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SOLVATED STRUCTURE-RETENTION RELATIONSHIPS OF PEROPYRENE-TYPE POLYCYCLIC AROMATIC HYDROCARBONS

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SUMMARY

The peropyrene-type class of polycyclic aromatic hydrocarbons (PAHs) resulting from the zinc dust fusion of perinaphthone and benzanthrone, contains several members whose degree of planarity is highly dependent on the solvent in which the sample is dissolved. The reversed-phase behavior of these compounds is profoundly effected by the changes in planarity. The nature of, and changes in, the UV-VIS spectra of these PAHs could be used to qualitatively predict which isomers would possess anomalous retention behavior.

INTRODUCTION

High-performance liquid chromatography (HPLC) has become one of the preferred methods for separation and analysis of polycyclic aromatic hydrocarbons (PAHs), whose occurrence in many types of carbonaceous material and etiological effects have made their analysis important. Most large PAHs (24 ring carbons or larger) are not volatile and so are not amenable to analysis by gas chromatography. The large number of potential isomeric species also limits other potential analytical techniques, such as mass spectrometry. The development of "full spectral" liquid chromatography detectors that collect UV-VIS or fluorescence spectra of the peaks as they elute has made HPLC an even more attractive technique^{1,2} since isomeric PAHs differ in their spectra³. Thus, the coupling of retention time with spectral information can make identification of isomer components or compounds with similar spectra much more precise.

The diversity of PAH classes exists because of the varying degrees of linear and condensed ring fusions that can occur⁴. In spite of this complication, prediction of retention times for similar PAHs in reversed-phase HPLC has been attempted and some trends are observed. A linear relationship between carbon number and retention time was reported by Sleight⁵ and Paeden *et al.*⁶ observed elution of large PAHs in a carbon black extract that approximately paralleled molecular weight. In both studies, the compounds were generally of similar PAH classes having planar structures. Retention order, therefore, could be approximated by ring number. In contrast, other work^{7,8} reports retention anomalies, explained in each instance by suggesting a non-planar structure in solution for the anomalous PAHs.

Observation of an anomalous elution order for some members of the peropyrene-type class of PAHs was reported in earlier work from this laboratory⁹. Zinc dust fusion of a perinaphthone-benzanthrone (1:3) mixture produced six major peropyrene-type PAHs. Using non-aqueous reversed-phase conditions, peropyrene (dibenzo [*cd,m*]perylene) and its benzo- and dibenzologues elute in the following order: first a nine-ring PAH; then, an eight-, a seven-, and, finally, 3-nine-ring compounds. The molecular weight of these compounds was 326, 376 and 426 daltons for the seven-, eight-, and nine-ring PAH, respectively. This work describes a further investigation of those observations, showing that this class of large PAHs has chromatographic retentions that are very dependent on the three-dimensional shape of the PAHs.

EXPERIMENTAL

The apparatus and details of the synthesis, isolation, and identification of these PAHs has been described in detail previously⁹. Briefly, a reversed-phase HPLC was coupled to a "full spectral" UV-VIS detector. The PAHs were identified by comparison with literature spectra or (for those PAHs which were not previously reported) by trapping the eluting peaks for field ionization mass spectrometry (FIMS). In the current work, a 25 × 1.0 cm I.D. Vydac 218TP5 (Separations Group, Hesperia, CA, U.S.A.) column was used, in addition to the 25 × 0.46 cm I.D. analytical columns reported previously. Isocratic mobile phases were premixed. All solvents were Burdick & Jackson (Alltech, Los Altos, CA, U.S.A.) distilled in glass and were used as received. UV-VIS spectra were collected by the Hewlett-Packard 1040A detector, except for the solvent-effect comparison spectra. Those were run on a Perkin-Elmer Lambda 3 system (60 nm/min scan-rate, 0.5 nm resolution, and five repetitive runs summed to increase signal to noise).

Size-exclusion chromatography (SEC) was performed on a DuPont 8500 HPLC system at ambient temperature, with either tetrahydrofuran (THF) or toluene as the mobile phase. Two column sets were used. The first consisted of two Waters Ultrastyrigel columns (50 cm × 0.46 cm I.D. each) of 50- and 10-nm pore sizes (Waters Assoc., Milford, MA, U.S.A.). The second set was Altex Microspherogel, 10- and 5-nm pore size (30 cm × 0.8 cm I.D. each) supplied by Beckman (Berkeley, CA, U.S.A.). The "full-spectral" detector was used with a monitoring wavelength of 440 nm, which was chosen because all the peropyrene-type PAHs absorbed significantly at that wavelength.

