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CAPILLARY COLUMN GAS CHROMATOGRAPHIC SEPARATION OF AMINO POLYCYCLIC AROMATIC HYDROCARBON ISOMERS

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SUMMARY

To date, only semi-detailed chemical analyses of biologically-active amino polycyclic aromatic hydrocarbons (amino-PAH) that occur in coal-derived materials have been achieved by capillary column gas chromatography. This is largely due to the increased number of possible isomers of the amino-PAH as compared to the analogous, non-functional, parent polycyclic aromatic compounds.

In this study, the separability of amino-PAH isomers on different capillary column stationary phases was investigated. Results are reported for a total of six stationary phases of varying selectivity and polarity. Binary stationary phase capillary column techniques were applied to optimize the resolution of nearly all of the three- and four-ring amino-PAH isomers.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) and nitrogen, oxygen and sulfur heterocyclic derivatives of PAH are the major classes of components found in coal-derived materials. In general, capillary column gas chromatography has served an increasingly important role in the detailed chemical characterization of these polycyclic aromatic compounds in complex coal liquids. Slightly polar gum phases such as SE-52 or SE-54 have been widely used as capillary column stationary phases for PAH separations because of their high efficiency and thermal stability[1,2]. Introduction of a heteroatom, such as nitrogen, either exocyclic or endocyclic to the parent PAH structure complicates capillary gas chromatographic analysis due to the increased number of possible isomeric compounds. For example, there are five possible nitrogen-containing endocyclic phenanthrene isomers: 1-, 2-, 3-, 4- and 9-azaphenanthrene.

In most cases, the conventional polymethylphenylsiloxane phases lack the selectivity and ability to achieve resolution of all possible isomers of substituted heterocyclic PAH. For these reasons the use of polar and/or selective phases to resolve isomeric heterocyclic PAH is currently receiving increased attention. For example, moderately polar stationary phases such as Superox 20M (polyethylene glycol) and methylpolysiloxane phases doped with liquid crystal components, such as N,N′-bis(*p*-

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butoxybenzilidene)-*a,a'*-bi-*p*-toluidine (BBBT) and N,N'-bis(*p*-methoxybenzilidene)-*a,a'*-bi-*p*-toluidine (BMBT), were recently used by Kong *et al.*[3] for the resolution of isomeric polycyclic aromatic sulfur heterocycles. Additionally, a mesogenic polysiloxane phase used by these same workers enabled the efficient separation of several isomeric PAH and sulfur-containing heterocyclic PAH[4]. Pluronic L64[5] (polyethylene-polypropylene glycol block copolymer) and Superox 20M[6,7] have also been applied successfully to the resolution of oxygen-containing phenolic aromatic compounds. Finally, new polysiloxane gum phases containing polarizable constituents such as phenyl[8] and cyanopropyl[9] appear promising for providing the added selectivity that these difficult isomeric separations require.

Of the numerous classes of PAH found in coal liquids, the amino polycyclic aromatic hydrocarbons (amino-PAH; a primary amino group attached exocyclic to the parent ring structure of the PAH) have recently been identified as the principal microbial mutagens in these materials (ref. 10 and references therein). Furthermore, microbial mutagenicity assays conducted with standard compounds of the three- and four-ring amino-PAH demonstrated that the biological potency of a given compound was a function of isomeric position[10]. To date, only semi-detailed chemical analyses of the amino-PAH in coal liquid isolates have been achieved via capillary column gas chromatography. The conventional polymethylsiloxane stationary phases that have been used in the past for the analysis of these amino-PAH isolates have proven inadequate, with several three- and four-ring amino-PAH coeluting[11,12].

This report deals specifically with the capillary column gas chromatographic resolution of the three- and four-ring amino-PAH isomers that have been isolated from coal-derived process materials; namely, the aminofluorenes, aminoanthracenes, aminophenanthrenes, aminopyrenes, and aminofluoranthenes. A variety of stationary phases are investigated here including methylpolysiloxane gum phases synthesized with varying amounts of cyanopropyl, phenyl, and biphenyl constituents, and a polyethylene glycol phase. The applicability of these stationary phases for resolving isomeric three- and four-ring amino-PAH is evaluated.

