SEPARATION AND CHARACTERIZATION OF THIA-ARENES AND HIGH MASS POLYCYCLIC AROMATIC HYDROCARBONS IN COAL TAR

SEPARATION AND CHARACTERIZATION OF THIA-ARENES AND HIGH MASS POLYCYCLIC AROMATIC HYDROCARBONS IN COAL TAR

By

Chun-Ling Li, M. Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements
for the Degree

Master of Science

McMaster University

MASTER OF SCIENCE (1997)	**MCMASTER UNIVERSITY**
(Chemistry)	**Hamilton, Ontario**

TITLE:	**Separation and Characterization of Thia-arenes and High Mass Polycyclic Aromatic Hydrocarbons in Coal Tar**
AUTHOR:	**Chun-Ling Li, M. Sc. (Lanzhou University), B.Sc. (Beijing Normal University)**
SUPERVISOR:	**Dr. B.E. McCarry**
NUMBER OF PAGES:	**XIV, 150**

ABSTRACT

Coal tar is a rich source of polycyclic aromatic compounds (PAC) which include hydrocarbons (PAH), sulfur-containing aromatics (PASH), nitrogen-containing aromatics and high molecular mass PAH. The separation of coal tar into four fractions was carried out on activated (170^0C, 48 hrs) neutral alumina. Low mass aromatics and aliphatics were eluted with hexane in fraction A1 (2.2% recovery), while PAH/PASH were eluted by benzene in fraction A2 (25%). High molecular mass PAH were eluted by dichloromethane in fraction A3 (5.5%) and nitrogen-containing aromatics were eluted by methanol in fraction A4 (21%). These fractions were characterized by normal phase liquid chromatography, GC-MS, probe mass spectrometry (for fractions A3 and A4) and LC-MS (for fraction A3).

While ratio of PASH to PAH in fraction A2 was rather unfavorable (25:1), the separation of PASH from PAH was attempted using two methods: an oxidation/reduction method and a ligand exchange method. The oxidation/reduction method of Lee was a complete failure. The $PdCl_2$-silica method of Nishioka was partially successful. Of the 25 thia-arenes identified in fraction A2, 12 eluted with the PAH while the other 13 were either partially (5) or fully (8) retained by the $PdCl_2$-silica gel column and then eluted later to give a sulfur-enriched fraction. This fraction was further separated using normal phase HPLC to afford sulfur-containing compounds with molecular masses between 184 amu and 258 amu which exhibited almost no contamination due to PAH. Thus, it would be possible to purify

about one-half of the PASH from a complex mixture such as coal tar.

Fraction A3 which contained high mass PAH was subjected to semi-preparative normal phase HPLC to afford several high molecular mass PAH fractions. The 326 amu PAH fraction was selected because it contributed to 10% of the genotoxic of the mixture. This fraction was further separated by normal phase HPLC and the subfractions were analyzed by reversed-phase HPLC with diode-array detection. Thirty-five peaks were collected from the reversed-phase HPLC analysis and were characterized by probe mass spectrometry and fluorescence spectroscopy (in part). Of the 35 peaks, 8 peaks were eluted in the mutagenic active range; these eight compounds will be evaluated using the Ames assay to determine which are active mutagens.

ACKNOWLEDGEMENTS

I would like to express my deepest and most sincere gratitude to Dr. Brian E. McCarry for his supervision, guidance, patience, and understanding throughout the course of this study.

I wish to thank Dr. D.W. Bryant and Dr. Chris H. Marvin for their helpful discussions and suggestions. I would like to thank my colleagues: Laurie Allan, Adrienne Boden for their constant help and support and for providing a very enjoyable working atmosphere.

I would also like to acknowledge the contributions of Dr. A. Legzdins and J. Villella, with all of whom I have had the pleasure of working.

The technical support of mass spectrometry lab, spectroscopy lab and the financial support of the Ontario Ministry of Environment and Energy and McMaster Eco-research program is greatly appreciated.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACN	Acetonitrile
amu	Atomic mass units
Avg.	Average
BeP	Benzo[e]pyrene
BaP	Benzo[a]pyrene
BbF	Benzo[b]fluoranthene
BkF	Benzo[k]fluoranthene
BjF	Benzo[j]fluoranthene
Conc.	Concentration
DEI	Direct electron impact
DCI	Direct chemical ionization
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
i.d.	Internal diameter
Int. Std.	Internal standard
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry

m/z	Mass to charge ratio
MW	Molecular weight
NPLC	Normal phase liquid chromatography
N.D.	Not detectable
N-PAC	Nitrogen-containing polycyclic aromatic hydrocarbons
PAC	Polycyclic aromatic compounds
PAH	Polycyclic aromatic hydrocarbons
PASH	Sulfur-containing polycyclic aromatic hydrocarbons
Per	Perylene
R.I.	Retention index
RPLC	Reversed phase liquid chromatography
S.I.M.	Selected ion monitoring
STD.	Standard deviation
Temp.	Temperature
US EPA	United States Environmental Protection Agency
UV	Ultraviolet

I. INTRODUCTION

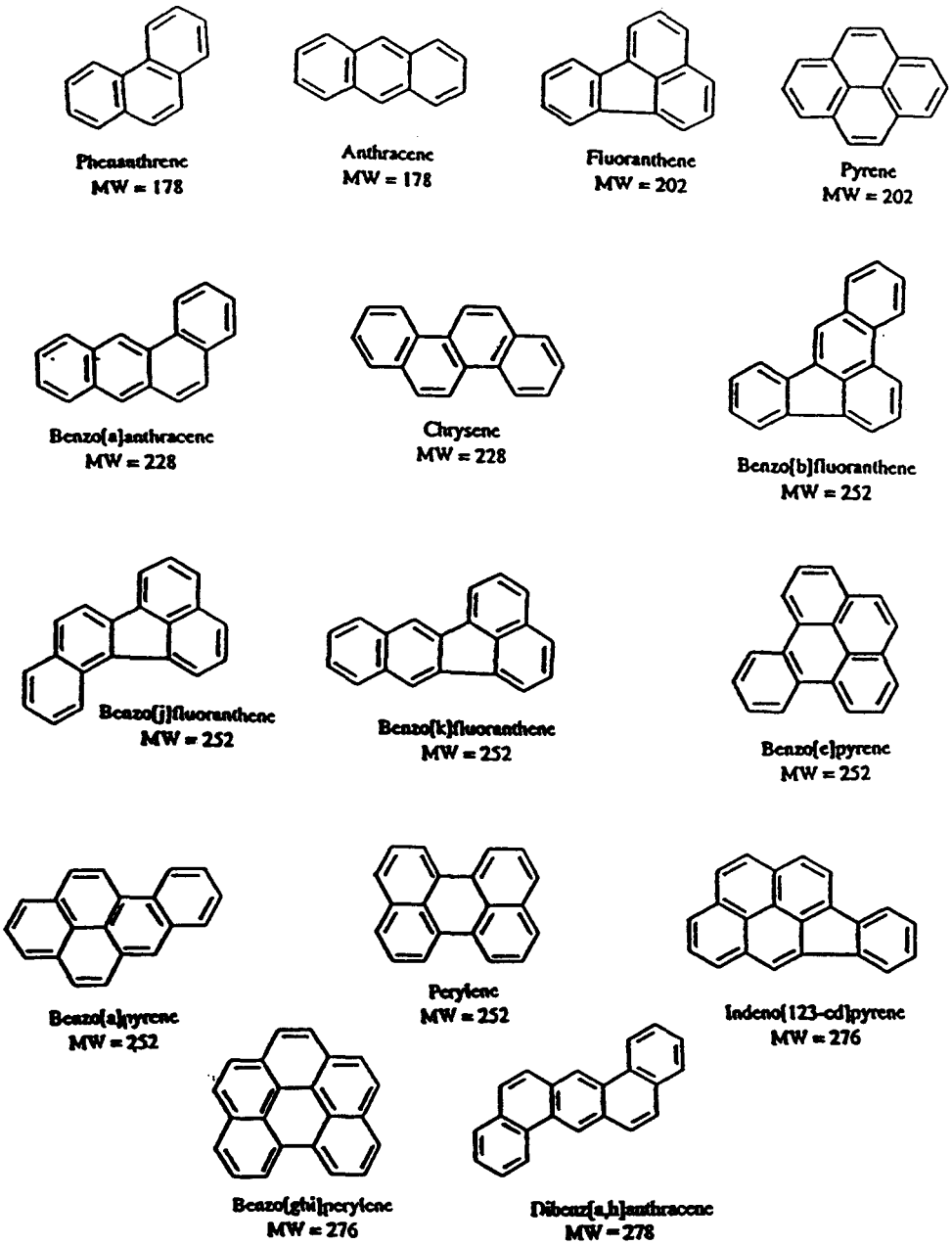
I. 1 Coal Tar and Its Health Effects

I. 1. 1 Coal Tar

Coal tar is a condensate derived from heating coal at high temperatures in the absence of air (1). Coal tar is produced by various industries; the steel industry produces coal tar as a by-product of the carbonization of coal in coke ovens. Distillation of crude coal tars produces various classes of coal tar derivatives such as naphthalene, creosote, anthracene oils and coal tar pitch, which have a variety of commercial uses (2). The organic chemical industry had its origins in using the products of coal tar refining as chemical feedstocks during the nineteenth century; this industry made a commercial impact by the production of the first synthetic dyestuffs (3). Tar processing grew, and before the Second World War, several industries were almost entirely dependent upon coal tar for their existence. For example, industries producing resins and plastics, dyestuffs, explosives, solvents, wood preservatives and disinfectants were all relied on coal tar as a feedstock. Since the war, coal tar has largely been replaced by cheaper petrochemical feedstocks, but now this position is changing again as crude oil becomes more expensive (4).