A standard mixture of several planar PAHs, pyrene, benzo[*ghi*]perylene (Chemical Service, West Chester, PA, U.S.A.), coronene, and ovalene (Aldrich, Milwaukee, WI, U.S.A.) was used for comparison of retention order and times. In addition, a fifth compound of this type, naphtho[8,1,2*abc*]coronene, was synthesized and used after purification. It was prepared from benzo[*a*]coronene, which had been synthesized from 37.6 g of coronene at a yield of about 35%⁸. The ring addition, producing naphtho[8,1,2*abc*]coronene, was accomplished by reacting maleic anhydride and chloranil with the benzo[*a*]coronene. The intermediate product was reduced to the PAH with copper powder in quinoline. Small amounts (50 mg, corresponding to an approximately 0.5% yield) of the yellow nine-ring PAH were isolated. The coronene used as starting material also contained small amounts of the eight-ring

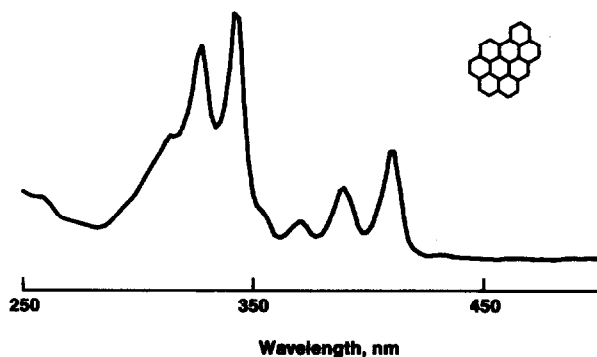


Fig. 1. Spectrum of naphtho[8,2,1abc]coronene.

PAH benzo[*pqr*]naphtho[8,1,2*bcd*]perylene¹⁰ as an impurity, and concern exists that this PAH could be the actual reacting species. However, no suitable supplies of this PAH are available for experimentation to see if this is the case. The UV-VIS spectrum of naphtho[8,1,2*abc*]coronene is shown in Fig. 1.

RESULTS AND DISCUSSION

After several preparative scale runs of the reaction mixture described in ref. 9 (using the larger diameter Vydac C₁₈ column), a decrease in resolution between two of the nine-ring dibenzoperopyrenes was seen. Cleaning the column by changing the mobile phase to 100% methylene chloride (from the 45% in methanol used as the final gradient condition) produced a large peak of previously adsorbed material. This material was collected and blown down under nitrogen and redissolved in methanol-methylene chloride (1:3). A gradient from 50 to 90% methylene chloride in methanol was then used to separate this fraction using the analytical Vydac column. One major and about a dozen minor components were seen (the minor peaks had absorbances, at any wavelengths from 250 to 600 nm, of 5% or less of the major peak's 475 nm maximum). The major peak in this fraction of strongly-retained material was trapped and FIMS showed a molecular ion at 376 daltons. It was, therefore, an eight-ring isomer of one of the components identified previously. UV-VIS spectra and structures for this compound, as well as the other major products of the reaction

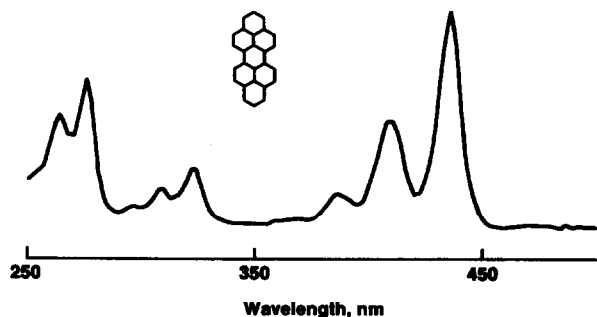


Fig. 2. Spectrum of peropyrene (dibenzo[*cd,m*]perylene).