EXPERIMENTAL

Standard amino polycyclic aromatic hydrocarbons were obtained from various sources. 2-Aminofluorene, 9-aminophenanthrene, 1- and 2-aminoanthracene, 1-, 2-, and 4-aminopyrene, and 3-aminofluoranthene were purchased from Aldrich (Milwaukee, WI, U.S.A.). The 9-aminofluorene was purchased from Aldrich as the hydrochloride salt and the free amino compound was obtained by precipitation in aqueous base followed by ether extraction and drying. 1-, 3- and 4-aminofluorene, 1-, 2-, 3- and 4-aminophenanthrene, 9-aminoanthracene, and 1-, 2-, 7- and 8-aminofluoranthene were not commercially available, but were obtained from specially synthesized[13] stock from Professor Milton Lee of Brigham Young University. The purity of each synthesized standard compound was verified by mass spectrometry and capillary gas chromatography. All compounds tested were more than 95% pure except for 9-aminoanthracene which was found to be extremely unstable, readily forming 9-iminoanthracen-10-one. Consequently, only freshly prepared standards of this compound were used and care was taken to store it in the cold and dark.

Amino-PAH isolates were obtained from solvent refined coal (SRC) process

distillates using adsorption chromatography fractionation, drivatization, and gel permeation chromatographic procedures that have been described previously[11]. Identifications of amino-PAH in the coal liquids were confirmed by gas chromatography–mass spectrometry.

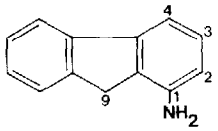
Capillary columns were prepared using 15–20-m lengths of 0.20-mm I.D. fused-silica capillary tubing (Hewlett-Packard, Avondale, PA, U.S.A.). To ensure maximum surface energy and wettability, and to expedite coating, no surface deactivation treatments were applied. Columns were statically coated at room temperature to an approximate film-thickness of 0.17 μm using the following stationary phases: SE-54 (5% phenyl, 1% vinyl polyphenylmethylsiloxane) and Superox 20M (polyethylene glycol) (Alltech Assoc., Deerfield, IL, U.S.A.); OV-17 gum (50% phenyl, 1% vinyl polyphenylmethylsiloxane) and OV-225 gum (25% cyanopropyl, 25% phenyl, 1% vinyl polyphenylmethylsiloxane) (Ohio Valley Specialty, Marietta, OH, U.S.A.); 76% cyanopropyl, 20% tolyl, 4% vinyl polysiloxane and 25% biphenyl, 1% vinyl polymethylsiloxane (newly synthesized phases of Professor Milton Lee of Brigham Young University). After coating, the polysiloxane stationary phases were stabilized by crosslinking with azo-*tert.*-butane[14].

Gas chromatography was accomplished using a Hewlett-Packard 5880A gas chromatograph equipped with a capillary split–splitless injection system and a flame ionization detector. Both isothermal and temperature programming modes of operation were used. In general, temperatures between 180°C and 220°C were used for isothermal analysis and temperature programming profiles of 50°C to 275°C at a rate of 3 or 4°C/min following 2 min at the initial temperature after injection. The injection system was operated in the splitless mode. Hydrogen was used as a carrier gas at a linear velocity of 100 cm/sec for the temperature programmed runs and 75 cm/sec for the isothermal runs. In all cases instrument sensitivity was adjusted to give full-scale peak deflection for less than 10 ng of sample component.

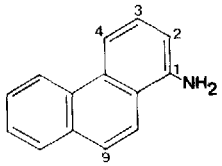
The retention time of each standard was obtained for each stationary phase from individual temperature programmed analyses (4°C/min). The resulting retention data were described in terms of a modified relative volatility term, α_{TP}, in which a ratio of the adjusted retention time was computed for each component as compared to the adjusted retention time of the first eluting isomer. Both modified capacity factor (k') values obtained under temperature programmed conditions and true isothermal k' values were computed. The elution time of methane was used for the dead time in k' calculations. Isothermal k' plots for various combinations of stationary phases were tabulated at 210°C. From these k' plots it was possible to determine optimum stationary phase combinations for maximum resolution of the solute components. These stationary phase combinations were achieved by either mixing the phases and then coating columns or by coupling columns in tandem. Connections were made using zero-dead-volume butt connectors (Supelco, Bellefonte, PA, U.S.A.).