Coal tar is a complex mixture of organic compounds, principally aromatic compounds, many of which are polycyclic aromatic compounds (PAC); this class includes

Figure 1. Examples of PAH which are recognized as priority pollutants



polycyclic aromatic hydrocarbons (PAH), sulfur-containing PAC (thia-arenes or PASH) and nitrogen-containing PAC (aza-arenes) (1, 4). The level of aromatic compounds in coal tar is determined mainly by the temperature of carbonization and type of oven and, to a lesser extent, by the coals used (3). For example, there is 38% of aromatics in the coal tar produced from low temperature carbonization (400-750^{0}C); while there is about 25% of aromatics in the coal tar produced from high temperature carbonization (900-1100^{0}C). The compositions of the aromatics are also different from one sample to another. Noel *et al.* (5) determined the concentration of PAH in two coal tars. They found the total PAH values (the sum of the concentrations of 14 measured PAH) were 12.7 and 21.5% respectively. In the coal tar Standard Reference Material (SRM 1597), the concentrations of PAH and PASH are 35.3 and 1.9% (6). It is these polycyclic compounds, particularly the PAH, which are believed to be the principal chemical substances responsible for the carcinogenic properties of coal tar and therefore it is these substances which are believed to pose the greatest potential threat to human health and to the environment. Figure 1 shows some of homocyclic PAH which are classified as "priority pollutants" by the US EPA.

Other compound classes such as aromatic acids and aromatic bases are also found in coal tar (1, 4). Aromatic acids, also called tar acids, is a general term for all the phenolic compounds in coal tar, of which the most important are phenols, the cresols and the xylenols. The level of tar acids ranges from 3 to 29%. Tar acids can be isolated from coal tar by

distillation and/or by extraction with base. The separated tar acid salts are acidified and then distilled into fractions which are converted chemically into weed-killers, disinfectants, insecticides, plasticisers, anti-oxidation agents, mineral flotation agents, resins and even vitamins (3).

The term aromatic base is generally meant to include pyridine and its alkyl homologs and also quinoline and alkyl homologs, which are but benzo derivatives of pyridine itself. In addition, aniline and homologs of the pyrrole series are also classed in the tar base category. Carbazole is the only coal tar pyrrole derivative which has attained industrial importance. The aromatic base content in coal tar ranges from 1.8 to 2.0% (3).

I. 1. 2 Health Effects of Coal Tar

As early as 1775, Dr. Percivall Pott (7) reported a rare form of scrotal cancer found exclusively in chimney sweeps. He speculated that the cancer was associated with some element contained in the soot from the coal burning chimneys or coal tar. Many researchers have identified carcinogenic substances in coal, in its products and in pollutants from coal processing. A wide range of carcinogens and potential carcinogens has been found, and their carcinogenicity has been postulated and/or confirmed on the basis of animal experiments and epidemiological studies (1, 2). The most important and abundant carcinogens in coal tar are among the PAH.

In 1921 PAH were first postulated as the agents primarily responsible for the carcinogenicity of coal tar (8). In 1930 Hiege (9) demonstrated the carcinogenicity of dibenz[a,h]anthracene; in 1933 benzo[a]pyrene (BaP) was isolated from coal tar and was shown to be a carcinogen. Subsequent research has established that dibenz[a,h]anthracene and benzo[a]pyrene are only two of the many carcinogenic PAH in coal tar. The World Health Organization's International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence that coal tars are carcinogenic in humans (10) and has classed coal tar as a Class I carcinogen.

Coal tar (and fractions prepared from it) have been shown to cause mutations in bacteria and to cause skin tumours in rats. When pharmaceutical grade coal tar is used in the treatment of psoriasis, it can cause a skin rash which is one manifestation of the toxic effects of coal tar on human beings. Skin effects include dermatitis, chronic tar dermatosis with permanent skin changes and phototoxic skin reactions such as intense burning sensations upon exposure to sunlight. Burning sensations of the eyes and photophobia also can occur (10).

I. 2 Coal Tar Contamination in Hamilton Harbour

Hamilton Harbour is a heavily industrialized region at the western end of Lake Ontario. Major sources of contaminants in the harbour include industrial effluents from the

steel mills and other industries, roadway runoff, and treated municipal sewage. The metropolitan area includes the cities of Hamilton, Ancaster, Dundas, Flamborough and Burlington with a combined population of about 500,000 people. The southeast shore of the harbour is dominated by the Steel Company of Canada (Stelco) and Dominion Foundry and Steel Company (Dofasco) steel mills; these are two of the largest integrated steel operations in the nation (Figure 2).

Sediments throughout Hamilton Harbour are contaminated to varying degrees with contaminants which include metals, oil and grease, polychlorinated biphenyls (PCBs) and PAH. PAH listed in Figure 1 are commonly identified in harbour sediments. Within the last 15 years, several researchers have attempted to quantitate PAH in sediments throughout Hamilton Harbour. Poulton (11) examined bottom sediments at various sites located in the harbour and in Windermere Arm. It was found that the total PAH values (the sum of the concentrations of six measured PAH) ranged from 8-50 μg of PAH per gram of sediment. These values were 2-3 orders of magnitude higher than in several Lake Ontario sediment samples.

Not surprisingly, the highest concentrations of PAH were found near the steel mills. Mayer *et al* (12) studied suspended sediments (collected by Westfalia centrifuge) as well as bottom sediments throughout the harbour. They reported total PAH values (in this case, the sum of 16 PAH) between 4-106 μg/g sediment for the suspended sediments and 26-90 μg/g