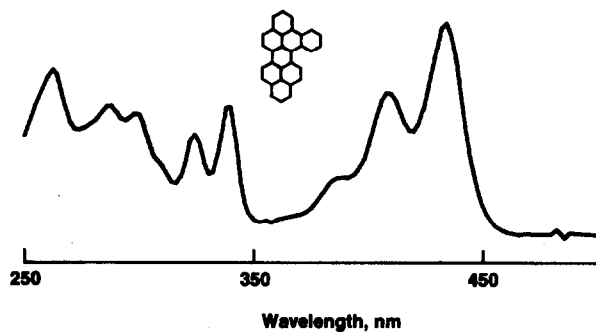


Fig. 3. Spectrum of tribenzo[a,cd,lm]perylene.

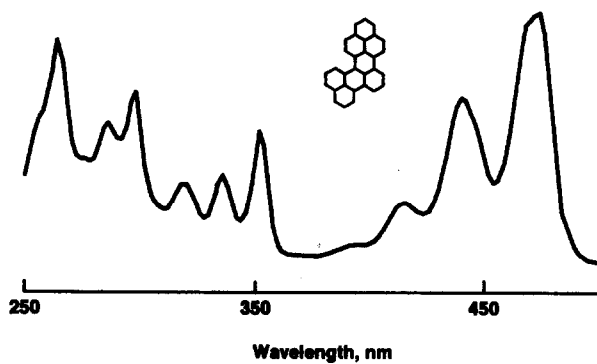


Fig. 4. Spectrum of phenaleno[1,9ab]perylene.

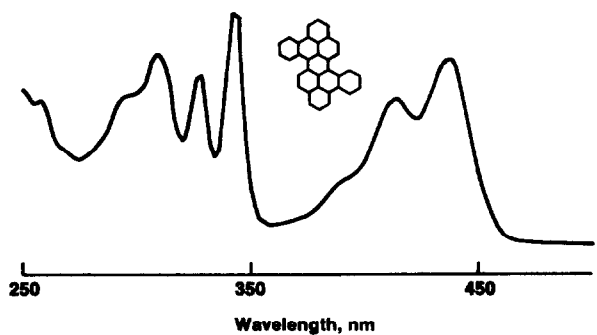


Fig. 5. Spectrum of tetrabenzo[a,cd,j,lm]perylene.

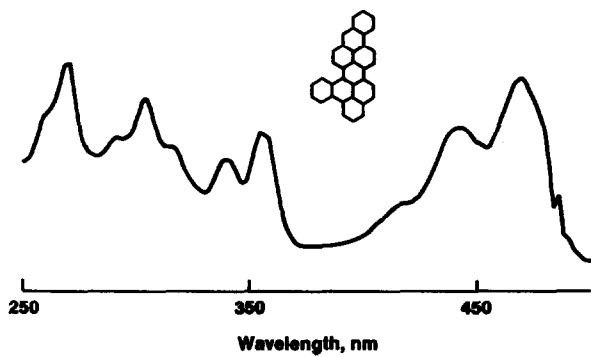


Fig. 6. Spectrum of benzo[*rs*]phenanthro[1,10,9*cde*]pentaphene.

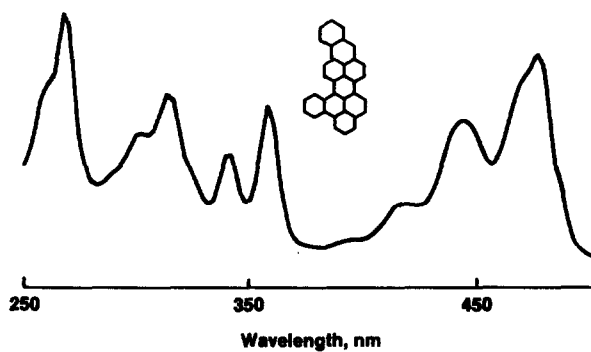


Fig. 7. Spectrum of dibenzo[*a,rs*]naphtho[8,1,2*cde*]pentaphene.