RESULTS AND DISCUSSION

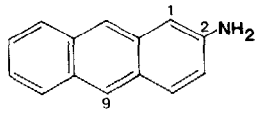
The molecular structures, names and abbreviations of the five isomeric groups investigated in this work are given in Fig. 1. Due to the very similar structural nature of these compounds, several coelute on conventionally used polymethylphenylsilox-



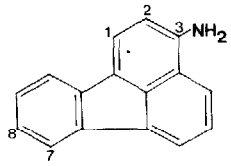
AMINOFLUORENES: AF



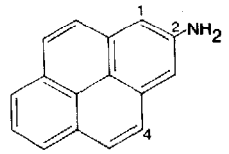
AMINOPHENANTHRENES: APh



AMINOANTHRACENES: AA



AMINOFLUORANTHENES: AFL



AMINOPYRENES: APy

Fig. 1. Structures, names and abbreviations of amino-PAH.

TABLE I

RELATIVE VOLATILITIES, α_{TP}, OF AMINOFLUORENES ON DIFFERENT STATIONARY PHASES

Compound	*SE-54*	*Biphenyl*	*Cyanopropyl*	*OV-225*	*OV-17*	*Superox*
1-Aminofluorene	1.014	1.016	1.039	1.031	1.015	1.026
2-Aminofluorene	1.040	1.036	1.064	1.053	1.039	1.054
3-Aminofluorene	1.024	1.020	1.039	1.031	1.022	1.034
4-Aminofluorene	1.000	1.000	1.000	1.000	1.000	1.000
[t_R actual (min)]	(27.10)	(37.58)	(39.11)	(35.17)	(33.98)	(42.88)
$\Delta\alpha_{TP}$	0.040	0.036	0.064	0.053	0.039	0.054
Δt_{real} (min)	1.08	1.34	2.50	1.85	1.33	2.32

TABLE II

RELATIVE VOLATILITIES, α_{TP}, OF THE AMINOANTHRACENES AND AMINOPHENANTHRENES ON DIFFERENT STATIONARY PHASES

Compound	*SE-54*	*Biphenyl*	*Cyanopropyl*	*OV-225*	*OV-17*	*Superox*
1-Aminophenanthrene	1.044	1.041	1.086	1.068	1.042	1.062
2-Aminophenanthrene	1.055	1.053	1.111	1.087	1.054	1.083
3-Aminophenanthrene	1.055	1.050	1.115	1.089	1.054	1.086
4-Aminophenanthrene	1.000	1.000	1.000	1.000	1.000	1.000
[t_R actual (min)]	(31.39)	(42.82)	(43.77)	(39.86)	(38.97)	(48.68)
9-Aminophenanthrene	1.044	1.045	1.086	1.068	1.043	1.065
1-Aminoanthracene	1.044	1.041	1.086	1.068	1.043	1.062
2-Aminoanthracene	1.065	1.063	1.119	1.095	1.063	1.091
9-Aminoanthracene	1.048	1.050	1.090	1.074	1.046	1.065
$\Delta\alpha_{TP}$	0.065	0.063	0.119	0.095	0.063	0.091
Δt_{real} (min)	2.05	2.70	5.22	3.80	2.45	4.45

TABLE III

RELATIVE VOLATILITIES, α_{TP}, OF AMINOFLUORANTHENES AND AMINOPYRENES ON DIFFERENT STATIONARY PHASES

Compounds	*SE-54*	*Biphenyl*	*Cyanopropyl*	*OV-225*	*OV-17*	*Superox*
1-Aminofluoranthene	1.028	1.025	1.050	1.043	1.028	–
2-Aminofluoranthene	1.022	1.021	1.036	1.030	1.021	–
3-Aminofluoranthene	1.037	1.034	1.060	1.052	1.038	–
7-Aminofluoranthene	1.000	1.000	1.000	1.000	1.000	–
[t_R actual (min)]	(38.17)	(51.06)	(53.26)	(48.33)	(46.42)	
8-Aminofluoranthene	1.022	1.018	1.034	1.027	1.021	–
1-Aminopyrene	1.055	1.055	1.062	1.061	1.059	–
2-Aminopyrene	1.049	1.051	1.056	1.053	1.053	–
4-Aminopyrene	1.043	1.047	1.041	1.043	1.047	–
$\Delta\alpha_{TP}$	0.055	0.055	0.062	0.061	0.059	–
Δt_{real} (min)	2.09	2.83	3.30	2.96	2.70	–