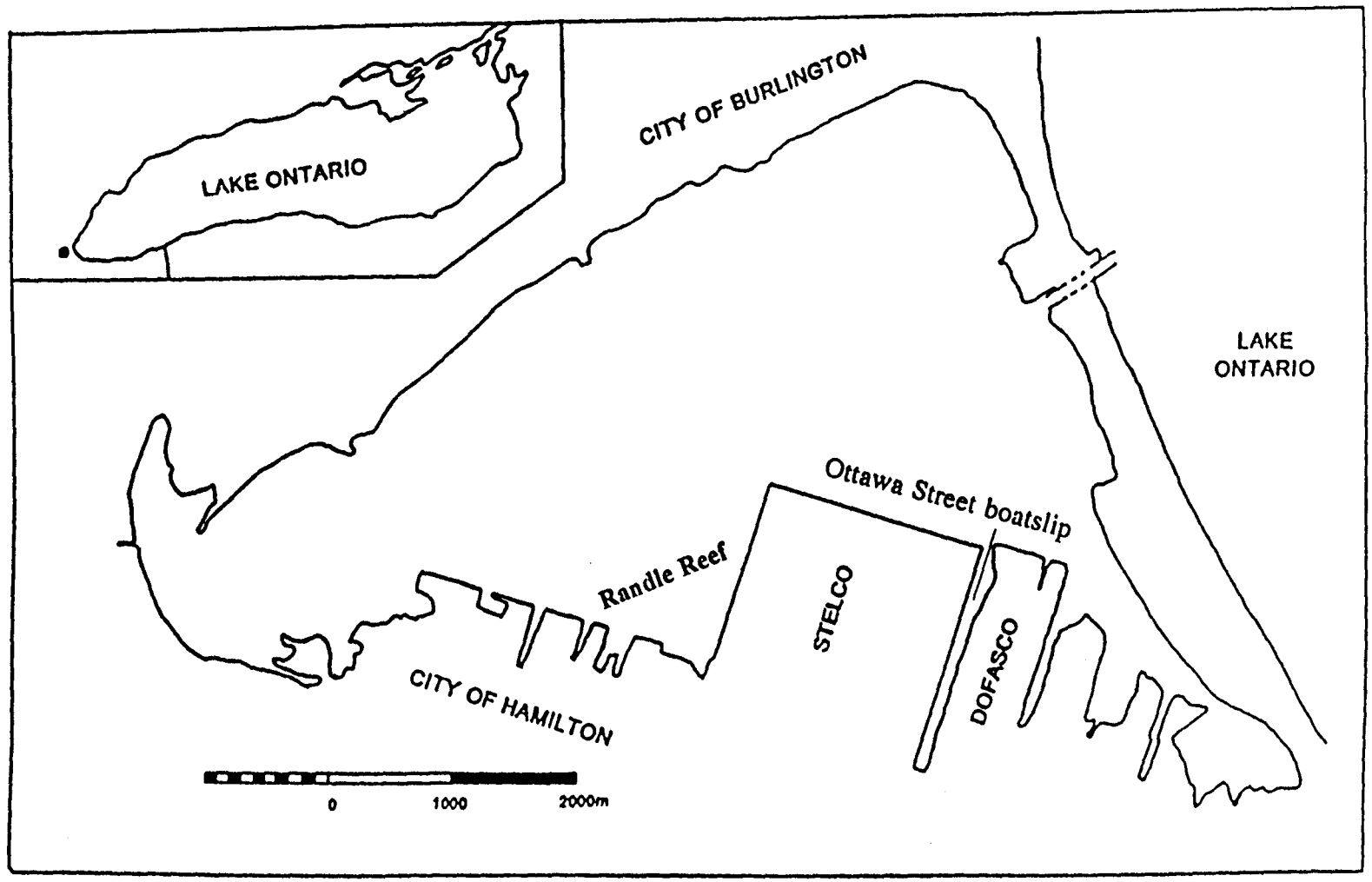


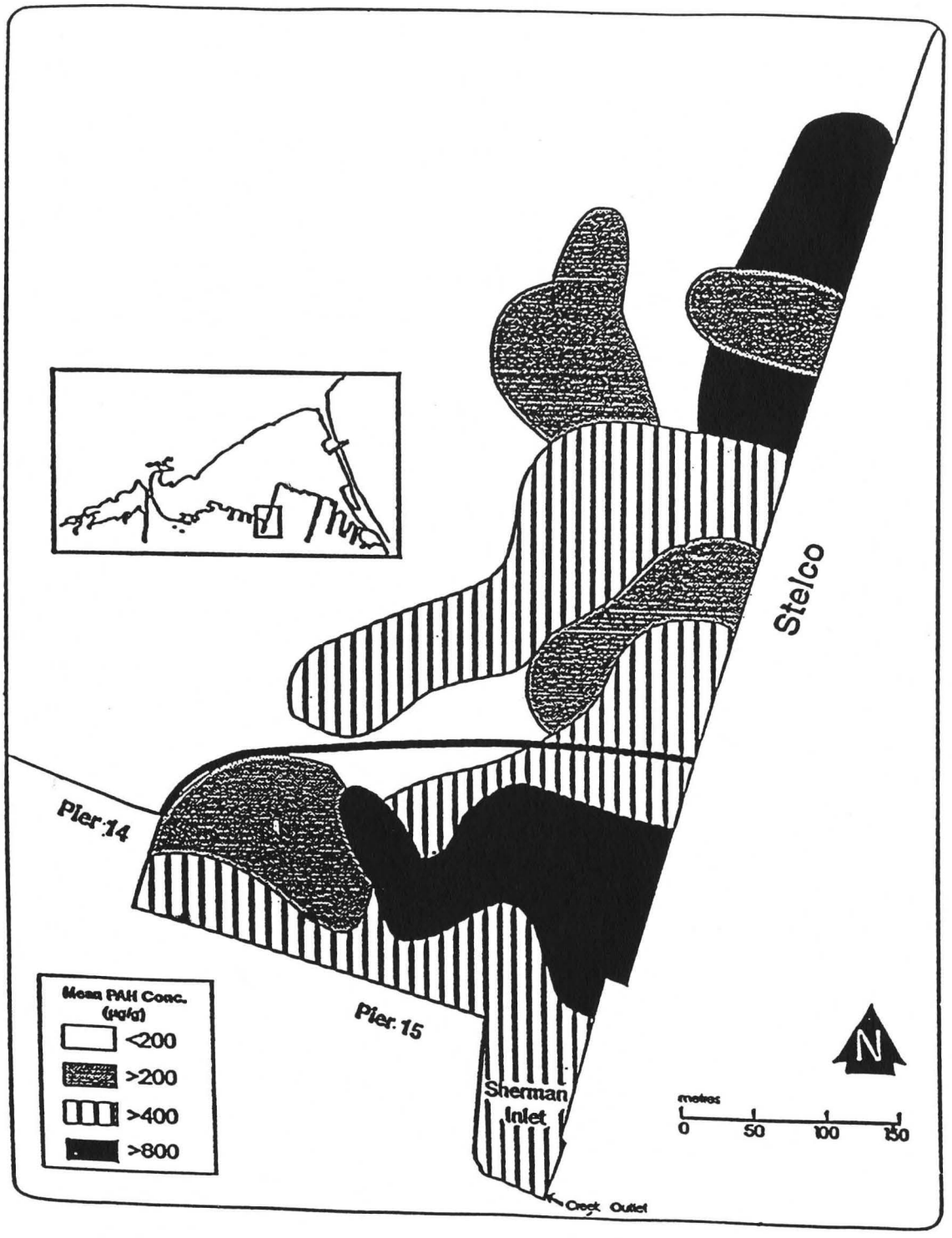
Figure 2. Map of Hamilton Harbour

sediment for the bottom sediments. Again, these values were 1-2 orders of magnitude higher than PAH concentrations in comparable sediments from the Great Lakes; the highest concentrations were observed near the industrial areas. The Ontario Ministry of the Environment and Energy (MOEE) studied the polycyclic aromatic hydrocarbon composition of six sediment samples collected from different sites in the harbour and determined that the highest concentrations were near Randle Reef (13).

The coal tar contamination in the sediments is the result of accidental or deliberate spills of coal tar into the harbour from areas near the coking operations of the steel mills. The highest concentrations of PAH are found near Stelco's coke ovens in sediments near Randle Reef and near Dofasco's coke ovens in sediments in the Ottawa street boat slip. Figure 3 shows PAH levels in bottom sediments collected near the Randle Reef " hotspot" (11). Levels of PAH exceeded 800 part per million in about a third of the samples taken to date within the site. Resuspension of these contaminated bottom sediments would result in the redistribution of coal tar throughout the harbour. Hamilton Harbour has been designated as an Area of Concern by the Water Quality Board of the International Joint Commission.

The coal tar contamination in sediments near Randle Reef was studied by Murphy (13) and Marvin *et al* (14, 15). They found a significant correlation between the PAH concentration and the acute toxicity and the gentoxicity of the sediments. Members from our research group have performed both chemical and biological analyses on organic extracts

Figure 3. Mean concentrations of PAH near Randle Reef [11].



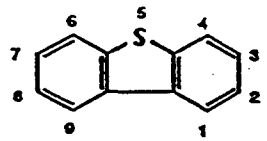
from coal tar contaminated bottom sediments collected near Randle Reef and from Sydney Harbour, Nova Scotia (14, 15). Sydney Harbour is close to the operations of the Sydney Steel Company and sediments near the old coking plant are very heavily contaminated with PAH (about 10,000 μg/g). The mutagenic responses of these sediments determined using the Ames assay (a microbiological reversion assay using a bacterial strain sensitive to PAH) were shown to correlate very well with the PAH content as determined by GC-MS analyses.

I. 3 Sulfur-containing Polycyclic Aromatic Compounds (PASH)

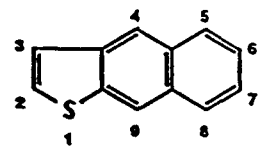
Sulfur-containing polycyclic aromatic compounds or thia-arenes (PASH) are found in many samples which contain PAH but at levels much lower than PAH. These compounds have been identified in petroleum and coal products, combustion emissions, sediments, air particulates, tobacco smoke, organisms inhabiting polluted water and other samples (16). The sulfur content of coal and crude oil varies from 0.2 to 12 wt% (17). For air quality reasons there is a considerable interest in the removal of sulfur from fossil fuels in order to decrease sulfur dioxide emissions. Some PASH may share the carcinogenic and/or mutagenic properties of PAH (16) and are therefore of relevance for health reasons.

Figure 4 shows the structures and molecular masses of some representative PASH which are present in environmental samples. PASH differ from PAH in that one of six-membered aromatic rings in a PAH is replaced by a five-membered thiophene ring in PASH.

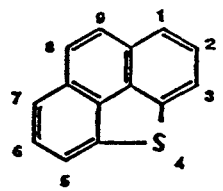
Figure 4. Examples of PASH in environmental samples



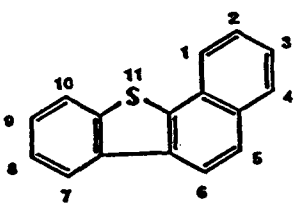
Dibenzothiophene
MW=184



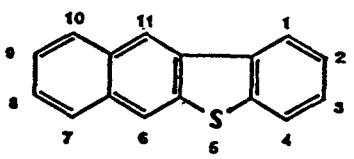
Naphtho[2,3-b]thiophene
MW=184



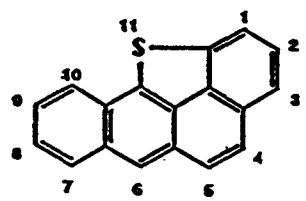
Phenanthro[4,5-bcd]thiophene
MW=208



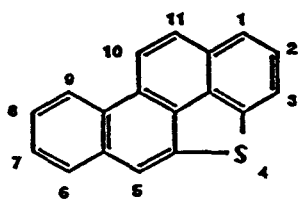
Benzo[b]naphtho[2,1-d]thiophene
MW=234



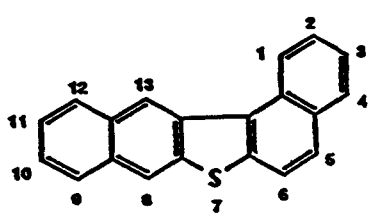
Benzo[b]naphtho[2,3-d]thiophene
MW=234



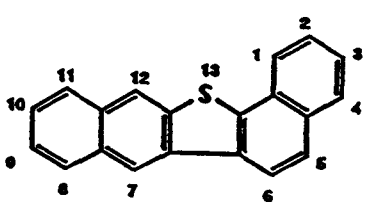
Benzo[2,3]phenanthro[4,5-bcd]thiophene
MW=258



Chryseno[4,5-bcd]thiophene
MW=258



Dinaphtho[2,1-b:2',3'-d]thiophene
MW=284



Dinaphtho[1,2-b:2',3'-d]thiophene
MW=284

The thiophene ring is aromatic and thus has similar properties to the benzene ring.

Although attempts have been made to analyze PASH for more than half a century, efforts have been hampered by the following factors:

1) PASH occur as minor or trace constituents relative to the levels of PAH. For example, a coal tar Standard Reference Materials (SRM) from the National Institute of Science and Technology, U.S.A., SRM 1597 was analyzed using gas chromatography-mass spectrometry (GC-MS). The ratio between the 3-ring PAH (molecular weight 178) to the 3-ring PASH (MW 184) was about 25:1 (6). Similarly, an urban air particulate reference material (SRM 1648) was found to have a ratio of the 4-ring isomers (MW 228 PAH to MW 234 PASH) about 7:1 (18).

2) The chromatographic properties of PASH are very similar to those of PAH.

3) The presence of the thiophene ring lowers the symmetry of a PASH compared to a comparable sized PAH; thus, there is a larger number of possible isomeric structures for PASH than for PAH. For example, in cata-condensed systems, there are five 4-ring PAH isomers, but thirteen 4-ring PASH isomers; there are twelve 5-ring PAH isomers, but fifty one 5-ring PASH isomers (19).

4) There are an even larger number of possible alkylated isomers among the PASH than among the PAH. For example, there are 29 monomethylated 4-ring PAH isomers but 130 monomethylated 4-ring PASH isomers (19).

The four points listed above make the analysis of PASH more challenging than the analysis of PAH. The identification and quantification of PASH in a complex environmental mixture is made more difficult due to a lack of authentic standards. Different approaches have been taken but none has proved to be completely successful. The first two problems listed above may, in principle, be overcome in one of two ways, either through the use of a sulfur-selective detection system or by a complete separation of the PASH from PAH. Both approach have been attempted.

A variety of sulfur-selective instrument detectors have been developed. The earliest method is that of Nishioka *et al.* (20), who employed gas chromatography (GC) with a flame-photometric detector (FPD) for the selective detection of sulfur compounds in heavy oils. A more recently developed GC technique, employing an atomic emission detector for the selective detection of sulfur-containing compounds, has also been applied to the problem (21). In all of these techniques, the ability of the chromatographic column to separate the PASH isomers (points 3 and 4 above) remains a problem.

