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THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PERO-PYRENE-TYPE POLYCYCLIC AROMATIC HYDROCARBONS

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SUMMARY

Various large polycyclic aromatic hydrocarbon ring structures of potential environmental and biological interest are synthesized and characterized. These compounds are then used as standards in the development of analytical high-performance liquid chromatographic separations.

INTRODUCTION

The environmental and etiological importance[1–3] of polycyclic aromatic hydrocarbons (PAHs) has made this class of molecules the subject of innumerable studies, and they have been found in a wide variety of samples. The overwhelming proportion of these studies has focused on PAH of 24 or fewer ring carbons (coronene, dibenzopyrenes) primarily because it has been assumed that large PAH (greater than 24-ring carbons) are either so sparingly soluble or so limited in mutagenic activity that no significant biological impact would result from exposure. In addition to the very limited solubility, a lack of standard compounds, low volatility characteristics, and the inability of previously available analytical methods (such as mass spectrometry) to provide isomer specificity have contributed to a dearth of analytical studies of large PAH.

Liquid chromatography offers an attractive separation/identification technique for large PAH. However, until recently, it too has suffered with both resolution and detection limitations. The large number of structural isomer possibilities inherent with large PAH required the development of smaller, more uniform particle-size commercial packings before the many PAH isomers found in soots, coal tar products, and other PAH-laden material could be resolved. While single-wavelength monitoring of the column eluent can detect a PAH peak, identification is tedious. Either stop-flow scanning or collection of the peak for later ancilliary analyses was necessary to establish correctly the peak identity[4,5]. In the case of many PAHs, the need for these off-line analyses has been overcome by use of a full spectral UV–visible detector. This detector is based on a photodiode array in which each photodiode collects the intensity at a particular wavelength and the total array collects the entire spectrum.

The typical UV spectrum of large PAHs contains much more structural infor-

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mation than that from most classes of organic compounds[6]. The benzenoid bands usually have five or more maxima and the corresponding intermediate minima. The location and relative intensities of these bands result in a unique spectral pattern for a specific ring system. Alkyl substitution of the ring does not greatly alter this unique pattern as bathochromic shifts of only a few nanometers are the only change generally observed[7]. The full-spectrum detector can efficiently exploit these unique spectral characteristics for rapid and accurate identification of parent ring structure.

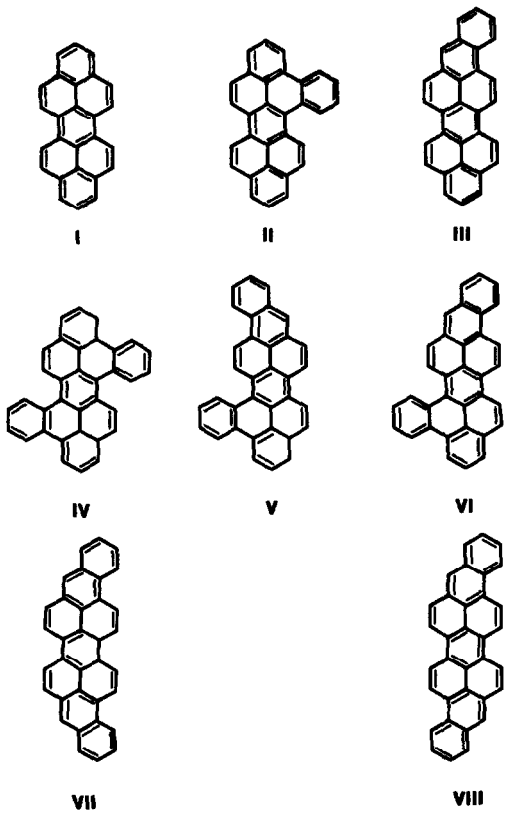
As mentioned previously, it has been assumed that large PAHs are nonmutagenic and/or so sparingly soluble that any biological impact would be minimal. However, the peropyrene series does not conform to these assumptions. Peropyrene itself has demonstrated carcinogenic activity[8], suggesting that molecular size or solubility character is not as great a barrier to mutagenic activity as previously assumed, if other molecular characteristics are disposed favorably toward it. Since the occurrence and levels of peropyrene (Compound I, dibenzo[*cd, lm*]-perylene) and its benzologues have not been extensively studied, the importance of this deviation from expected behavior is unknown. Peropyrene itself has been found in carbon black[5] and coal tar[9] and is suspected to occur in chimney soot, automobile exhaust, and other airborne particulates[10,11].