ane capillary column stationary phases. For example, 1-aminoanthracene, 1-aminophenanthrene, and 9-aminophenanthrene all coelute on SE-52. Hence, five stationary phases of varying selectivity and polarity were studied in an attempt to achieve better resolution of the three- and four-ring amino-PAH isomers. For comparison, all analyses were also conducted with crosslinked SE-54.

Since numerous measurements were made, and it would be impractical to show chromatograms for the separation of each isomeric group on each stationary phase,

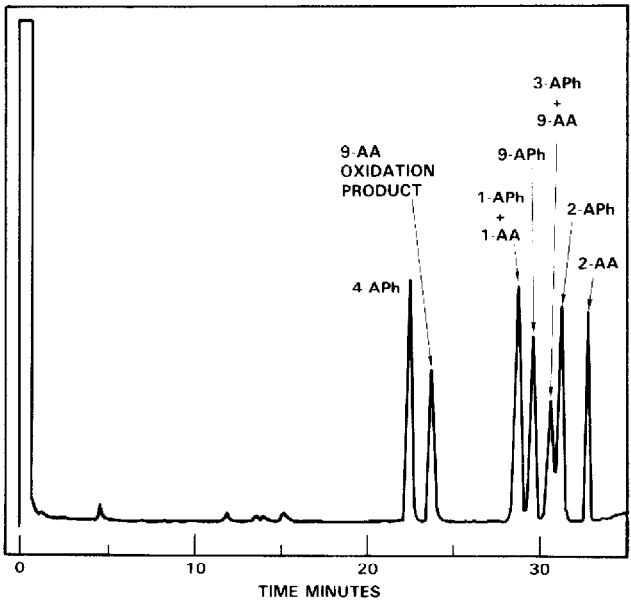


Fig. 2. Capillary gas chromatogram of an isothermal separation of aminophenanthrene and aminoanthracene isomers on the biphenyl stationary phase. Conditions: 16 m × 0.20 mm I.D., fused-silica capillary column; hydrogen carrier flow at 75 cm/sec; isothermal temperature, 175°C. For compound abbreviations see Fig. 1.