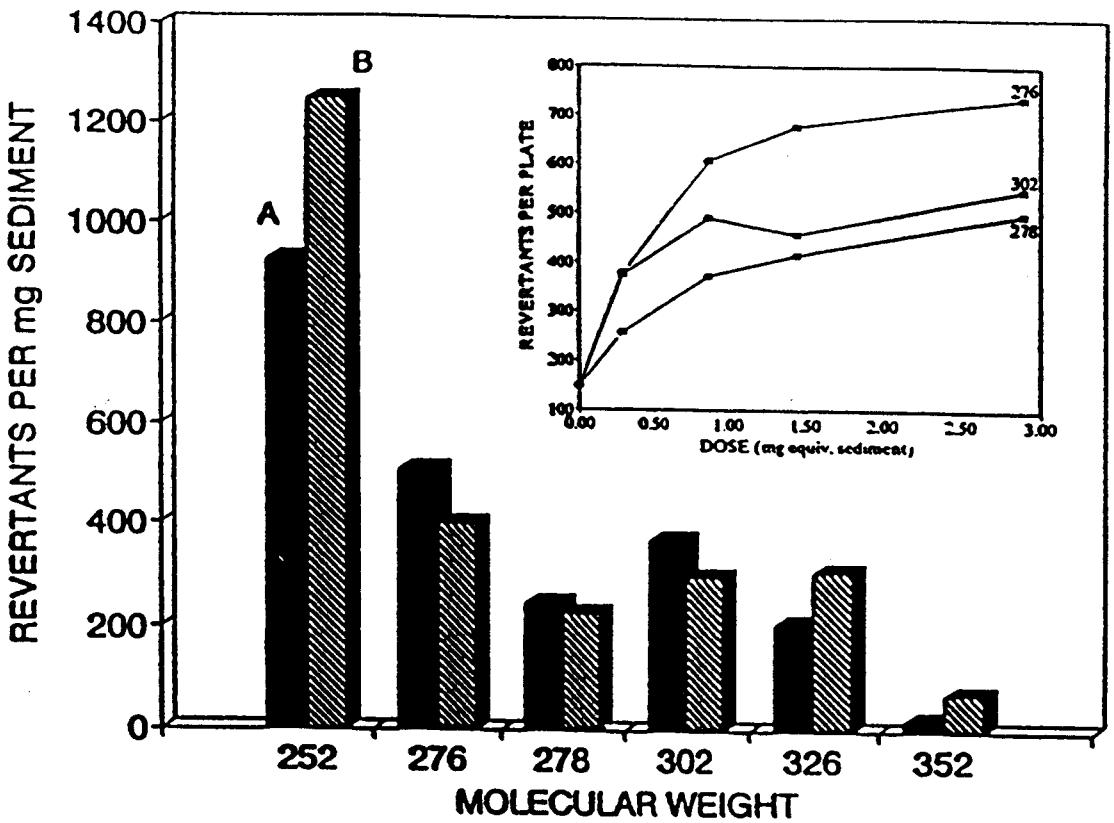
Another strategy for the analysis of PASH involves their separation as a class of compounds from PAH. Both ligand-exchange chromatographic approaches and oxidation/reduction procedures have been used and will be discussed in Section **I. 5 (Methodology).**

I. 4 High Molecular Mass PAH in Coal Tar

Polycyclic aromatic hydrocarbons with molecular masses greater than 300 amu are prevalent in a variety of matrices including carbon blacks, petroleum still bottoms, coal tar, asphalts and urban air particulates. A number of these compounds have been demonstrated to be potent mammalian carcinogens. Grimmer *et al* (22) estimated that PAH containing 6 and more rings were potentially responsible for over 25% of the carcinogenic potential of diesel particulate, gasoline-engine particulate and coal combustion particulate.

Members of our research group have identified genotoxic PAH in extracts of coal tar-contaminated sediments and urban air particulate material by using a bioassay-directed fractionation methodology (23, 24, 25). In these studies, a PAH-rich fraction from a coal tar-contaminated sediment was separated by normal phase HPLC into subfractions containing individual molecular mass classes of PAH; these fractions were then subjected to GC-MS analyses and to biological tests using the Ames *Salmonella* assay. Subfractions containing PAH with molecular masses between 252 and 326 amu exhibited positive mutagenic responses (Figure 5); other fractions, e.g., the 178, 202 and 228 amu fractions, showed almost no response in this assay. The 302 and 326 molecular mass PAH accounted for 15% and 10 %, respectively, of the total biological activity of the sample. This work showed that these classes of PAH are significant contributors to the genotoxic effects of complex PAH mixtures. The 302 and 326 amu classes of PAH encompass molecules containing 6, 7 and

Figure 5. Mutagenic potencies of PAH fractions separated from coal tar-contaminated sediment by NPLC.



Bar diagram showing the relative mutagenic potencies of the PAH fractions separated using normal phase HPLC, and then bioassayed with strain YG1025 (TA100-like strain) in the presence of 4% (bar A) and 10% (bar B) rat liver supernatants. Inset shows dose-response curves exhibited by some of the PAH fraction (with molecular masses identified by the accompanying numbers) when assayed in the presence of 4% S9.

8 rings. It is curious that these fractions accounts for 25% of the Ames assay response of coal tar and that Grimmer (22) estimated that PAH in this size range accounted for 25% of the skin tumors in rats caused by coal tar PAH.

In order to identify those PAH in the 326 amu class which were responsible for the Ames bioassay response, a sample of this 326 amu fraction was analyzed by reversed phase HPLC and the eluent collected in 30-second fractions; each fraction was then analyzed using the Ames bioassay (23). Figure 6a showes a histogram of the mutagenic activity data for the 326 MW PAH fraction analysis while Figure 6b is the reversed phase liquid chromatogram (RP-HPLC) for this fraction. The resulting "mutachromatogram" for the 326 MW fraction identified components which eluted in the 28-29.5 and the 31.5-32 minute ranges, the two shaded areas on the chromatogram, as being responsible for the majority of the mutagenic activity of this sample. Identification of the specific compounds responsible for the biological responses in the two shaded areas is one of goals of this thesis.

Despite the demonstrated biological importance of some higher molecular mass PAH, the determination and identification of these compounds is seldom undertaken. The reasons for this include:

1) The large number of possible isomers for the high molecular mass PAH places greater demands on chromatographic resolution and spectroscopic specificity. The resolution of conventional GC and HPLC is inadequate for the analysis of most PAC mixtures that

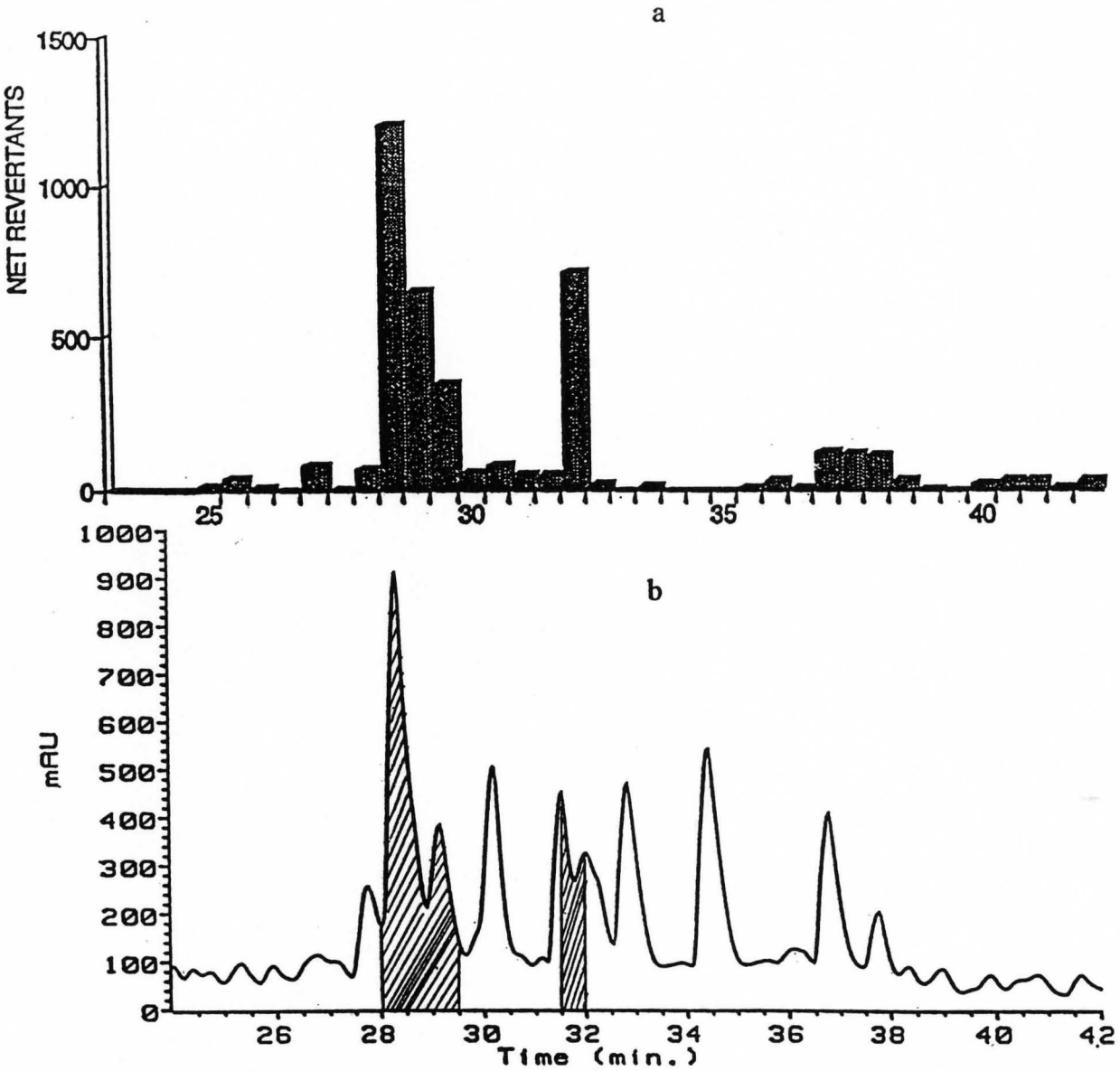


Figure 6a. Mutachromatogram of the 326 amu PAH fraction

Figure 6b. RP-HPLC chromatogram for the 326 amu PAH fraction

contain components with molecular masses greater than 300 amu.

2) High molecular mass PAH are generally present in environmental mixtures at much lower levels than lower molecular weight PAH.

3) There is a paucity of readily available authentic standards of higher molecular mass PAH for obtaining UV/Vis absorption spectra, fluorescence spectra, retention index data and biological/toxicological studies.

4) The biological effects of high molecular mass PAH have commonly been thought to be of lesser importance than those of the 16 priority pollutant PAH (as designated by the US EPA and Environmental Canada).

The biological responses of high molecular mass PAH derived from coal tar demonstrates the need for the development of methodologies that are capable of resolving and identifying these compounds.