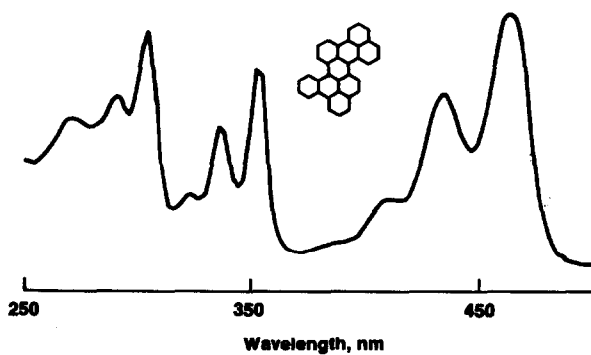


Fig. 8. Spectrum of dibenzo[*a,cd*]naphtho[1,8*jk*]perylene.

TABLE I

PAHs ELUTED BY USING A METHYLENE CHLORIDE GRADIENT AND DETECTED BY UV-VIS ABSORBANCE

Peak No.	Figure	No. of rings	Name
1	5	9	Tetrabenzo[<i>a,cd,j,lm</i>]perylene
2	3	8	Tribenzo[<i>a,cd,lm</i>]perylene
3	2	7	Peropyrene(dibenzo[<i>cd,lm</i>]perylene)
4	6, 7	9, 9	Benzo[<i>rst</i>]phenanthro[1,10,9 <i>cde</i>]pentaphene Dibenzo[<i>a,rsi</i>]naphtho[8,1,2 <i>cde</i>]pentaphene
5	8	9	Dibenzo[<i>a,cd</i>]naphtho[1,8 <i>jk</i>]perylene
6	4	8	Phenalenol[1,9 <i>ab</i>]perylene

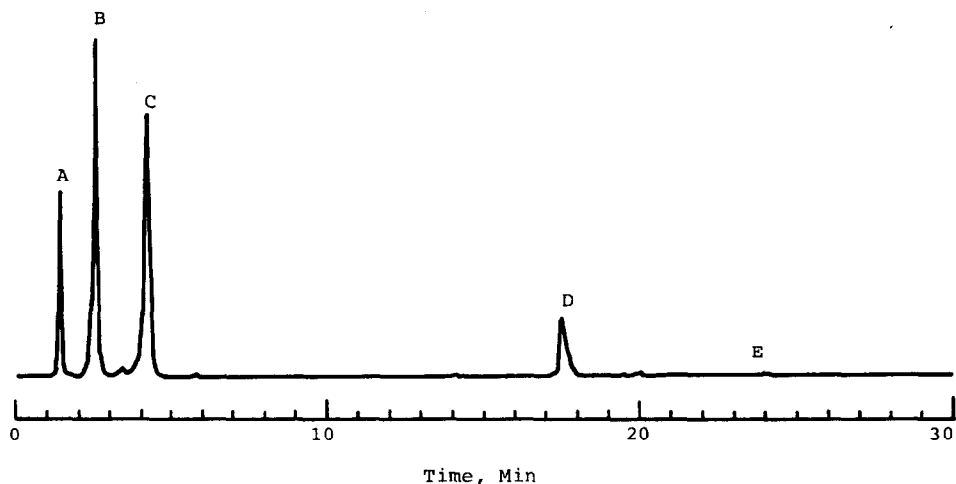
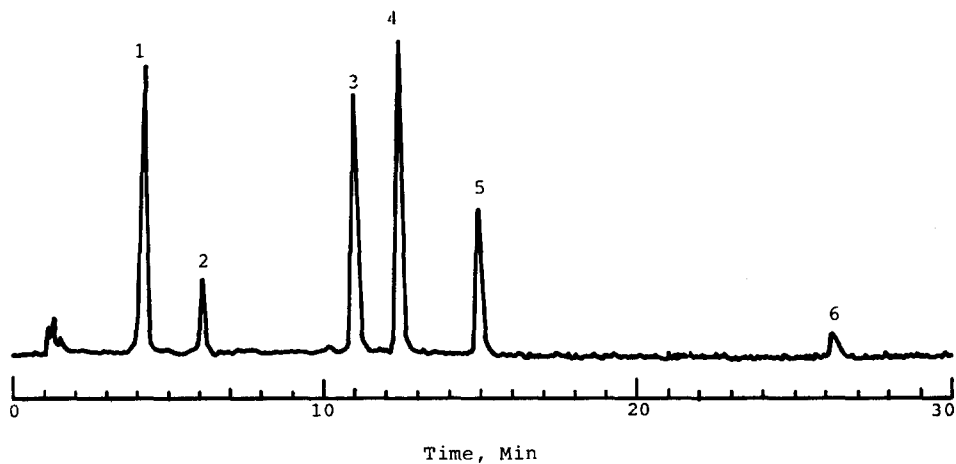