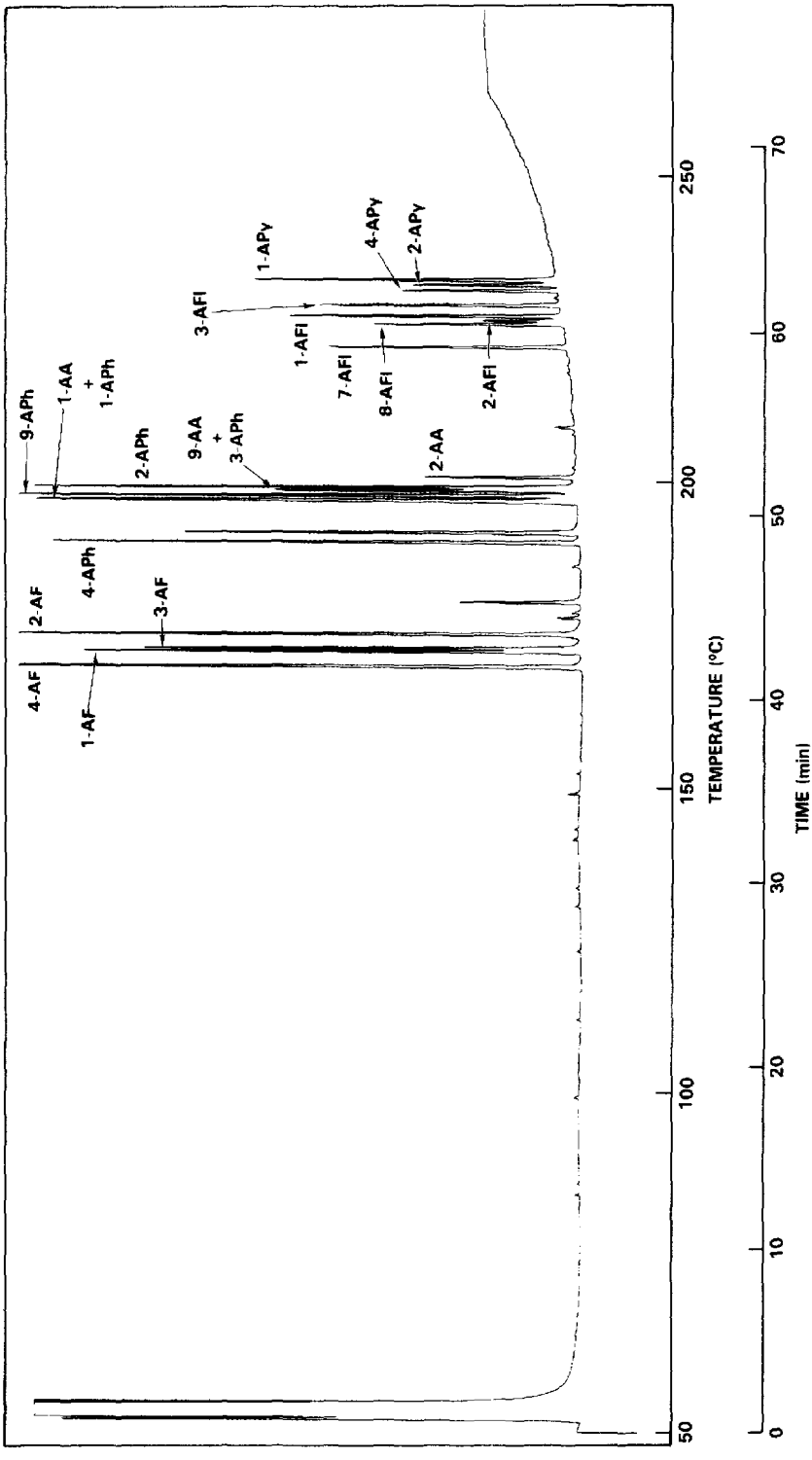


Fig. 3. Capillary gas chromatogram of a temperature programmed programmed separation of all five amino-PAH isomer groups on the biphenyl stationary phase. Conditions: capillary column same as Fig. 2; temperature programmed from 50°C to 275°C at 4°C/min following a 2-min isothermal time after injection; hydrogen carrier gas at a linear velocity of 100 cm/sec. See Fig. 1 for compound abbreviations.

a parameter was sought that could be easily used for the evaluation of these data. A term that could be used to describe the relative elution order and separability of the amino-PAH isomers on each phase was required. The relative volatility of each compound with respect to a reference appeared to be a reasonable comparison method. Therefore, a modified relative volatility term, α_{TP} (α obtained under temperature programmed conditions rather than isothermally), was defined to summarize the retention information.

Table I lists the relative volatilities of the four aminofluorene isomers tested on each of the six stationary phases. The reference for these particular α_{TP} measurements was the earliest eluting compound of this isomer group (*i.e.*, 4-aminofluorene) and was arbitrarily designated a value of 1.000. The absolute retention times of 4-aminofluorene on each phase are also given and, if desired, can be used to generate the actual retention time of each compound on each phase. For baseline resolution of two components to occur, a difference in α_{TP} values of greater than or equal to 0.010 units had to be observed. However, partial separation of components could be observed with a difference in α_{TP} of slightly less than 0.003.

The four aminofluorene isomers were separable on SE-54, biphenyl, Superox, and OV-17. The Superox phase retained the aminofluorene isomers the longest while they were retained the least on SE-54. The cyano-containing phases provided the largest separability in terms of Δt_{real} (time between first and last eluting isomer), but unfortunately the 1- and 3-aminofluorenes coeluted on both the cyanopropyl and OV-225 columns.