A major difficulty in studying large PAHs is the absence of commercially available standards to act as calibrants and to provide a reference spectrum under the experimental conditions. We report here the synthesis and characterization of various large PAH ring structures for use as standards and the development of various high-performance liquid chromatographic (HPLC) techniques for their analysis.

EXPERIMENTAL

A series of syntheses was performed to produce peropyrene and its benzo- and dibenzologues (Compounds I–VIII). All the syntheses were based on the use of the zinc dust melt for either reduction of large aromatic ketone systems or for the fusion of smaller aromatic ketones into large PAH ring structures[12].

Initially, the reduction of three large cyclic diketones was studied to evaluate optimum reaction conditions. Pyranthrene and Compounds VII and VIII (violanthrene and isoviolanthrene) were obtained by reduction of the corresponding diketones. (All ketones were obtained from Tokyo Kasei, except where noted.) It was found that 50 g of starting material, when mixed to proportions of 1:1:1:5 mole fraction ratio of ketone, zinc dust (J. T. Baker), sodium chloride (Mallinckrodt), and zinc chloride (J. T. Baker) resulted in large quantities (0.1–1.0 g) of the desired PAH. The mixture was first heated to 250°C for 5 min to homogenize the melt and activate the zinc dust, then the temperature was raised to 330–340°C. The melt was vigorously stirred and heated for 25 min. A color change from black to orange or red indicated the extent of reaction. After cooling, the material was exhaustively extracted with *o*-xylene. (All solvents used were supplied by Burdick & Jackson). This crude extractant was partially purified by passage through a preparative-scale column (85 × 4 cm I.D.) packed with 100–120 mesh silica (W. R. Grace) previously activated at 200°C for 3 h. The *o*-xylene eluted material (PAH fraction) was collected while unreacted starting material and salts remained on the column. The PAH fraction was dried and redissolved in cyclohexane–toluene (1:1) and chromatographed on a 75



I Dibenzo[*cd,lm*]perylene (peropyrene)
II Tribenzo[*a,cd,lm*]perylene
III Benzo[*rst*]naphtho[2,1,8*cde*]pentaphene
IV Tetrabenzo[*a.cd,j.lm*]perylene
V Dibenzo[*a,rst*]naphtho[2,1,8*cde*]pentaphene
VI Benzo[*rat*]phenanthro[1,10,9*cde*]pentaphene
VII Benzo[*rst*]anthra[9,1,2*cde*]pentaphene (violanthrene)
VIII Benzo[*rst*]phenanthro[10,1,2*cde*]pentaphene (isoviolanthrene)

× 1 cm I.D. column of Woelm activity Grade 1 basic alumina. A fraction was collected by elution with cyclohexane–toluene (1:1) and another with methylene chloride as the eluent. The latter fraction contained the PAH of interest and was reduced in volume to 25 ml. This fraction was then passed twice through Florisil Sep-Paks (Water Assoc.) to remove any traces of unreacted diketone. A reversed-phase separation was then performed with a Waters Preparative 500A chromatograph on a Prep-Pak octadecyl bonded-phase column. A step gradient from methanol to methanol–methylene chloride (1:1, v/v) was used (5% steps every liter, flow-rate 250 ml/min) and fractions of 500 ml each were collected.

Each fraction was examined by reversed-phase HPLC and field-desorption mass spectrometry (FDMS). The analytical HPLC system consisted of a Du Pont 8000 gradient system, a Valco C6U injector, and a Hewlett-Packard 1040A photodiode array detector. Data analysis was done on a Hewlett-Packard HP 85 micro-

computer using the Infometrix MCR2 software package, an upgraded version of the original operating program. Flexible disc storage of the data was accomplished using an HP 82901M disc drive. The mass spectrometer utilized was a VG Analytical ZAB with an INCOS data system.