Fig. 9. Comparison chromatograms, upper figure: peropyrene-type PAHs, peaks are identified in Table I. Lower figure: planar PAHs; A, pyrene; B, benzo[*ghi*]perylene; C, coronene; D, naphtho[8,1,2*abc*]coronene and E, ovalene.

mixture are shown in Figs. 2-8. Table I lists these compounds by the elution order observed using a methylene chloride gradient (20-80% linearly in 35 min). Peak 4 is an isomeric pair, which could not be totally separated chromatographically, but could be resolved spectrally (this was the isomeric pair used to evaluate the column resolution). Under isocratic conditions, the separation of these isomers was not as large as that seen in the previous gradient-elution studies⁹. The molecular weights of these compounds were 326, 376, and 426 for the seven-, eight-, and nine-ring PAHs. IUPAC names for the compounds are also given in the table.

When the standard mixture of planar PAHs was chromatographed under an identical gradient to that run with the peropyrene mixture described above, elution times that span the whole chromatogram resulted. Fig. 9 shows these comparison runs. The peropyrene-types also eluted throughout, but some of these large PAHs had elution times very similar to much smaller PAHs. Tetrabenz[*a,cd,j,m*]perylene was the extreme example. It eluted at almost the same time as coronene, which has ten fewer ring carbons; and with mobile phases that had higher methylene chloride concentrations, its relative retention was even smaller. With a mobile phase of 35% methylene chloride it eluted before benzo[*ghi*]perylene.

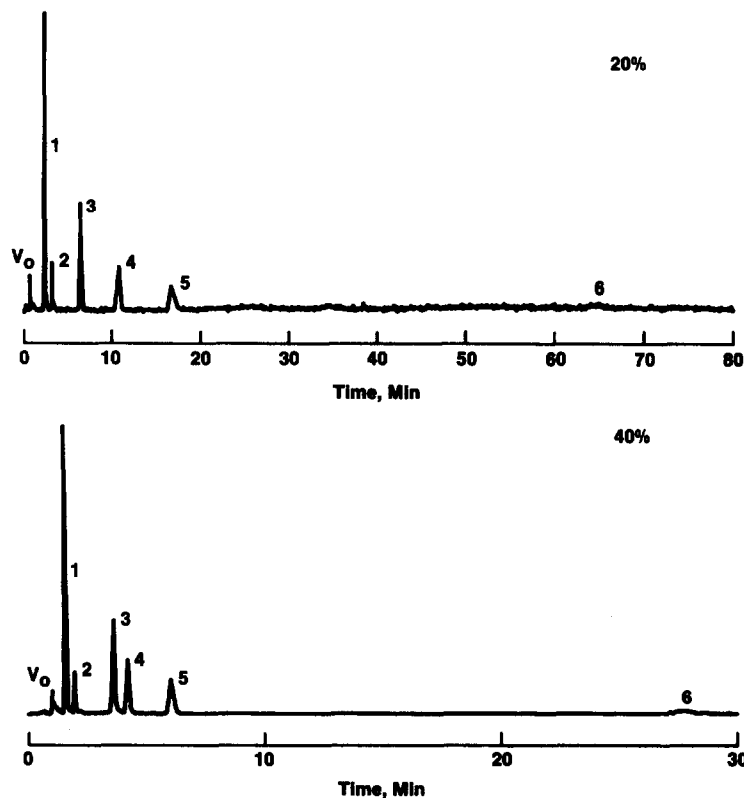


Fig. 10. Separation of the peropyrene-type PAH mixture with isocratic mixtures of methanol and methylene chloride as the mobile phase. Percentages refer to the concentration of methylene chloride. Flow-rates were: 20%, 3.5 ml/min; 40%, 2.5 ml/min.

The elution order anomalies observed for the peropyrene types were examined more closely with a series of isocratic runs in which the methylene chloride concentration was successively increased. The resulting chromatograms are Figs. 10–13. The flow-rate was decreased as the solvent strength was increased in order to facilitate detection and resolution of the peaks.