I. 5 Methodology

I. 5. 1 Separation Schemes for Coal Tar Analysis

In order to identify individual components in complex mixtures such as coal tar, some type of clean-up or prefractionation is required prior to the analysis step. Some separation schemes have been developed which use solvent partitioning methods (26, 27); others use adsorption chromatography approaches (28, 29). Recently, planar chromatography was used

to fractionate coal tar (30).

A solvent partitioning procedure (26, 27) for PAH analysis of coal tar involves dissolving coal tar in iso-octane and then partitioning this solution sequentially with 1 M HCl, 1 M NaOH and dimethylsulfoxide. Interfering compounds such as phenols and nitrogenous bases were removed by the HCl and the NaOH, respectively. The resulting fractions were then analyzed for PAH by liquid chromatography or gas chromatography.

Chromatographic methods have been widely used in fractionation of coal tar. Royer *et al.* (28) reported using gel chromatography on Sephadex LH-20 to fractionate of coal tar. The primary fractions were subfractionated by liquid chromatography on silica gel. The subfractions were subjected to GC-MS analysis and bacterial mutagenicity (Salmonella) assays.

Several problems exist with the previously described separation methods. First, most schemes are complicated, time-consuming and tedious. Some methods use expensive materials; large volumes of high purity solvents are necessary for each chromatographic or partitioning step. Solvent extraction and partitioning of polar types of compounds can cause formation of stable emulsions which contribute to the loss of components and inefficient separations. Finally, many extraction schemes are often plagued by incomplete chemical class separations due to partioning between two phases resulting in overlap of compound types into adjacent fractions.

Compared to the separation methods described above, the chromatographic methodology developed by Later *et al.* (29) was simpler and less time-consuming. This chromatographic method was originally applied to the chemical class separation and characterization of organic compounds in synthetic fuels. Fractionation of the sample into discrete chemical classes was performed by adsorption column chromatography using neutral aluminum oxide. Elution of the column by four solvents, hexane, benzene, chloroform/ethanol, and tetrahydrofuran/ethanol afforded four fractions: aliphatic hydrocarbons, neutral PAH, nitrogen-containing PAC and hydroxylated PAH. This neutral alumina method has been used by other research groups in the fractionation of coal tar (30) and solvent refined coal (31) into chemical classes. It has been modified and adapted previously by our research group for separation of PAC from various complex environmental samples (24, 32, 33). This modified neutral alumina method was used in the fractionation of coal tar in this study.

I. 5. 2 Methods for Separation of PASH from PAH

Sulfur-containing organic compounds are often present as minor components in complex mixtures which contain aromatic compounds. In this work we sought to find or develop a method for the isolation of a sulfur-rich fraction from coal tar so that we could

perform chemical and biological analyses of these compounds. Two general approaches have been used to accomplish the separation of PASH from PAH: ligand-exchange chromatography (34) and an oxidation/reduction approach (35).

Ligand-exchange chromatography procedures to separate PASH from PAH have used silica gel primarily, coated with salts of metals known to coordinate with sulfur compounds; These salts include those of mercury (36), copper (37), silver (38), palladium (34) and zinc (39). Procedures using these salts have been shown to be effective for the isolation of aliphatic sulfides, but are generally less applicable for the separation of thiophenic compounds. However, the method developed by Nishioka (34) which uses palladium chloride-treated silica gel was reported to be successful in the separation of PASH from PAH. After review of the above methods, Nishioka's procedure was selected for evaluation in this study.

An oxidation/reduction procedure has also been reported by Lee *et al.*(35) to separate PASH from PAH. The idea behind this method is that the PASH are easily oxidized to sulfones which are more polar than PAH and thus should be easily separated chromatographically from PAH. Reduction of these sulfones should restore the original thiophenes, thereby effecting a separation of PASH from PAH. Oxidation of the PASH can be accomplished with a variety of reagents, including hydrogen peroxide in a mixture of acetic acid and benzene. After chromatographic separation of the polar sulfones from the

non-oxidized PAH, reduction of the sulfones with lithium aluminum hydride (LAH) was reported to give the sulfur aromatics. Recently, Andersson (19) examined this approach in more detail and showed that oxidation of PASH with Lee's conditions produced not only the desired sulfones but also a variety of oxidized PAH species. Some PAH were completely consumed by these oxidation conditions. Furthermore, reduction of aromatic sulfones with LAH was often accompanied by side-reactions and poor yields of PASH. Unfortunately, this work (19) came to our attention during the course of our own attempts to use the Lee's approach.

I. 6 Analytical Methods for the Determination of PAC

Capillary column gas chromatography is one of the most common methods for the separation and determination of polycyclic aromatic compounds (40, 41). In the last decade, almost all GC analytical work has been done using fused silica wall-coated open tubular capillary columns (42); glass capillary columns are no longer used because they are too fragile and packed GC columns do not provide sufficient efficiencies for these complex mixtures. The two most important general detectors used for GC, the flame ionization detector (FID) and the mass spectrometer (MS), have been used extensively in those studies. The sulfur-selective detectors have also been used in the detection of PASH (21).

High performance liquid chromatography (HPLC) is the second common technique

for the analysis of polycyclic aromatic compounds. UV absorption and fluorescence detection are the most widely used LC detectors for the measurement of PAH (43). UV/Visible diode-array detectors allow the collection of UV/Visible spectra of eluting components during a LC run and the matching of these spectra to library spectra. The fluorescence detector offers far greater sensitivity and, more importantly, greater selectivity than a UV detector (43). By selection of the appropriate excitation and emission wavelengths, a high degree of specificity in the detection of specific compounds can be achieved.

The combination of mass spectrometry and liquid chromatography (LC-MS) has been applied to the analysis of PAH and, in particular, high molecular mass PAH. A number of LC-MS methods including moving belt LC-MS (44), particle beam LC-MS (45) and heated pneumatic nebulization atmospheric pressure chemical ionization (APCI)-LC-MS (46) have been used in the analysis of high molecular weight PAH. Anacleto *et al.* (46) compared the performance of the three aforementioned methods and found that (APCI)-LC-MS afforded the best combination of detection limits and linear dynamic range for PAH in a coal tar standard reference material. Marvin *et al.* (47) analyzed the high molecular weight PAH from a variety of environmental matrices by using (APCI)-LC-MS; the operating parameters and mobile phase were optimized conditions for the analysis of high molecular weight PAH.

Probe electron impact ionization mass spectrometry (EIMS) and chemical ionization mass spectrometry (CIMS) are another very useful technique in identifying PAH, especially the high mass PAH. For example, the LC peaks can be collected, evaporated and then analyzed by probe mass spectrometry. One limitation of mass spectrometry is that it can not readily differentiate between isomers of the same molecular mass.

Other spectrometric techniques like ultra-violet visible or fluorescence spectrophotometry are also important for the characterization of PAH. The UV absorbance spectra and fluorescence emission spectra of PAH are unique fingerprints and are useful.

I. 7 Research Objectives

Although it is well documented that some polyclic aromatic hydrocarbons and some nitrogen-containing PAH are potent mutagens and carcinogens in animals, little is known about potential of PASH to exert similar effects. Members of our group have examined a variety of environmental samples in order to identify those compounds which may have potential to cause the long-term health effects (14, 15). Using a bioassay-directed fractionation approach, the extracts were separated into compound classes and were evaluated as mutagens using a bacterial genotoxicity assay (Ames assay). In these studies, it was found that benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and benzo[e]pyrene were responsible for the mutagenicity of the 252 amu

PAH fraction, while indeno[1,2,3-cd]pyrene and benzo[ghi]perylene were responsible for the mutagenicity of the 276 amu PAH fraction. However, the determination of the genotoxicities of the PASH that correspond to these PAH, such as the 258 amu PASH and 284 amu PASH which are similar in structure to the 252 and 276 amu PAH, has not been undertaken. Very few PASH standards are available commercially. In order to determine the mutagenic potencies of PASH compare to PAH, it will be necessary to isolate PASH from a source in which PASH are relatively abundant. The main task will be to separate the PASH from the predominant PAH. It was felt that some of the multi-dimensional chromatographic methods developed in our group would be useful in this project.

By using the aforementioned bioassay-directed fractionation approach, members of our group also tested the mutagenic activities of molecular weight 326 amu PAH (Figure 5, Figure 6a, 6b). The biological responses of 326 amu PAH demonstrate the need for the development of methodologies that are capable of resolving, isolations and identifying these compounds.

My research project was directed in the two following general directions:

(1) To develop a methodology to separate PASH from PAH in coal tar.

(2) To separate and identify the genotoxic compounds in the 326 amu PAH fraction.

To accomplish these general objectives the research was directed to the following specific stages:

(a) to separate coal tar using open column alumina chromatography and analyze the resulting coal tar fractions using various chromatographic and spectroscopic techniques.

(b) to develop analytical methods for the analysis of PAH and PASH in coal tar fractions, including GC-MS and LC-MS methods.

(c) to use or develop a methodology to separate PASH from PAH.

(d) to explore multi-dimensional chromatographic approaches for the separation of coal tar into individual compound classes and individual compounds.

(e) to isolate and fractionate the molecular mass 326 PAH fraction with the goal of obtaining individual compounds.

(f) to examine the PASH and 326 amu PAH isolated above using the Ames microbiological reversion assay.

II. EXPERIMENTAL

II. 1 Gases and Solvents

High purity nitrogen and helium were purchased from Canada Liquid Air Ltd. (Toronto, Ont.). HPLC grade hexane was obtained from BDH Chemicals (Toronto, Ont.); HPLC grade acetonitrile, dichloromethane, methanol and acetone were purchased from Caledon Laboratories (Georgetown, Ont.). Benzene (Fisher Scientific, Fairlawn. New Jersey) and toluene (Caledon, Georgetown, Ont.) were distilled before use. The department's distilled water was purified further by a Milli-Q Water Purification System (Waters Associates, Milford, MA).

II. 2 Chemicals

Hydrogen peroxide (BDH Inc. Toronto), lithium aluminum hydride (BDH Chemicals Ltd., England), Palladium chloride (99%, Aldrich) and benzonitrile (Eastman Kodak Co.) were used directly. PAC standards were obtained from a variety of sources and used as received. The Hamilton coal tar sample was used as received from a steel company.

