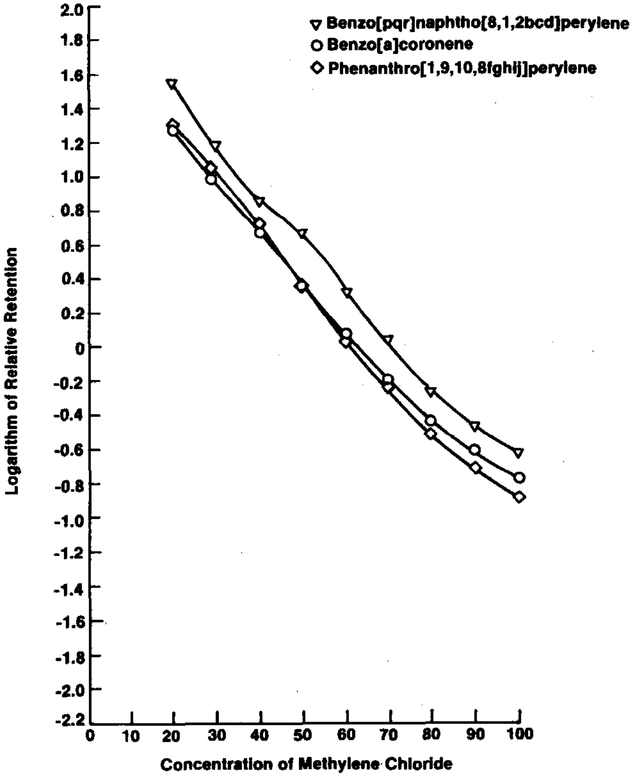


Fig. 3. Plots of the logarithm of the relative retention *versus* percentage dichloromethane for the hydrocarbons with 26 carbons.

Fig. 4. Plots of the logarithm of the relative retention *versus* percentage dichloromethane for the hydrocarbons with 28 carbons.