The reversed-phase system initially used was a Vydac 201TP5 column (Separations Group) with various proportions of methanol and methylene chloride. This solvent pair was chosen because other strong solvents (such as isopropanol) did not solubilize the PAH as well as methylene chloride, and solvents which did suffered from various shortcomings (excessive UV absorbances, peroxide formation, high viscosity, etc.). Methanol proved to have a higher separating capability than acetonitrile when used with methylene chloride gradients. Fifteen different manufacturers' reversed-phase columns were evaluated and this column had the greatest resolution capability. It was, however, replaced by a Vydac 218TP5 column in later work because of possible secondary effects seen with some samples. These effects will be described later. This second column is more efficiently endcapped than the 201TP5 column. The mobile-phase gradient was 85:15 to 60:40 methanol–methylene chloride. The samples were dissolved in methanol–methylene chloride (1:1, v/v). The solubility of peropyrene and the benzo- and dibenzologues was found to be higher than expected. In methanol, the solubilities were in the range of 0.5–2.0 mg/ml and were in the 0.01–0.25 mg/ml range in water–methanol (10:90) and 10 ppm in water–methanol (50:50).

For Compounds I–VI, individual syntheses were performed using the zinc dust fusion method. Peropyrene (I) was made by starting with perinaphthone (IX) (Aldrich). Compounds V–VII were produced by using 3-bromo-7*H*-benzo[*de*]anthracene-7-one (Pfaltz and Bauer) as the starting material. Compound IV was also produced in large quantity by an isomer specific fusion[13]. Copper dust was used in this melt rather than zinc dust, and the reaction temperature was 360–370°C. The two benzoperopyrenes (II and III) were produced by using a mixture of ketones IX and X for the fusion material.

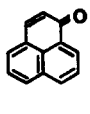
Preparative fractions determined from HPLC and FDMS analysis to contain a predominance of a particular compound were combined. These combined fractions were microsublimed[14] on an apparatus consisting of a 1.25-m thermal gradient from 350°C to 175°C. This was produced by an oven that had a resistance wire heating element wound at various densities. The separation tube was a glass tube (2 m × 2.5 mm I.D.).

Another set of HPLC and FDMS analyses were then done on the microsublimed fractions to detect contamination by another PAH or any ketone or side reaction impurities.

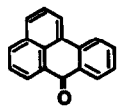
RESULTS

Seven PAHs were obtained in large quantities (> 50 mg) and in purities of greater than 95% (Compounds I, II, IV–VIII). A small amount of Compound III was also obtained. Spectra of the previously known compounds matched those in the literature[15].

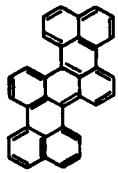
Compounds II and III have not been previously reported in the literature. Compound II (yellow-orange needles, mp 295–300°C) has a very strong bluish-green



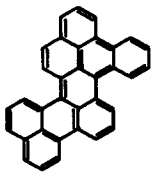
IX



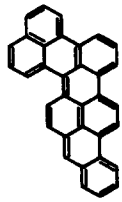
X



XI



XII



XIII

IX Perinaphthone (Phenalene-1-one)
X 7H-Benzo[*de*]anthracene-7-one
XI Tetrabenzo[*de,hi,op,st*]pentacene
XII Dibenzo[*a,cd*]naphtho[1,8*jk*]perylene
XIII Benzo[*rst*]phenaleno[1,2*de*]pentaphene

fluorescence, and the UV–visible spectra of it and Compound III are given in Fig. 1. All spectra were obtained during the chromatographic run. Structural assignments for these two were based on the predicted spectral characteristics from annellation theory of PAH spectra[16]. Compound II, tribenzo[*a,cd,lm*]perylene, should have the shorter wavelength absorbances when compared with the isomeric Compound III, benzo[*rst*]naphtho[2,1,8*cde*]pentaphene.

One chromatographic peak (brownish-yellow plates and an intense green fluorescence) did not match any of the spectra of the five isomeric dibenzoperopyrenes or another known product, tetrabenzo[*de,hi,op,st*]pentacene (XI). Mass spectra showed the molecular ion to be 426, and so it could not be a more fused PAH. Possible structures include XII and XIII. The former is more likely from the annellation trends and rules as well as from the reactivity of 7H-benzodeanthracene-7-one. Compound XIII has been reported, and its absorption maxima do not match those of the unknown peak[17]. Since no spectrum was given in that work, the pattern is not known. Solvent shifts cannot be ruled out as the cause for this difference and this structure is still possible for the peak seen. The UV–visible spectrum of this product is given in Fig. 2.