II. 3 Instrumentation

Gas chromatography-mass spectrometry (GC-MS) experiments were performed on

a Hewlett-Packard Model 5890 Series II gas chromatograph with electronic pressure control and interfaced to a Hewlett-Packard Model 5971A mass selective detector (Hewlett-Packard Co., Mississauga, Ont.). All injections were executed either manually or with an autosampler (Hewlett-Packard 7673A Automatic Injector). Reversed and normal phase HPLC were carried out on a Hewlett-Packard model 1090 liquid chromatograph with a built-in diode-array detector and ChemStation data system (Hewlettt-Packard Co., Mississauga, Ont.). Fluorescence spectra were obtained using a Perkin-Elmer model LS-5 fluorescence spectrophotometer controlled by a microcomputer equipped with CFS (version 3) software.

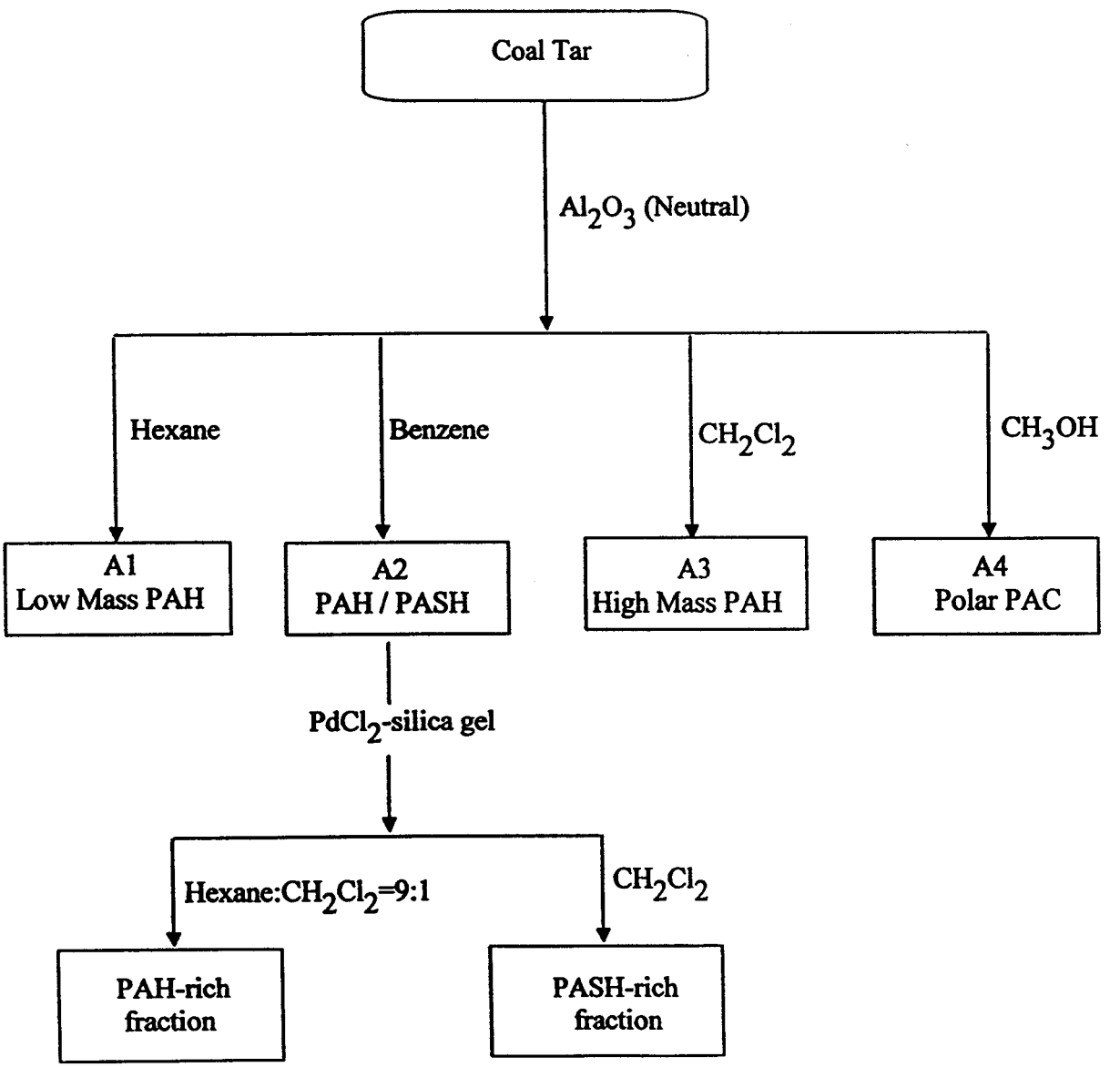
II. 4 Chromatographic Separation of Coal Tar

A schematic diagram of the separation procedure for the characterization of coal tar is shown in Figure 7.

II. 4. 1 Neutral Alumina Chromatographic Separation of Coal Tar

The alumina chromatography procedure, first described by Later *et al.* (29), has been modified by our research group for separation of PAC from various complex mixtures (24, 32, 33). The following procedure was adapted and scaled up for the separation of 10 g of coal tar.

Figure 7. Scheme for separation of coal tar by chemical class



Neutral alumina (Fisher, Brockman activity I, 80-200 mesh), which had been heated for at least 48 hours at 170^{0}C, was allowed to cool to room temperature immediately before being weighed for use in the following protocol. Crude coal tar (10 g) was dissolved in dichloromethane (100 mL), into which magnesium sulfate was added in order to dry the sample. After filtration to remove magnesium sulfate, neutral alumina (50 g) was added to the solution and the solvent evaporated to dryness at reduced pressure. The coated alumina was then poured on top of an alumina column (200 g, 5 cm X 40 cm). Elution of the column with hexane (800 mL) afforded fraction A1 (0.226 g, 2.26 % of coal tar); elution with benzene (2500 mL) afforded fraction A2 (2.45 g, 24.5% of coal tar). The third fraction (A3, 0.554 g, 5.54% of coal tar) was obtained by elution with dichloromethane (2300 mL) while fraction A4 (2.11 g, 21.1% of coal tar) was obtained by elution of the column with methanol (1540 mL). The remaining 47% of the coal tar was not recovered.

The A2 fraction was fractionated further by the procedures described below. The A3 fraction was further fractionated by semi-preparative NPLC and analyzed by reversed phase HPLC and LC-MS.

II. 4. 2 Separation of PASH from PAH by Oxidation/Reduction Procedure

The oxidation, reduction and column separations were performed according to Lee's procedure (35). Coal tar fraction A2 (1 g) was taken up in 25 mL benzene and 25 mL glacial

acetic acid, and the mixture was refluxed. Hydrogen peroxide (5 mL, 30%) was added to the refluxing mixture over a period of 30 min. and refluxing was continued overnight. The mixture was allowed to cool before it was washed five times with 25 mL portions of distilled water to remove any excess H_2O_2 and acetic acid. The water washings were then back-extracted with benzene (3 X 25 mL).

The oxidized product was separated by silica gel column chromatography to give a non-polar (unoxidized) fraction and a more polar (oxidized) fraction. The reaction mixture was adsorbed by solvent evaporation onto silica gel (2 g) which was then applied to 20 g of silica gel in a column. Instead of using benzene and benzene/methanol (1:1) to elute the silica column as in Lee's procedure (35), hexane/dichloromethane (9:1) was first used to elute the silica gel column followed by dichloromethane/methanol (1:1). Elution of this column with 100 mL hexane/dichloromethane (90/10) afforded unoxidized compounds, most of which were polycyclic aromatic hydrocarbons. The oxidized fraction was subsequently collected by elution with 100 mL dichloromethane/methanol (1:1).

The dichloromethane/methanol solution was then taken to dryness and suspension was formed by adding 20 mL of anhydrous ethyl ether to the residue. This suspension was reduced by adding it dropwise to a refluxing suspension of 13 mL of ethyl ether containing 250 mg of finely powdered lithium aluminum hydride. Addition was continued for 1 hour with stirring. The mixture was refluxed for an additional 2 hours, after which water was

added dropwise to decompose the $LiAlH_4$. The ether portion was separated from the inorganic precipitate and excess water. The precipitate was then washed twice with 10 mL portions of ethyl ether and methylene chloride. All ethyl ether and methylene chloride solutions were combined and evaporated to a volume of 2 mL which was then adsorbed onto 1 g of silica gel.

The sulfides were separated from the hydroquinones (formed by reduction of oxidized PAC) by column adsorption chromatography on 10 g of silica gel and elution with benzene. The first 10 mL volume contained the sulfur heterocycles, which was evaporated in a rotary evaporator to the appropriate volume for GC-MS.

II. 4. 3 Separation of PASH from PAH by Ligand-exchange Chromatography

II. 4. 3. 1 The Preparation of $PdCl_2$-silica gel and $Pd(PhCN)_2Cl_2$-silica gel

Silica gel (100 g, 230-400 mesh, Merck) was mixed with $PdCl_2$ (5 g) in 200 mL of water. The brown-coloured suspension was evaporated at reduced pressure, then dried at 95^0C overnight and finally activated at 170^0C for more than 24h before use. The colour of the dried $PdCl_2$-silica gel was dark brown.

$Pd(PhCN)_2Cl_2$ was synthesized as described by Kharasch *et al.* (48). $PdCl_2$ (0.5 g, 2.8 mmole) was dissolved in benzonitrile (7.5 mL) at 100^0C. The reaction was held at the temperature for 20 minutes. The resulting solution was filtered and cooled, yielding an

orange, heavy precipitate, which was collected by filtration. More precipitate was obtained when the filtrate was treated with low-boiling petroleum ether. After washing the precipitate with petroleum ether, the combined precipitates were dried over calcium chloride under reduced pressure for one hour. The yield was 0.43 g, 1.1 mmole, 41%.

For the preparation of $Pd(PhCN)_2Cl_2$-silica gel, a solution of $Pd(PhCN)_2Cl_2$ (0.1 g) in dichloromethane (20 ml) was treated with silica gel (10 g). The solvent was evaporated at reduced pressure to give a light brown coloured product which was divided into two parts; one half was used to make a column directly, while the other half was dried and activated at 170^0C for 24h.

II. 4. 3. 2 The Column Separation Procedure

The following procedure was used for both the $PdCl_2$-silica gel column separation and $Pd(PhCN)_2Cl_2$-silica gel column separation.