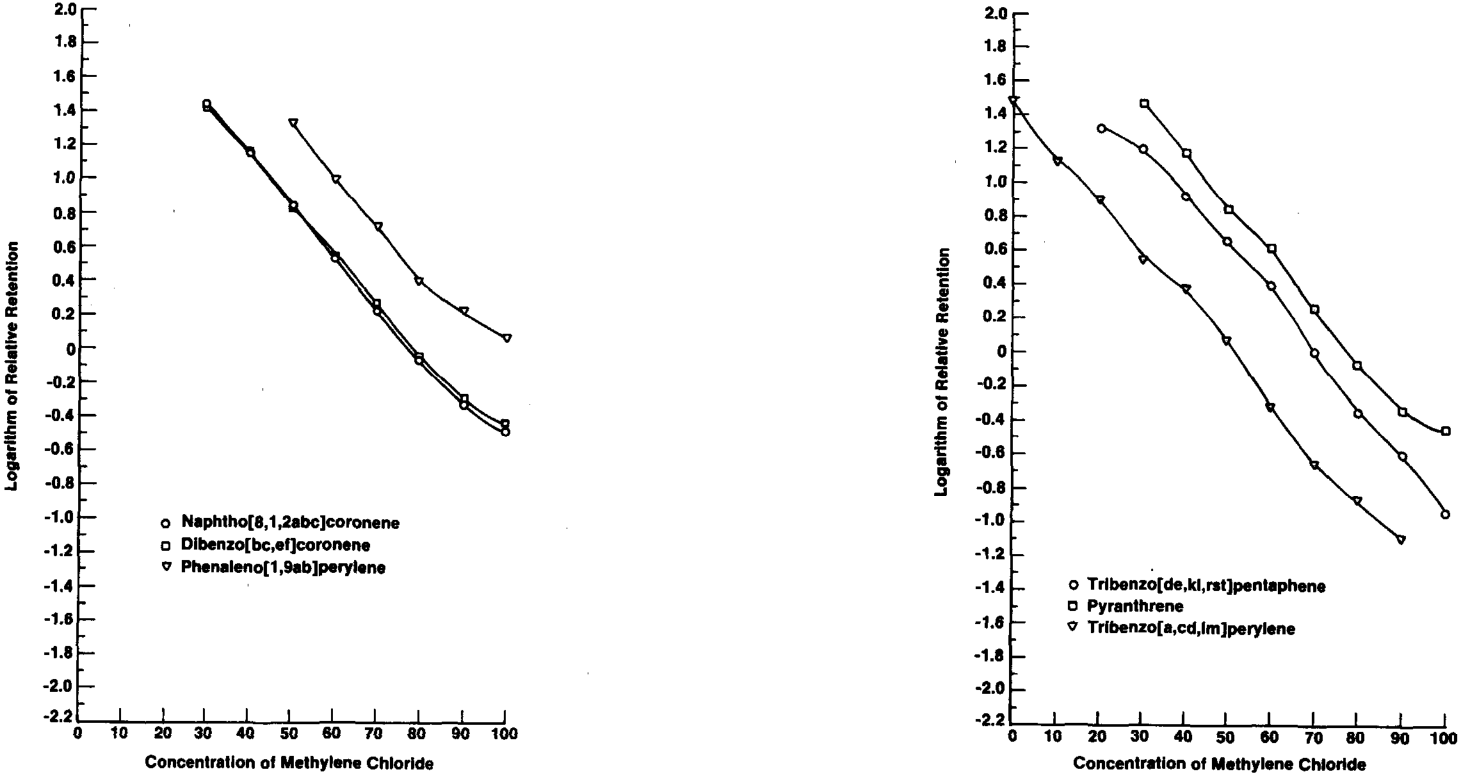


Fig. 5. Plots of the logarithm of the relative retention *versus* percentage dichloromethane for the hydrocarbons with 30 carbons.