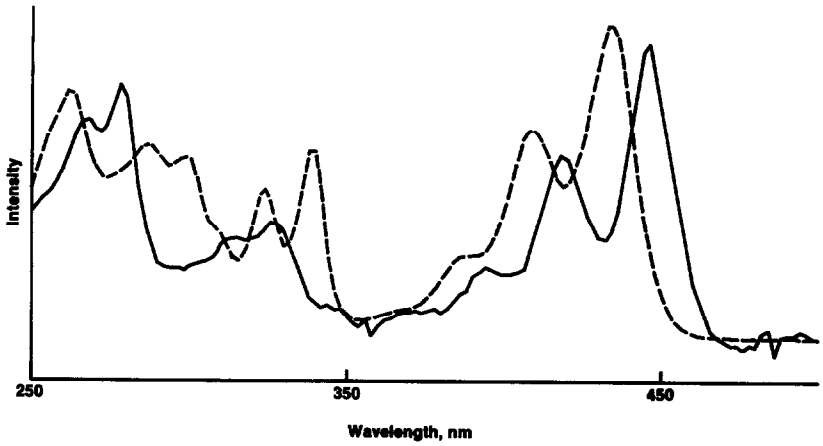


Fig. 1. Spectra of tribenzo[*a,cd,lm*]perylene (Compound II) (---) and the isomeric benzo[*rst*]naphtho[2,1,8*cde*]pentaphene (Compound III) (——).

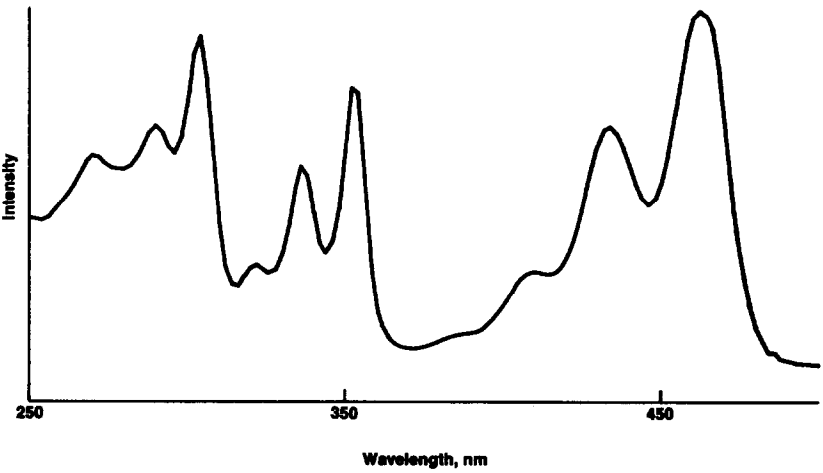


Fig. 2. Spectrum of dibenzo[*a,cd*]naphtho[1,8*jk*]perylene (Compound XII).

The chromatogram of a crude reaction product mixture is shown in Fig. 3. An apparent steric effect was seen with the Vydac columns. The elution order was IV, II, III, I, VI, V, VII and VIII. Normally, the elution order of these compounds could be predicted from the number of carbon atoms or aromatic rings. Small deviations from the predicted order are expected, but large deviations such as those seen with the Vydac columns are quite unexpected. Elution of the 9-ring compound, tetrabenzo[*a,cd,j,lm*]perylene (IV), before either of the 8-ring compounds, and the elution of peropyrene after the 8-ring compounds indicate that a much different separation mechanism than simple two-phase partition is operating. All other columns tested in this study consistently separated by carbon number. Examples of this type of behavior are shown in Fig. 4.

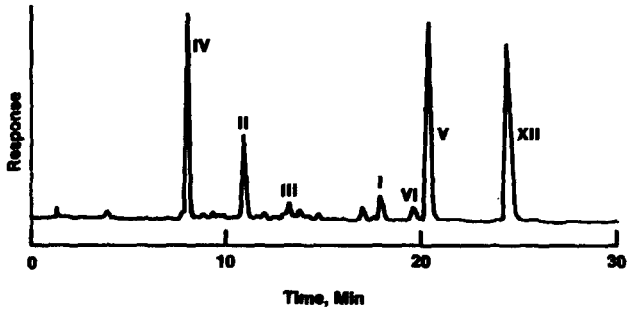


Fig. 3. Chromatogram of mixed ketone reaction mixture, peaks correspond to compounds in text; column: Vydac 218TP5 with gradient conditions as given in the text.