Silica gel impregnated with $PdCl_2$ (5 g) was introduced into a glass column (10 mm i.d.) which contained dichloromethane and hexane (1:9 v/v). Coal tar fraction A2 (100 mg) was dissolved in dichloromethane (5 mL), to which was added $PdCl_2$-silica gel (0.5 g). The solvent was removed by evaporation at reduced pressure and the resulting $PdCl_2$-silica gel was then put to the top of the column. Instead of using chloroform/hexane (1:1) to elute two fractions from the column as in the original method (34), hexane/dichloromethane (90/10,

60 mL) was used to elute the column to afford A2-P1 before dichloromethane (60 mL) was added to the column to afford a second fraction (A2-P2). Next, both fractions were worked-up in the same way to remove any residual Pd. Each fraction was reduced in volume to 20 mL by rotary evaporation, transferred into a separatory funnel and treated with 1 mL of 0.05 M NaCN solution. The organic layer was collected and further extracted with water (2 X 5 mL). The organic phase was dried over anhydrous $MgSO_4$ and then filtered into a 10 mL volumetric flask.

On shaking fraction A2-P2 with aqueous NaCN, the Pd complex was decomposed and the dark brown colour disappeared; however, no colour change was observed in A2-P1.

II. 5 Analytical Methods

II. 5. 1 Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

Two capillary columns were used in this work. Initially, all analyses were performed on a 60 m X 0.25 mm i.d. DB-5ms (J&W Scientific) capillary column (0.25 μm film thickness). Later, analyses were performed on a 30 m X 0.25 mm i.d. DB-17ht column (0.15 μm film thickness, J&W Scientific), once it had been demonstrated that the performance of the DB-17ht column was better suited to these analyses. Samples were injected in toluene solution with an injection volume of 1 μL. For full scan analyses, scan parameters were set to scan the range between m/z 50 and m/z 550 with a threshold of 100 units.

The temperature program used depended on the GC column and sample being analyzed. The details of the solvent delays, column oven temperature programs and carrier gas flow rates for the DB-5ms and DB-17ht columns are summarized in Table 1. Method A was used for analyses performed on the DB-5ms column while method B was used for the DB-17ht column. The temperature programs listed in Table 1 were used for the analysis of PAC in coal tar fractions A2, A3 and A4. However, when analyzing fraction A1 using Method B, an initial temperature of 40^0C was used because this fraction contained volatile and low mass compounds; the injection solvent for this analysis was hexane.

Both full scan analyses and selected-ion monitoring (SIM) programs were used for analyses of fraction A2, while only the full scan method was used for the analyses of fractions A1, A3 and A4. Selected-ion monitoring programs varied with the sample analyzed, column type and column length. Table 2 and Table 3 show the grouping of ions for the DB-5ms column and the DB-17ht column, respectively. For the analyses performed on the DB-17ht column, more parent ions and fragment ions were included compared to the DB-5ms protocol.

The internal standard method was used for quantitative analyses. The quantitation was based on the calculation of the peak area ratio between the peak of interest and the known amount of internal standard. Benz[a]anthracene-d_{12} was used as internal standard for analyses on the DB-5ms column. Two deuterated PAH, pyrene-d_{10} and perylene-d_{12} were

Table 1. Protocols for GC-MS Analysis Methods A and B

	Method A	Method B
Column	**DB-5ms**	**DB-17ht**
Length	60 m	30 m
Internal Diameter	0.25 mm	0.25 mm
Film Thickness	0.25 um	0.15 um
Carrier Gas	He	He
Flow Velocity*	29 cm/sec	29 cm/sec
Solvent Delay	8.0 min.	7.0 min.
Oven Temp. Program	initial temp. 130^{0}C 130-300^{0}C at 1.6^{0}C/min., hold at 300^{0}C for 25 min.	initial temp. 90^{0}C 90-300^{0}C at 2.5^{0}C/min., hold at 300^{0}C for 20 min.
Total Run Time	131.25 min.	104 min.
MS Analysis	full scan or SIM program in Table 2	full scan or SIM program in Table 3

* Flow velocity was kept constant by electronic pressure control.

Table 2. Ion Groupings Used in Selected Ion Monitoring (S.I.M.) Program for Method A (60 m DB-5ms Column)

S.I.M. Ion Group	Time (min.)	Ions Monitored (m/z)
1	8.00 - 29.00	128, 134, 142, 147, 148, 152, 154, 166, 181
2	29.00 - 50.60	139, 152, 178, 180, 184, 191,192, 197, 198, 205, 206, 211, 212
3	50.60 - 64.00	163, 176, 202, 208, 215, 216, 221, 222, 229, 230, 235, 236
4	64.00 - 84.00	189, 202, 228, 229, 230, 234, 235, 236, 240, 242, 247, 248, 261, 262
5	84.00 - 98.00	213, 226, 252, 258, 265, 266, 279, 280, 284
6	98.00 - 131.25	142, 239, 252, 276, 278, 284, 297, 298, 300, 302, 311, 312

Table 3. Ion Groupings Used in Selected Ion Monitoring (S.I.M.) Program for Method B (30 m DB-17ht Column)

S.I.M. Ion Group	Time (min.)	Ions Monitored (m/z)
1	7.00 - 35.00	128, 129, 139, 141, 142, 151,152,153, 154, 165, 166, 178, 179, 184,188, 189, 191, 192, 197, 198
2	35.00 - 43.00	152, 180, 191, 192, 197, 198, 202, 203, 205, 206, 208, 211, 212, 219, 220, 225, 226, 229, 230,
3	43.00 - 50.00	163, 191, 202, 203, 205, 206, 208, 212, 213, 215, 216, 219, 220, 221, 222, 225, 226, 229, 230, 231
4	50.00 - 56.00	117, 189, 202, 215, 216, 221, 222, 226, 227, 228, 229, 230, 234, 235,236, 243, 244
5	56.00 - 59.00	117, 189, 202, 226, 227, 228, 229, 230, 234, 235, 236, 239, 240, 241, 242, 243, 244, 247, 248, 255, 256
6	59.00 - 61.00	117,189, 201, 202, 217, 230, 234, 239, 240, 241, 242, 243, 244, 247, 248, 255, 256, 261, 262, 275, 276
7	61.00-63.00	201, 217, 230, 239, 240, 241,242, 244, 247, 248, 253, 254, 255, 256, 258, 261, 262, 269, 270, 275, 276

8	63.00-65.00	201, 217, 230, 239, 240, 241, 242, 247, 253, 254, 255, 256, 258, 261, 262, 267, 268, 269, 270, 275, 276
9	65.00 - 69.00	213, 252, 253, 254, 255, 256, 258, 261, 262, 265, 266, 267, 268, 269, 270, 271, 272, 275, 276
10	69.00 -70.00	213, 252, 253, 254, 258, 260, 264, 265, 266, 267, 268, 269, 270, 271,272, 275, 276, 280, 285, 286
11	70.00 - 72.00	213, 252, 253, 258, 260, 264, 265, 266 268, 269, 270, 271, 272, 275, 276, 279 280, 285, 286, 299, 300
12	72.00 - 76.00	239, 252, 265, 266, 268, 269, 270, 271 272, 275, 276, 278, 279, 280, 284, 285 286, 293, 294, 299, 300
13	76.00 - 79.00	239, 271, 272, 276, 277, 278, 279, 280, 284, 285, 286, 288, 289, 290, 292, 293, 294, 299, 300, 303, 304
14	79.00 - 81.00	239, 261, 271, 272, 276, 277, 278, 279, 280, 284, 285, 286, 289, 290, 292, 293, 294, 299, 300, 303, 304, 306
15	81.00 - 104	150, 239, 261, 276, 277, 278, 284, 285, 286, 289, 290, 292, 293, 294, 299, 300, 302, 303, 304, 306, 317, 318

added to samples as internal standards for analyses carried out on the DB-17ht column prior to GC-MS analysis. Pyrene-d_{10} was used to quantify the three-ring and four-ring PAC while perylene-d_{12} was used to quantify the five and higher ring PAC.

II. 5. 2 Normal Phase HPLC Analysis

Two Whatman Partisil PAC columns (5 μm packing, 4.6 mm X 25 cm for analytical applications and 10 μm packing, 9.4 mm X 25 cm for preparative work, Whatman, Clifton, NJ) and an amino precolumn (1.5 cm X 3.2 mm i.d. Brownlee Labs, Santa Clara, CA) were used for all normal phase HPLC analyses. The solvent gradient programs described below were used for both analytical and preparative applications with the following exceptions: loop size (20 μL, analytical and 100 μL, preparative), flow rates (1.0 mL/min., analytical and 4.2 mL/min., preparative).

The HPLC operating conditions were as follows: diode array UV absorption detection over the wavelength range from 230 to 440 nm; column temperature 40^{0}C. Gradients A and B (see below) used the same hexane/dichloromethane gradient profile; however, in gradient A the column was eluted with acetonitrile after the hexane/dichloromethane gradient in order to elute residual polar compounds from the column. Gradient A required longer times between analyses for column equilibration than did Gradient B.

In cases which required repetitive injections to prepare sufficient material, Gradient

B was used for all of the injections except for the last injection which was made using Gradient A. Thus, all of the polar material from the previous injections eluted in the acetonitrile wash at the end of the program following the last injection.

Gradient A: (elapsed time, composition of mobile phase): initial, 95% hexane and 5% dichloromethane; 10 min., 95% hexane and 5% dichloromethane; 35 min., 70% hexane and 30% dichloromethane; 55 min., 30% hexane and 70% dichloromethane; 65 min., 100% dichloromethane; 70 min., 100% dichloromethane; 75 min., 100% acetonitrile; 80 min., 100% acetonitrile; 85 min., 100% dichloromethane; 100 min., 95% hexane and 5% dichloromethane; 130 min., 95% hexane and 5% dichloromethane.

Gradient B is a modification of Gradient A with the following changes: 70 min., 100% dichloromethane; 75 min., 95% hexane and 5% dichloromethane; 110 min., 95% hexane, 5% dichloromethane.

Gradient C was used to increase the retention time range of the 326 molecular weight group of isomers to a period of roughly eight to ten minutes, allowing collection of nine 60-second fractions.

Gradient C: initial, 88% hexane and 12% dichloromethane; 30 min., 80% hexane and 20% dichloromethane; 32 min., 88% hexane and 12% dichloromethane; 40 min., 88% hexane and 12% dichloromethane.