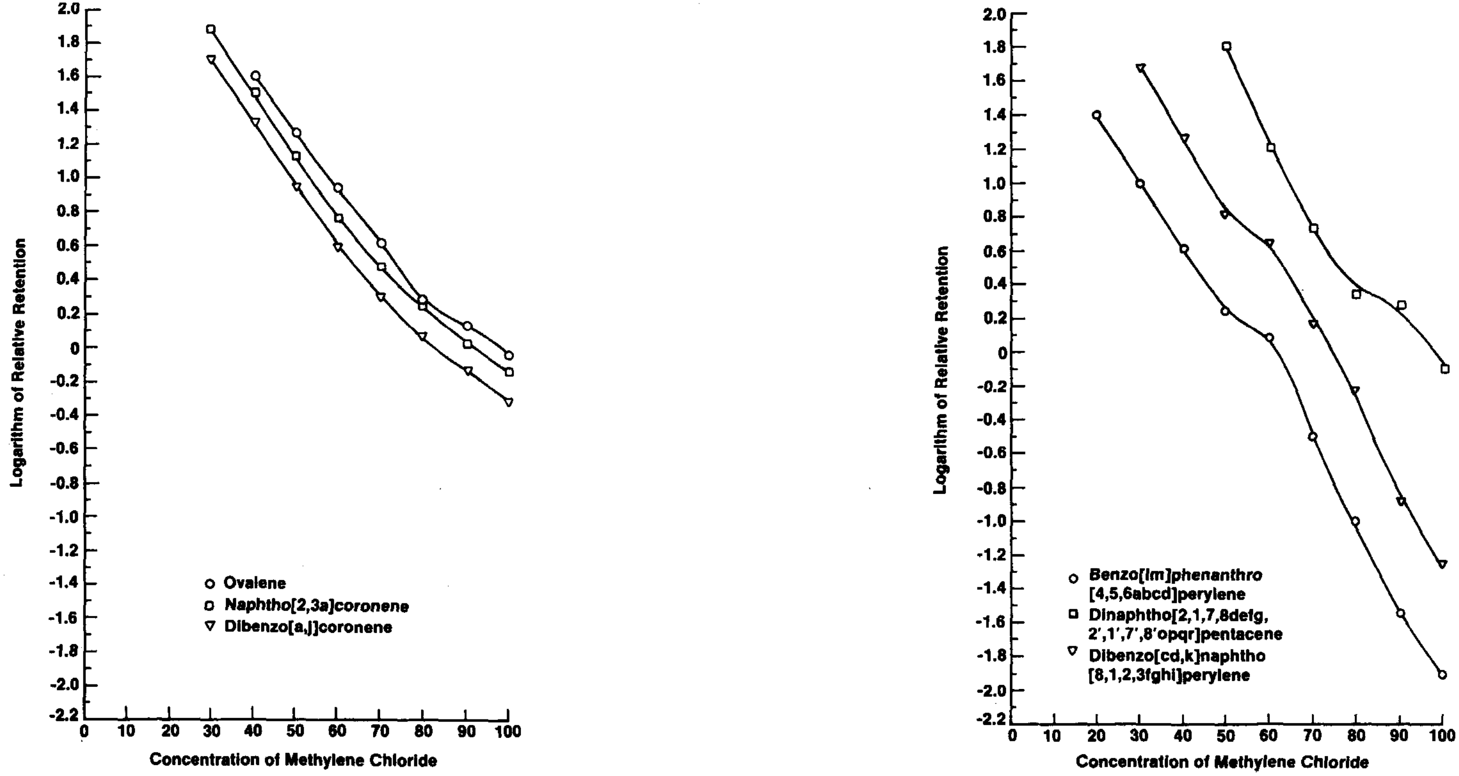


Fig. 6. Plots of the logarithm of the relative retention *versus* percentage dichloromethane for the hydrocarbons with 32 carbons.

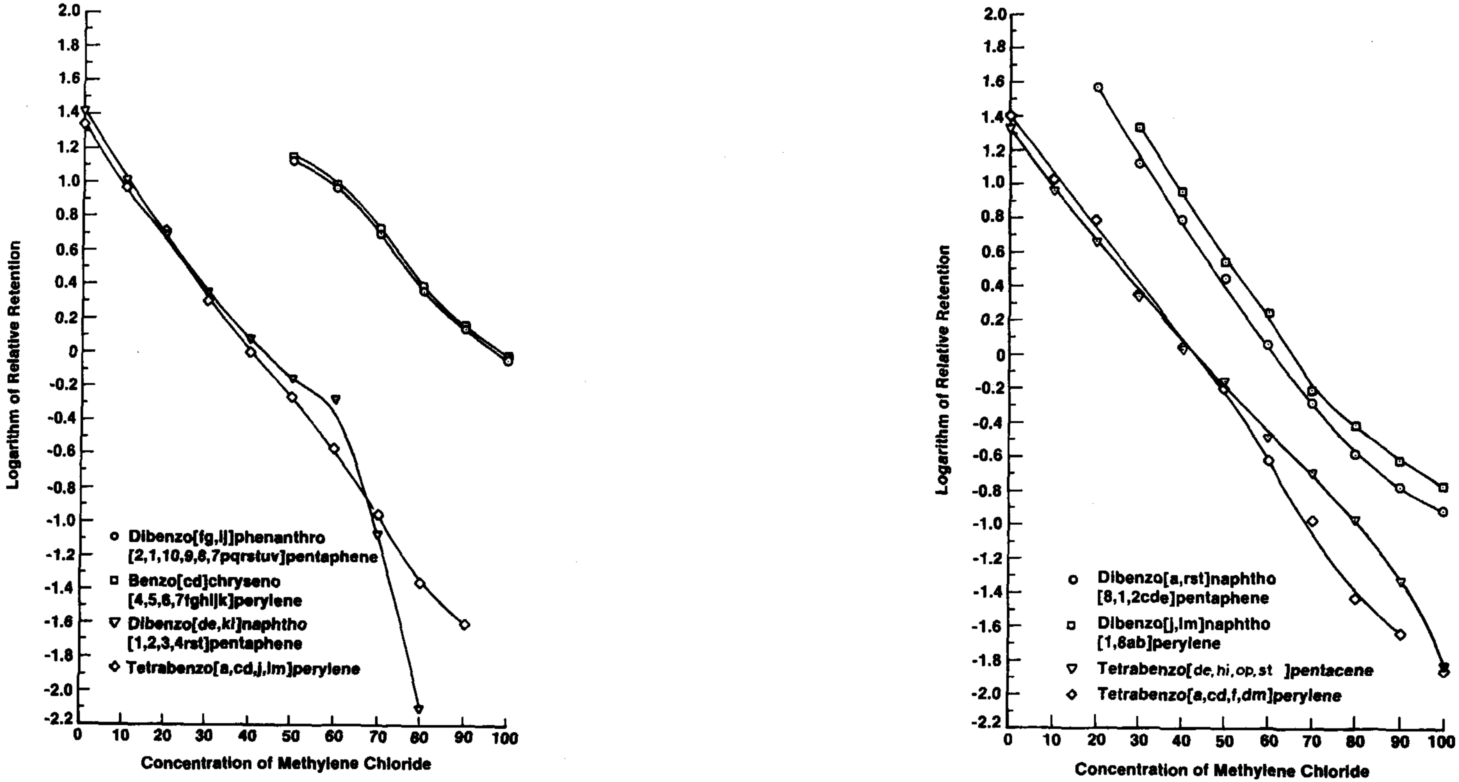


Fig. 7. Plots of the logarithm of the relative retention *versus* percentage dichloromethane for the hydrocarbons with 34 carbons.

sets so that comparisons could be easily made between isomers or PAHs with the same number of carbons.