At lower concentrations of methylene chloride, peropyrene eluted as the third major peak. Two of the nine-ring isomers (Peaks 4 and 5) eluted later. As the level of strong solvent (methylene chloride) was raised, these two isomers eluted with smaller relative retentions, until Peak 4 coeluted with peropyrene at about 50% methylene chloride and became the third peak at higher concentrations. A similar trend was observed for the other nine-ring isomer, with its crossover concentration at about 85% methylene chloride. No other drastic changes in elution of the other compounds were seen.

When other strong reversed-phase solvents were used, similar behavior was observed. Chloroform proved a stronger inducer of this anomalous retention than methylene chloride. In contrast, THF had a less pronounced effect on elution order, even though it is a stronger reversed-phase solvent and caused these PAHs to elute

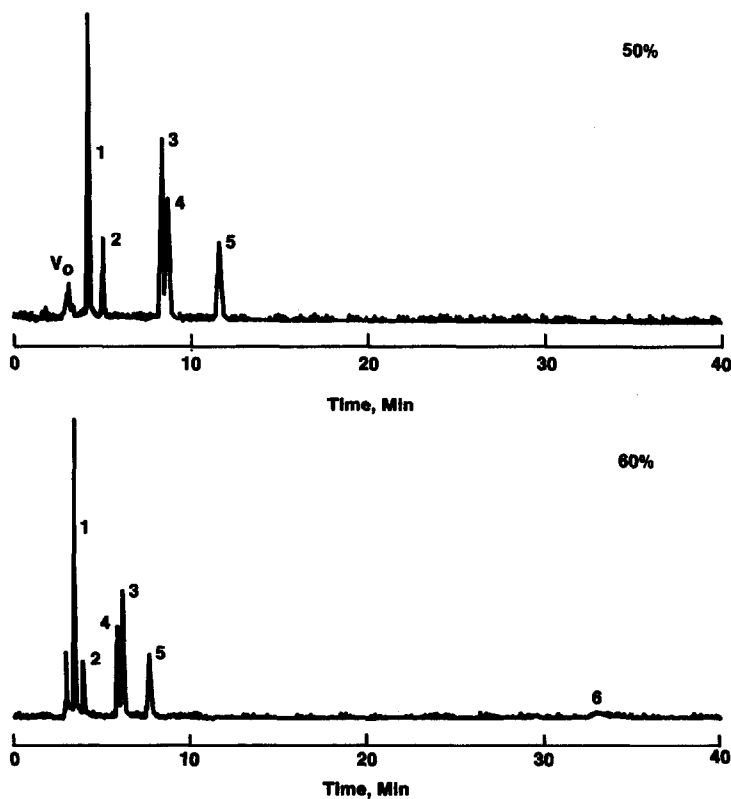


Fig. 11. Separation of the peropyrene-type PAH mixture with isocratic mixtures of methanol and methylene chloride as the mobile phase. Percentages refer to the concentration of methylene chloride. Flow-rate was: 1 ml/min.