Similar comparisons and evaluations can be made for the other three- and four-ring amino-PAH compounds in Table II and III, respectively (4-aminophenanthrene and 7-aminofluoranthene were the first eluting compounds in these isomeric groups and were assigned values of 1.000). For the aminophenanthrenes and aminoanthracenes, no single column was capable of resolving all eight isomers. The

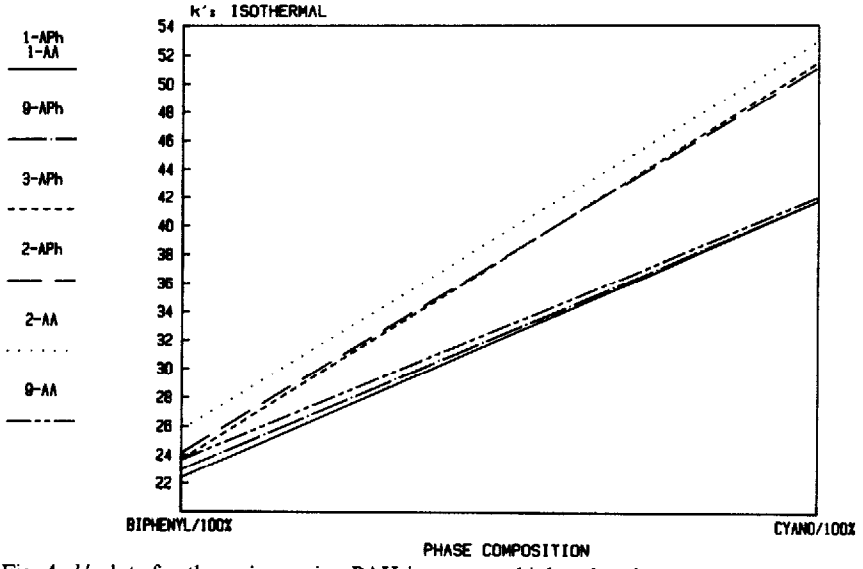


Fig. 4. k' plots for three-ring amino-PAH isomers on biphenyl and cyanopropyl stationary phases obtained isothermally at 210°C.

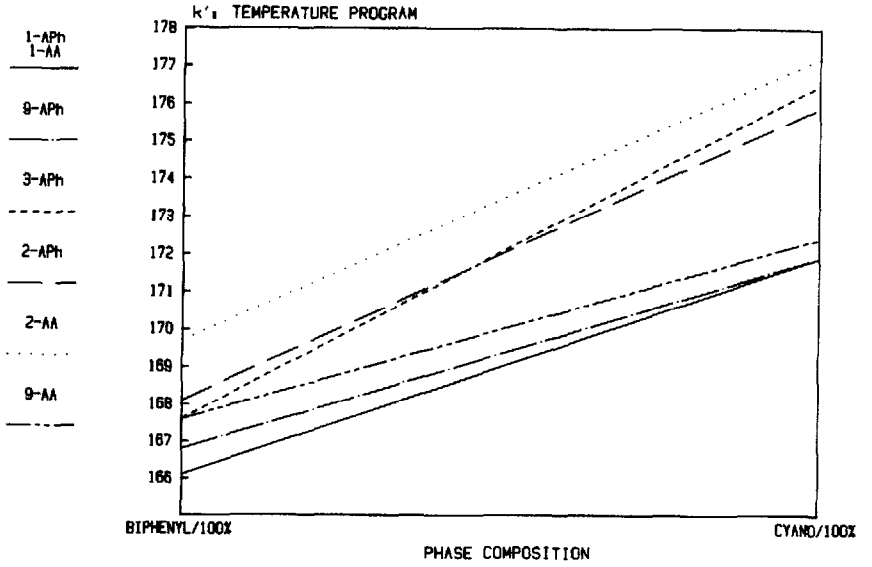


Fig. 5. Modified k' plots for three-ring amino-PAH isomers on biphenyl and cyanopropyl stationary phases obtained under a temperature program of 4°C/min.