As was seen in our earlier work on bonded-phase HPLC of the peropyrene-type PAHs[5,6], several of the PAHs studied in this work were eluted much earlier than other PAHs with equivalent carbon numbers. The most extreme examples (Fig. 7) were dibenzo[*de,kl*]naphtho[1,2,3,4*rst*]pentaphene, tetrabenzo[*a,cd,j,lm*]perylene, tetrabenzo[*de,hi,op,st*]pentacene, and tetrabenzo[*a,cd,f,lm*]perylene (compounds 26, 27, 30, and 31) which were eluted much earlier than the other PAHs with 34 carbons. This behavior was similar to that seen in our earlier normal-phase chromatograms[7].

In addition to the earlier-than-expected elution seen for the 34-carbon PAHs, the compounds with 32 carbons also showed a wide range of retention times (Fig. 6). In our earlier studies, only three PAHs of this carbon number were available (ovalene, dibenzo[*a,j*]coronene, and naphtho[2,3*a*] coronene. All three of these compounds appeared to be planar from their chromatographic and spectrometric behaviors in normal-phase chromatography and, therefore, their elution behavior was not expected to be different on reversed-phase packings. Three different PAHs of that carbon number were added to the set of model compounds for this work (compounds 21–23). These were of much different shapes and expected degrees of intramolecular steric strain (as evidenced by characteristics in their UV–visible spectra). They were, therefore, added to the standard sets to see whether these spectral properties would be reflected in anomalous chromatographic behavior.

These two carbon-number groups of PAHs had such wide ranges of elution times that serious overlaps resulted with the retention times of PAHs having different carbon numbers. This supports the idea that the three-dimensional shape of the PAHs affects their chromatographic behavior as well as their spectral characteristics.

The solvent-induced non-planarity of some of the PAHs is more evident in this work than in the earlier normal-phase chromatography on aminopropyl- and nitropropyl-bonded columns due to the higher concentrations of dichloromethane that can be used with polymeric reversed-phase stationary phases. In the earlier normal-phase chromatography, mixtures of only up to 25% dichloromethane were used because the retention times became too short for accurate measurements. These concentrations are, of course, much lower than the 100% dichloromethane used in this study. Hence, the solvent-induced non-planarity would be expected to be much smaller in the earlier work.

However, in one part of the normal-phase chromatography studies, a much more retentive tetranitrofluorenoimidopropyl (TENF) column was used. This column allowed elution with pure dichloromethane. If the elution order on that column is compared to those found for the Vydac reversed-phase column, there is an almost perfect correspondence. In addition to the very early eluted compounds (26, 27, 30, and 31), the other fourteen PAHs eluted from the TENF column were also eluted in identical order to that of the Vydac column. The other thirteen PAHs were so strongly retained by the TENF column that they were not eluted in a reasonable time: the peaks were too broad for accurate measurement of the peak maxima (these compounds also were strongly retained on the Vydac packing, but retention time measurements were possible).

The large number of elution order changes found when low concentrations of dichloromethane were changed to higher ones indicates that analysis of complex samples containing a large number of PAHs could be even more difficult than previously thought. Identification of any particular peak can only be accomplished if its retention behavior and absorbance spectrum are identical to those of a standard of known structure. In instances where both of these criteria cannot be met, the identification of a peak can only be tentative or if other data (such as the molecular weight from liquid chromatography–mass spectrometry) were available, then a proposed structure could be assigned. This cannot be accomplished with single-wavelength absorbance or fluorescence detectors. More informative detectors, such as the photodiode-array UV and fluorescence detectors or mass spectrometers, must be used in concert if complete structural assignments are to be made.