The results for the IBM octadecyl column are similar to most of the chromatograms obtained (Fig. 4a). Elution order proceeded by the number of rings, even if no isomer specific separation occurred. Several columns, such as the Du Pont Zorbax ODS column, showed an apparent mixed mechanism separation. This behaviour typically generated a chromatogram much like Fig. 4b. The Zorbax column

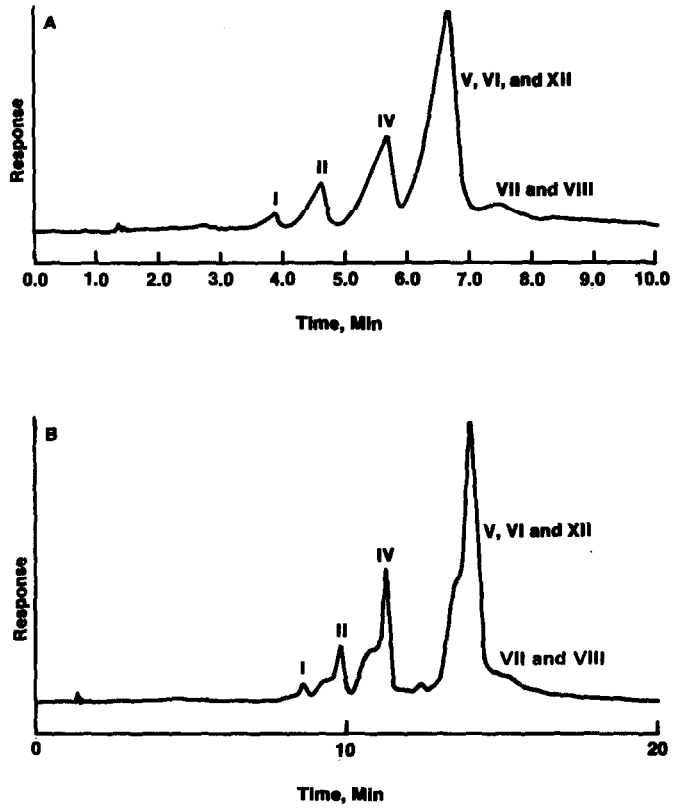


Fig. 4. (a) Chromatogram of mixed ketone product on IBM octadecyl column (25 × 0.46 cm I.D.), 5-μm packing), gradient of 5–30% methylene chloride in methanol. (b) Identical run with Du Pont Zorbax ODS column (25 × 0.46 cm I.D., 5-μm packing).

uses silica with a mean pore diameter of 6-7 nm, and the C_{18} derivatization reduces that by 1 nm or so[18]. The length of the large PAH presently under study typically runs 3–4 nm, so steric exclusion effects are probable. The Vydac columns cannot suffer from this particular problem because their mean pore diameter is 29–31 nm[19]. Another major difficulty with most of these columns was the low retention of PAH under the solvent conditions chosen. If a weaker sample solvent was used, severe peak broadening resulted because of mobile-phase, sample-solvent mismatch.

The spatial geometry of large PAHs in solution is not known. Ring number is not the only criteria for their overall geometry and, thus, their chromatographic behavior[20]. These molecules are probably similar to perylene in being nonplanar[21], either twisting or folding to keep the rings away from each other. ^{13}C and 300-MHz ^{1}H NMR suggest this possibility, but no concrete conclusions can be made. The UV spectra of Compounds II–IV suggest some folding, as the absorption bands are very broad with shallow minima, while those of Compound I are much sharper. This difference has been claimed[18] to be a reflection of the nonplanar structure of perylene and similar molecules relative to pyrene (which is planar).

CONCLUSIONS

HPLC with full-spectral detection has been shown to be useful for large PAH analysis. The full-spectrum detector easily differentiates isomeric species and with a secondary supporting technique such as mass spectrometry, specific compound identification is possible. The HPLC of these large PAHs is not as straight-forward as smaller PAHs because of secondary effects (such as steric exclusion) that may depend on three-dimensional conformations of the molecule.

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