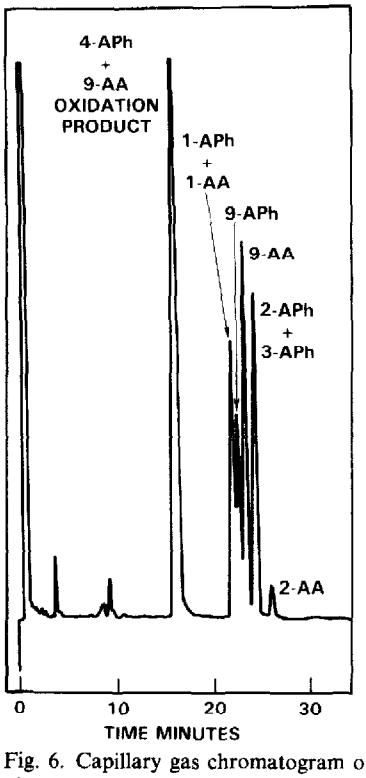
biphenyl and Superox columns provided the best separation for the aminophenanthrenes, with resolution of all five isomers. Although Superox resolved the aminophenanthrenes, this phase did not successfully elute the four-ring amino-PAH (see Table III) under these conditions and was, therefore, not considered further as a viable alternative for these separations. The biphenyl phase appeared to provide the best resolution of a mixture of all eight aminophenanthrene and aminoanthracene isomers; however, the 1-aminoanthracene and 1-aminophenanthrene did coelute, as did the 9-aminoanthracene and 3-aminophenanthrene (see Fig. 2). The cyanopropyl phase separated five of the eight isomers with 1- and 9-aminoanthracene and 1-aminophenanthrene coeluting in a single peak. Since the 9-aminoanthracene experiences a retention shift between the biphenyl and cyanopropyl phases, this suggests that a combination of these phases might enhance this separation (discussed later).

The aminofluoranthenes and aminopyrenes posed less of a problem in terms of isomer resolution than the three-ring amino-PAH. Two factors were of concern in this separation: (1) resolution of the coeluting 2- and 8-aminofluoranthene, and (2) maintaining adequate separation of the aminopyrene–aminofluoranthene groups. Perusal of Table III suggests that the biphenyl phase again provided the best separation. An example of the separation achieved for all five amino-PAH isomer groups using the biphenyl stationary phase is shown in Fig. 3.

Since the biphenyl and cyanopropyl stationary phases provided the best overall resolution of the amino-PAH isomers, a binary mixture of these phases was prepared. In order to determine the optimum binary phase composition that would maximize resolution, k' data (obtained at 210°C) for each isomer on both phases were plotted as illustrated in Fig. 4. A similar plot is shown in Fig. 5 in which modified k' values obtained from temperature programmed conditions were used. Since temperature programming is necessary in complex mixture analysis, it seemed more practical and

applicable to collect these modified k' values. Optimum elution temperatures for maximum efficiency are also obtained automatically during temperature programming. While these values are not strictly true k' values, it is evident from comparing Figs. 4 and 5 that essentially the same information was obtained. These plots suggest that a phase composition of 8–10% cyanopropyl in biphenyl would resolve 9-aminoanthracene from 9-aminophenanthrene while still maintaining the resolution between 2- and 3-aminophenanthrene. The larger the vertical spacing between the isomer k' lines (corresponding to relative volatility) the greater the separation The addition of only small amounts of cyanopropyl quickly changed the selectivity of the biphenyl phase (*e.g.*, steep slope of 3-aminophenanthrene line). A chromatogram of the eight three-ring isomers obtained on a column coated with a binary mixture of 10% cyanopropyl in biphenyl is shown in Fig. 6. The 9-aminoanthracene and 9-aminophenanthrene are resolved, but resolution is lost for the 2- and 3-aminophenanthrene pair and these compounds coeluted. This is probably due to the cyanopropyl concentration being slightly higher than optimal.