If reversed-phase, gradient-elution chromatography is used for analysis of mixtures containing large PAHs it must be used judiciously. Complex and varied elution behavior can result from retentions based on several simultaneously changing factors, because the varying solvent composition will affect the various PAHs in different ways. Compounds that are planar at the start of the chromatographic run, for example, could become non-planar at higher concentrations of the strong solvent, resulting in an elution which is not in the expected order. The order of elution is affected by both the shape and rate of change of the gradient and by the mobile phase proportions at both the start and end of the gradient, and not just the solvents used. After each run, reequilibration of the column to the starting conditions must be complete. If it was not, then both elution order and relative retention times would not be reproducible. Each analysis of a PAH-laden material must be tailored individually to the types of PAHs found in the sample. This would involve some "scouting" runs to determine the complexity of the sample and if different types of elution were possible. The identification and quantitation of each species must be accomplished through careful consideration of all the factors that control retention and spectral response.

The problem in attaining reproducible chromatograms also make the quantitation of some of the PAHs more difficult. If the mobile phase composition is not highly reproducible, the response factors of those compounds showing changes in planarity will vary because the absorbance maxima of their spectra change in intensity with different proportions of the strong solvent[6,7]. The magnitude of this change can be up to 30–40%. An example is shown in Fig. 8 for identical concentrations of compound 30 at 5 and 85% dichloromethane. Although the response changes seen through variations in chromatographic conditions would be smaller, they would limit accuracy and reproducibility.

The non-planarity of some PAHs does make some separation schemes possible that would ordinarily not work. For two that are eluted close together, the degree of separation could be improved by the usual change to different weak and strong solvents or by a decrease in solvent strength. However, a unique means of improving resolution is possible if one of the two unresolved compounds is a planar PAH and one a PAH that becomes increasingly non-planar in stronger solvent mixtures. In this case, the separation would also be accomplished by using a stronger solvent mixture, contrary to the usual schemes for improving peak separation. The isolation of compound 21 from its reaction mixture was accomplished by this strategy. It was eluted very close to dibenzo[*cd,lm*]perylene, compound 6, at low or medium concentrations of dichloromethane. However, with a mobile phase of 80% dichloromethane