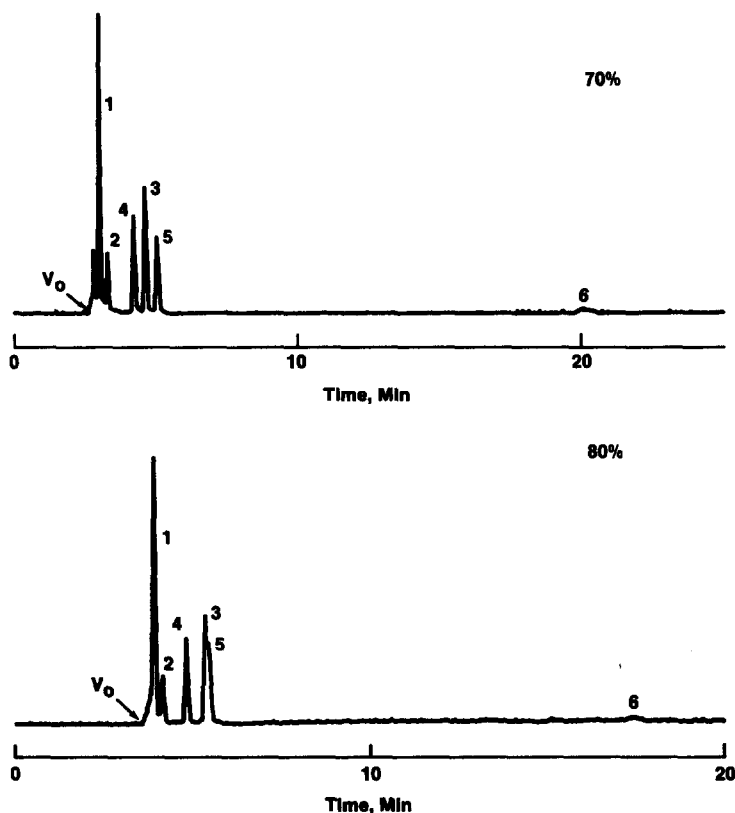


Fig. 12. Separation of the peropyrene-type PAH mixture with isocratic mixtures of methanol and methylene chloride as the mobile phase. Percentages refer to the concentration of methylene chloride. Flow-rates were: 70%, 0.9 ml/min; 80%, 0.8 ml/min.

at earlier times for identical concentrations. Also showing this type of behavior to lesser degrees were ethyl acetate, acetone, and isopropanol.

The dependence of elution order on the solvent selected for the separation could be explained by changes in the shape of the PAHs with solvent. The degree of planarity of PAHs can be estimated by certain characteristics in their UV-VIS spectra¹¹. Non-planarity is evidenced by a decrease in the resolution between the PAH spectral bands. The non-planar PAHs have shallow valleys between the band peaks. To evaluate the possibility that changes in planarity were responsible for retention behavior observed above, a small amount of each PAH of interest was dissolved in 1 ml of methylene chloride and half was diluted with methanol and the other portion was diluted with methylene chloride. No major differences in the pairs of spectra were seen, except for the two nine-ring isomers (Peaks 4 and 5). Along with the expected solvent induced shift of the band maxima, the shape of some of the bands changed. Fig. 14 compares the spectra of Peak 4 that occurred in these two solvents. This result suggests that a change in planarity of the two nine-ring isomers with solvent does indeed occur.

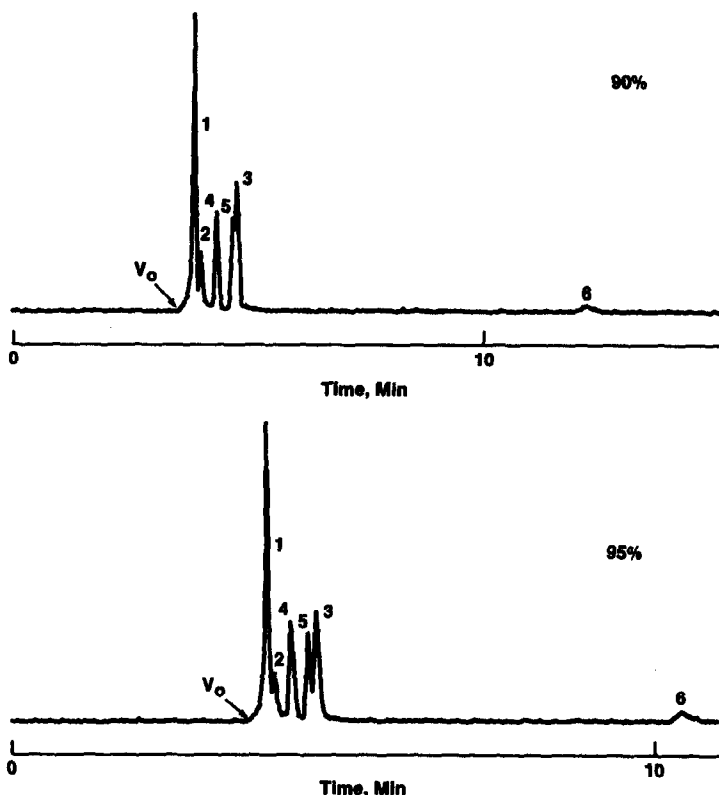


Fig. 13. Separation of the peropyrene-type PAH mixture with isocratic mixtures of methanol and methylene chloride as the mobile phase. Percentages refer to the concentration of methylene chloride. Flow-rate was: 0.7 ml/min.