Another approach to obtain mixed phase effects is to couple columns of dif-



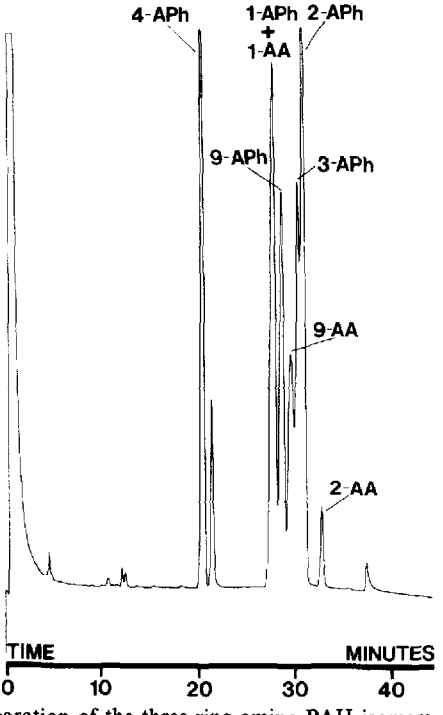


Fig. 6. Capillary gas chromatogram of an isothermal separation of the three-ring amino-PAH isomers obtained on a 10% cyanopropyl in biphenyl stationary phase mixture. Conditions: the same as Fig. 2 except for isothermal at 190°C. For compound abbreviations see Fig. 1.

Fig. 7. Capillary gas chromatogram of an isothermal separation of the three-ring amino-PAH isomers obtained with a cyanopropyl–biphenyl coupled column (*ca.* 8% cyanopropyl by length). Conditions: 1.2 m × 0.20 mm I.D. fused-silica capillary column coated with cyanopropyl stationary phase coupled to a 16 m × 0.20 mm I.D. fused-silica capillary column coated with biphenyl stationary phase; hydrogen carrier gas at 75 cm/sec; isothermal temperature, 180°C. For component abbreviations see Fig. 1.

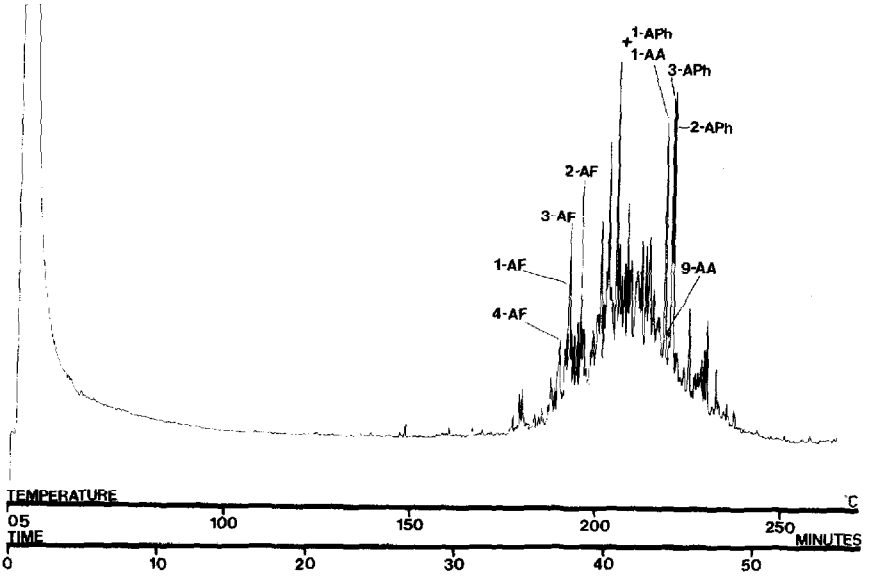


Fig. 8. Capillary gas chromatogram of an amino-PAH fraction of a coal liquid. Conditions: same column as described in Fig. 7; temperature programmed from 50°C to 275°C at 4°C/min following a 2-min isothermal time after injection; hydrogen carrier gas at a linear velocity of 100 cm/sec. Compound abbreviations the same as Fig. 1.

ferent phases in series. Consequently, 1.2 m (*ca.* 8% by length) of cyanopropyl column was connected to a biphenyl column (16 m). A chromatogram obtained on this coupled column is shown in Fig. 7. The slightly lower concentration of cyanopropyl successfully resolved (partially) the 2- and 3-aminophenanthrene and the 9-aminoanthracene and 9-aminophenanthrene isomers. An application of this column is demonstrated in Fig. 8 where a chromatogram of the amino-PAH isolate of a coal liquid is shown.

This work demonstrates a practical approach to the separation of coeluting isomeric compounds. In addition to using currently available stationary phases, an approach is suggested and demonstrated for "tailor-making" stationary phases of proper composition and polarity for resolving a specific separation problem.

ACKNOWLEDGEMENTS

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