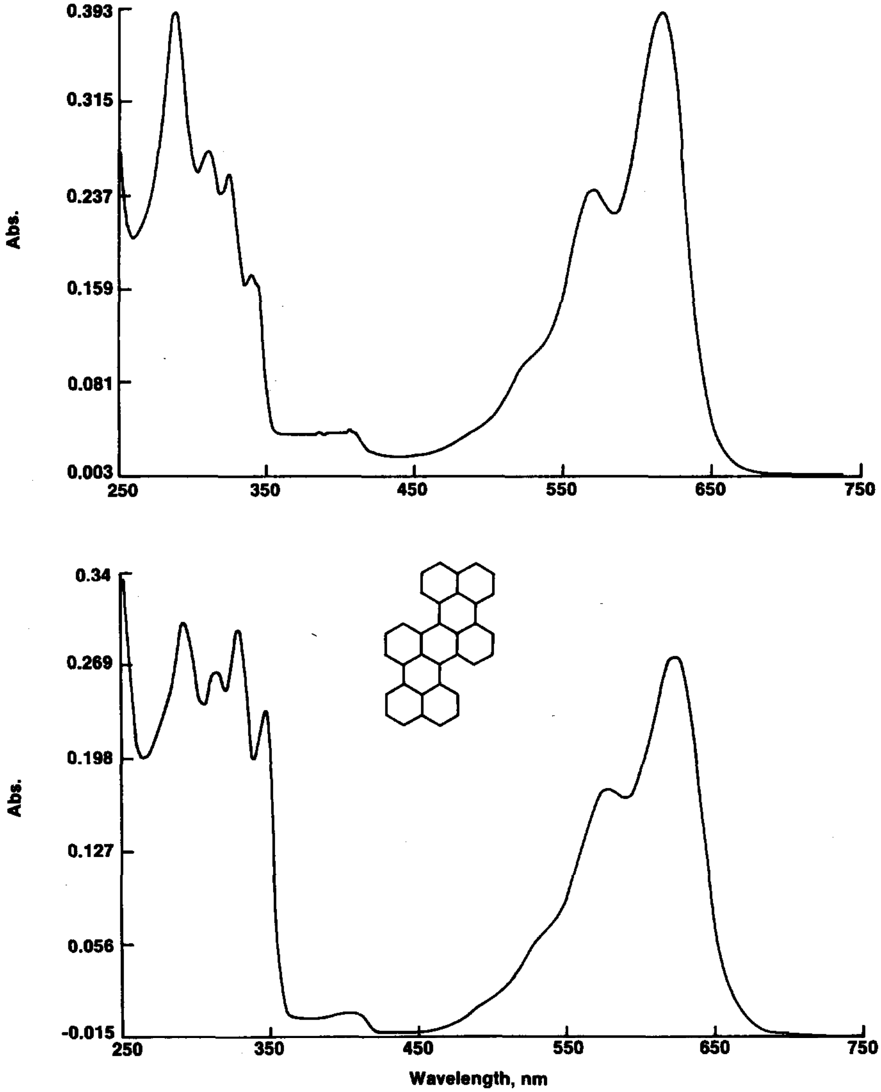


Fig. 8. Spectra of compound 30 showing intensity and bandshape changes. (A) 5% Dichloromethane; (B) 85% dichloromethane.

in methanol, it was eluted much earlier than the other lower-molecular-weight PAHs, and in this way, several milligrams of pure compound 21 were isolated (Fig. 9).

The UV–visible spectra of those PAHs that have the largest degree of anomalous retention behavior are also those which exhibit the shallowest absorbance minima (Fig. 10), as was seen in our earlier work[6,7]. The idea proposed by other groups that retention is controlled by the shape of the PAH is correct to a certain degree. The retention of a particular PAH is controlled by its shape, but only because the shape controls the amount of intramolecular strain that can occur and, thus, it de-

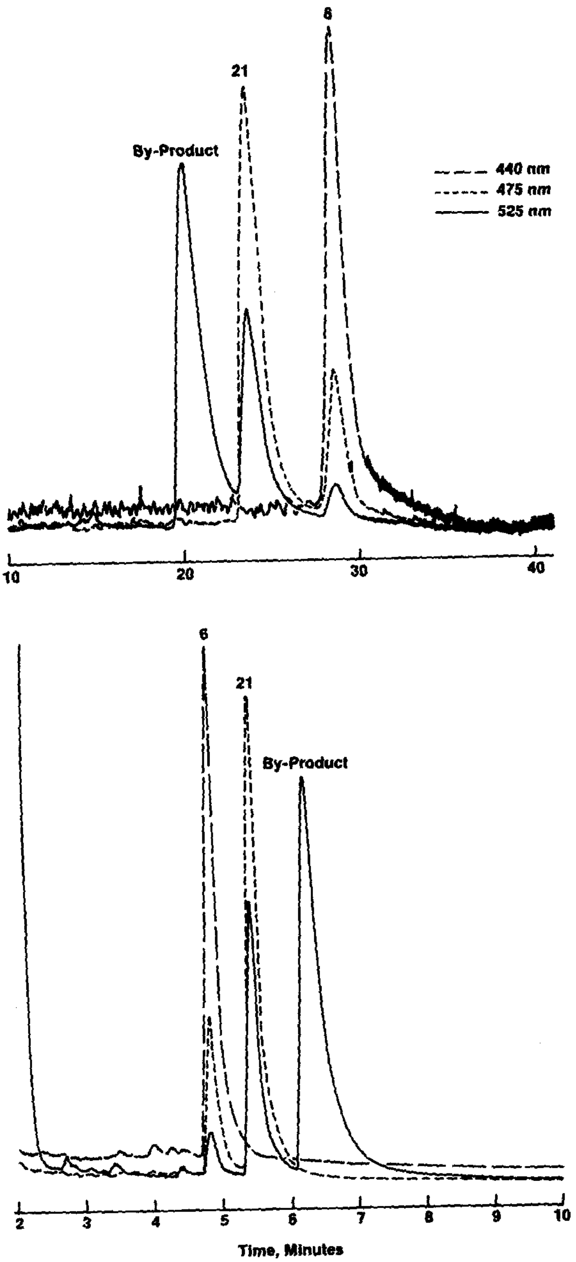


Fig. 9. Elution order reversal for compounds 6 and 21, and a larger by-product when dichloromethane concentration is raised.

termines the degree of planarity of the PAH. Within any one PAH class (of the three types of elution behavior classes), the second most important factor influencing the strength of retention is the active profile of the PAH, *i.e.*, the effective shape of the PAH that interacts with the bonded phase. This is only true for PAHs of very similar