Size-exclusion spectral studies

It was not possible to completely separate the peropyrene-types from each other by SEC, but it did provide further evidence that these PAHs do not have similar behaviors in different solvents. The use of the "full-spectral" detector allowed the small separations to be seen, even though the various peropyrene-types were not resolved. For example, peropyrene could be monitored with its sharp 325-nm band. The behavior of these PAHs in the two mobile phases used was different. In THF, the two "anomalous" nine-ring compounds did not elute before peropyrene, as would be expected from their number of rings but eluted after it. This would suggest that when dissolved in THF, they are smaller in volume, and hence, could permeate the column pores better than peropyrene. When toluene was substituted for THF, there was a much smaller degree of separation. In toluene, peropyrene coeluted with tetra-benzo[*a,c,d,j,lm*]perylene which was the last eluting PAH in both solvents. When the UV-VIS spectra of the same PAHs that had suggested differences in their shapes in methanol and methylene chloride were run in THF and toluene similar, but smaller, spectral changes were seen.

Comparing the nature of the UV-VIS spectral bands of these large PAH to

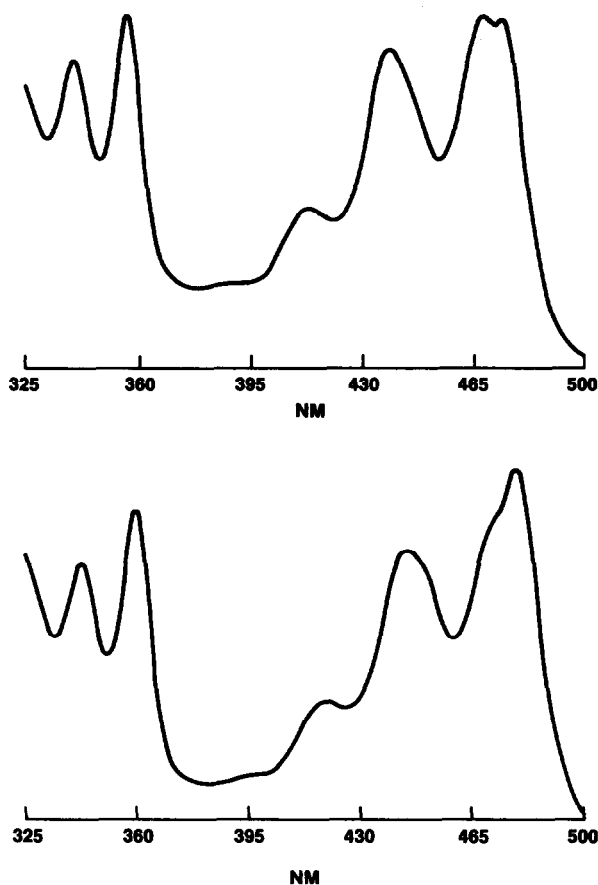


Fig. 14. Spectra of benzo[rsz]phenanthro[1,10,9cde]pentaphene with differing mixtures of methylene chloride in acetonitrile as the solvent. Upper figure: 5% methylene chloride. Lower figure: 80% methylene chloride.

the reversed-phase elution behavior produced the following observation: The large PAH with the shallowest minima in the absorption spectrum (indicating the highest degree of non-planarity) was tetrabenzo[*a,cd,j,lm*]perylene, the same PAH that eluted earliest under reversed-phase conditions even though it has nine rings. This large PAH was also the latest eluting compound in the THF size-exclusion separation, consistent with the proposition that it assumes a more compact non-planar structure in certain solvents. Additionally, spectra of the two other nine-ring compounds demonstrating odd reversed-phase elution behavior showed bands which were less sharp (and minima which were less shallow) than planar PAHs, again indicating reversed-phase retention behavior based on structural changes. In contrast, the spectra of peropyrene and the eight-ring isomer (phenaleno[1,9ab]perylene) that eluted very late under reversed-phase conditions had the sharpest bands, indicating significant structural planarity in solution. When the reversed-phase retention behavior of these large PAHs was compared to the retention behavior of known planar PAHs (pyrene, benzo[*ghi*]perylene, coronene, and ovalene), reasonable agreement in ex-

pected retention time was observed, supporting the spectral evidence for structural planarity.

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