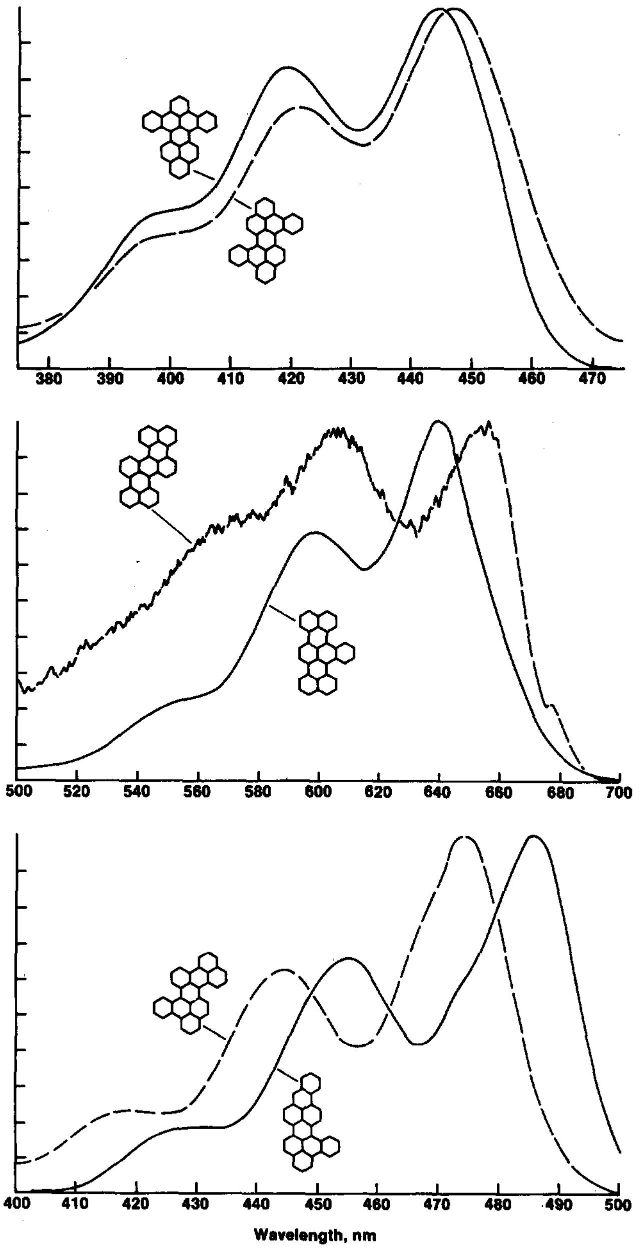


Fig. 10. Comparisons of the longest wavelength maxima for the 34-carbon PAHs. Note that increasing retention parallels decreasing shallowness of the valley between maxima.

shapes and planarities (the PAHs with longer axes are retained longer). For the planar PAHs, the shape of the PAHs is the predominant factor that determines the strength of interaction, the length of the molecule being the major contributor to increased retention.

Secondary effects, such as those due to solvent–bonded-phase interactions, complicate the separation even further. The logarithmic plots of relative retention seem to indicate that this secondary interaction between mobile and stationary phases occurs for the Vydac packing at 60–70% dichloromethane concentration. The planar PAHs, such as coronene and ovalene, have some curvature of their plots in this range. This type of change in the separation mechanism could explain an observation made in our earlier work[6].

Tetrahydrofuran (THF) is a stronger solvent than dichloromethane for elution of PAHs from the Vydac column. When methanolic mobile phase mixtures with the same proportions of dichloromethane and THF were used, the peropyrene-type PAHs were eluted earlier in THF. However, the elution order was not changed compared to lower concentrations of strong solvent, as it was with dichloromethane. The shallowness of the absorbance minima in the spectra of the PAHs in THF mobile phases also shows that the interaction with THF does not cause the PAHs to become as non-planar as dichloromethane does.

Although the Vydac column showed a high degree of retention behavior based on the three-dimensional structures of the PAHs, this type of behavior was also seen on several other polymeric phases including Brownlee Labs. 224 polymeric, Chemcopak, and Shandon octadecyl columns.

The retention of a PAH is mainly controlled by the degree of non-planarity of the molecule and the effective surface area it presents to the bonded phase. These are functions of a complex set of interactions between solutes, solvents, and the bonded phase. Some of the more important solvent–solute interactions are not only reflected in the chromatographic behavior but are also seen in various spectral absorbance characteristics of the solvated PAHs.

ACKNOWLEDGMENTS

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