II. 5. 3 Reversed Phase HPLC Analysis

Reversed phase HPLC was used for analysis of fractions prepared by alumina chromatography and by normal phase analyses. A 5 μm Vydac reversed phase analytical column (25 cm X 4.6 mm i.d., Separations Group, Hesperia, CA) was used. Sample were injected using a 20 μL loop, and the operation conditions were identical to those for normal phase HPLC. Gradient D was used to analyze the NPLC fractions containing 326 amu PAH. Gradient E was used for the LC-MS analysis of coal tar fraction A3.

Gradient D: initial, 80% acetonitrile and 20% water; 30 min, 100% acetonitrile; 45 min, 85% acetonitrile and 15% dichloromethane; 60 min, 50% acetonitrile and 50 % dichloromethane; 65 min, 100% acetonitrile; 70 min, 80% acetonitrile and 20% water; 80 min, 80% acetonitrile and 20% water.

Gradient E: initial, 100% acetonitrile; 10 min, 100% acetonitrile; 35 min, 85% acetonitrile and 15% dichloromethane; 45 min, 45% acetonitrile and 55% dichloromethane; 60 min, 100% dichloromethane; 65 min, 100% dichloromethane; 70 min, 100% acetonitrile; 80 min, 100% acetonitrile.

II. 5. 4 Low Resolution Mass Spectrometry

Direct inlet probe electron impact (DEI) and chemical ionization (DCI) mass spectrometry were performed using a VG ZAB-E mass spectrometer (VG Analytical,

Altrincham, U.K.). Methane and ammonia (Matheson, Whitby, Ontario) were used as reactant gases in the CIMS experiments. Details of various parameters for the operation of the mass spectrometer in each ionization mode are listed in Table 4. A PDP 11/24 data system (Digital Equipment Co.) was used for data acquisition and analysis.

II. 5. 5 Liquid Chromatography-Mass Spectrometry (LC-MS)

The LC-MS experiments were performed using a Hewlett-Packard Model 1090 liquid chromatography interfaced with a Fisons Platform benchtop quadrupole instrument. Gradient E was used on a 5 μm 25 cm X 4.6 mm i.d. Vydac reversed phase analytical column. The column temperature was maintained at 35^{0}C. The mass spectrometer was operated in the atmospheric pressure chemical ionization (APCI) mode. The source temperature was 120^{0}C and the APCI probe temperature was maintained at 500^{0}C. The full scan mass range was 170 to 520 amu with a scan duration of 2.96 seconds. The mass spectrometer operation parameters were optimized prior to the LC-MS experiments by constant infusion into the source of a 1 μg/mL standard solution of dibenzo[a,l]pyrene (302 amu) in 100% acetonitrile at 1 mL/min.

Table 4. Experimental Conditions Used for Probe Mass Spectrometry

Operating Parameter	Electron Impact Ionization	Chemical Ionization
Electron Energy	70 eV	50 eV
Emission	100 uA	100 uA
Source Temperature	200^{0}C	200^{0}C
Probe Temperature: Initial Temperature	30^{0}C	30^{0}C
Temperature Programming	4^{0}C/sec	4^{0}C/sec
Final Temperature	300^{0}C	300^{0}C
Accelerating Voltage	6 KV	6 KV
Source Pressure	10^{-6} torr	
Ion Ratios		CH_4 CIMS: CH_5^+:$C_2H_5^+$=1:1 NH_3 CIMS: NH_4^+:$(NH_4 \bullet NH_3)^+$=1:20
Resolution (10% valley definition)	1000	1000

III. RESULTS AND DISCUSSION

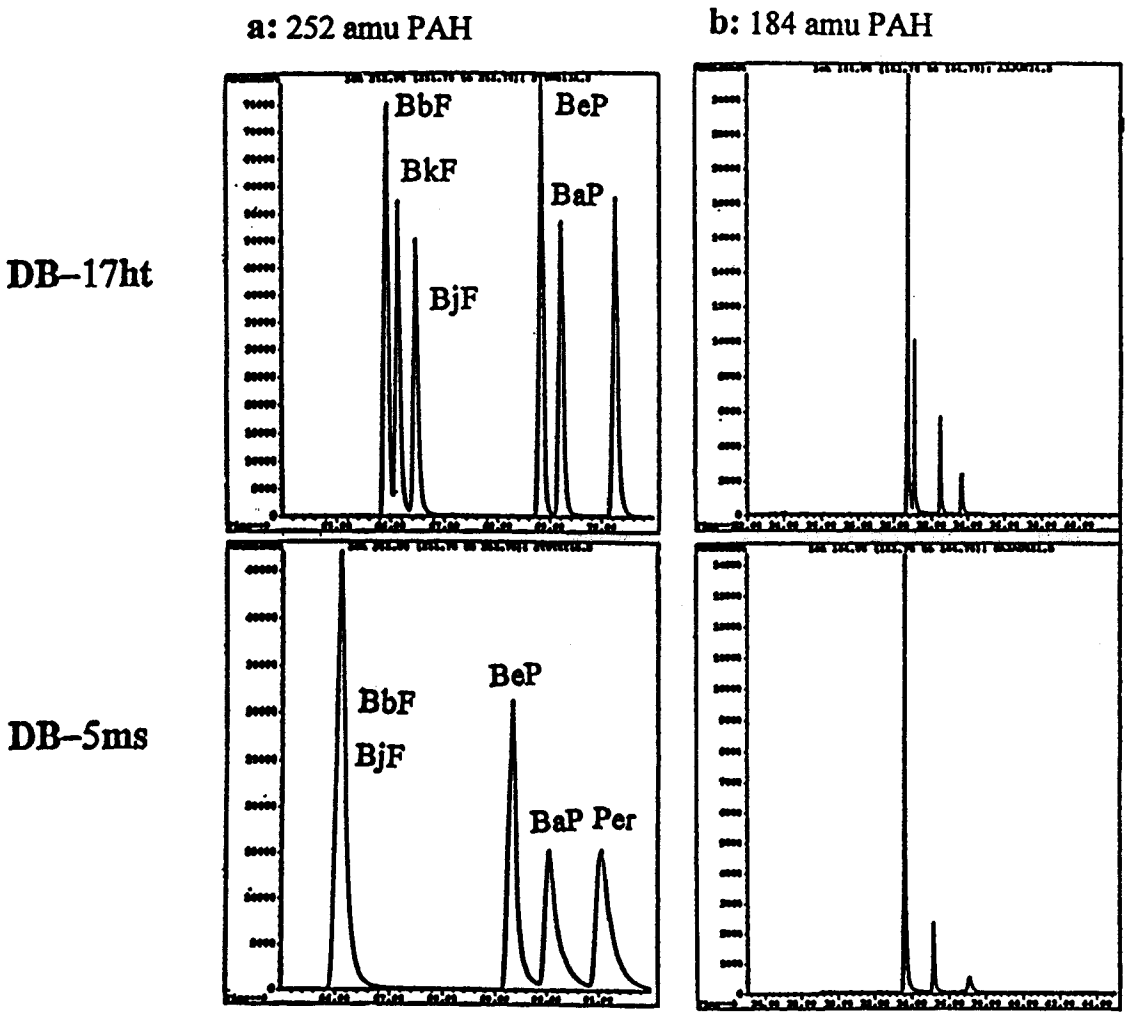
III. 1 GC-MS Method Development

Gas chromatography-mass spectrometry (GC-MS) was used for both the qualitative and quantitative analyses of PAH and PASH. The GC-MS methods described in Table 1 (Experimental section **II. 5. 1**) were developed based on the following criteria for the identification of PAH and PASH: (1) obtaining good chromatographic resolution, (2) obtaining reproducible retention index data for these compounds and (3) obtaining good and selective detection of the target compounds. These criteria will be discussed in the following section in detail.

III. 1. 1 GC Column Comparison

Both DB-5ms and DB-17ht columns were used in this study. The difference between the two columns is the degree of substitution of phenyl groups for methyl groups in the polysiloxane stationary phase. The stationary phase of a DB-5ms column consists of 5% phenyl-methylpolysiloxane whereas a DB-17ht column uses a stationary phase with 50% of phenyl-methylpolysiloxane. Figure 8 is the comparison of relative separations of selected classes PAH and PASH on DB-17ht and DB-5ms columns. Figure 8a compares the separation of certain 252 amu PAH; DB-17ht was able to separate benzo[b]fluoranthene and

Figure 8. Comparison of relative separations of some PAH and PASH on 60 m DB-5ms and 30 m DB-17ht columns



benzo[j]fluoranthene. Figure 8b compares the separation of the four 184 amu PASH in a coal tar sample; two 184 amu isomers coeluted on the DB-5ms column. It was clear from these and other examples that the 30 m DB-17ht column performed much better in the separation of PAH and PASH than did a 60 m DB-5ms column. In the early stages of this study, the 60 m DB-5ms column was used. However, once the capabilities of the DB-17ht column had been discovered, all subsequent analyses were performed on the DB-17ht column.

III. 1. 2 Determination of Retention Indices of PAC

The retention of compounds on a gas chromatographic (GC) column is a function of many parameters, including column length, column diameter, carrier gas flow, etc.; thus, absolute retention times are rarely reported in the context of comparing data between laboratories. Furthermore, in studies involving analyses of complex mixtures, the extremely large number of possible isomers coupled with the unavailability of standard reference compounds in many laboratories makes standardization of retention data almost essential. Various schemes have been devised to normalize GC retention data including the relative retention and retention index approaches (49, 50, 51).

The linear retention index system developed by Lee *et al.* (50, 51) was adopted by our laboratory for chromatographic analyses of polycyclic aromatic compounds from complex

environmental samples. The system was designed based on the temperature-programmed capillary gas chromatographic analysis of polycyclic aromatic compounds. Naphthalene (2-rings), phenanthrene (3-rings), chrysene (4-rings), and picene (5-rings) were selected as retention index internal standards and were assigned retention index values of 200, 300, 400 and 500, respectively. The structures of these compounds (except naphthalene) are shown in Figure 9; phenanthrene is compound 8, chrysene, compound 25 and picene, compound 41. These four compounds were selected because it was noted that their relative elution under a constant temperature programme rate was extremely uniform. The retention index of any compound is calculated as the linear interpolation of its retention time between the retention times of two standard compounds which bracket the compound of interest. The following equation is used to calculate retention index of a compound:

$$I = 100Z + 100\,(t_x - t_z)\,/(t_{z+1} - t_z) \qquad (1)$$

where z and z+1 are the numbers of aromatic rings in the bracketing standards.

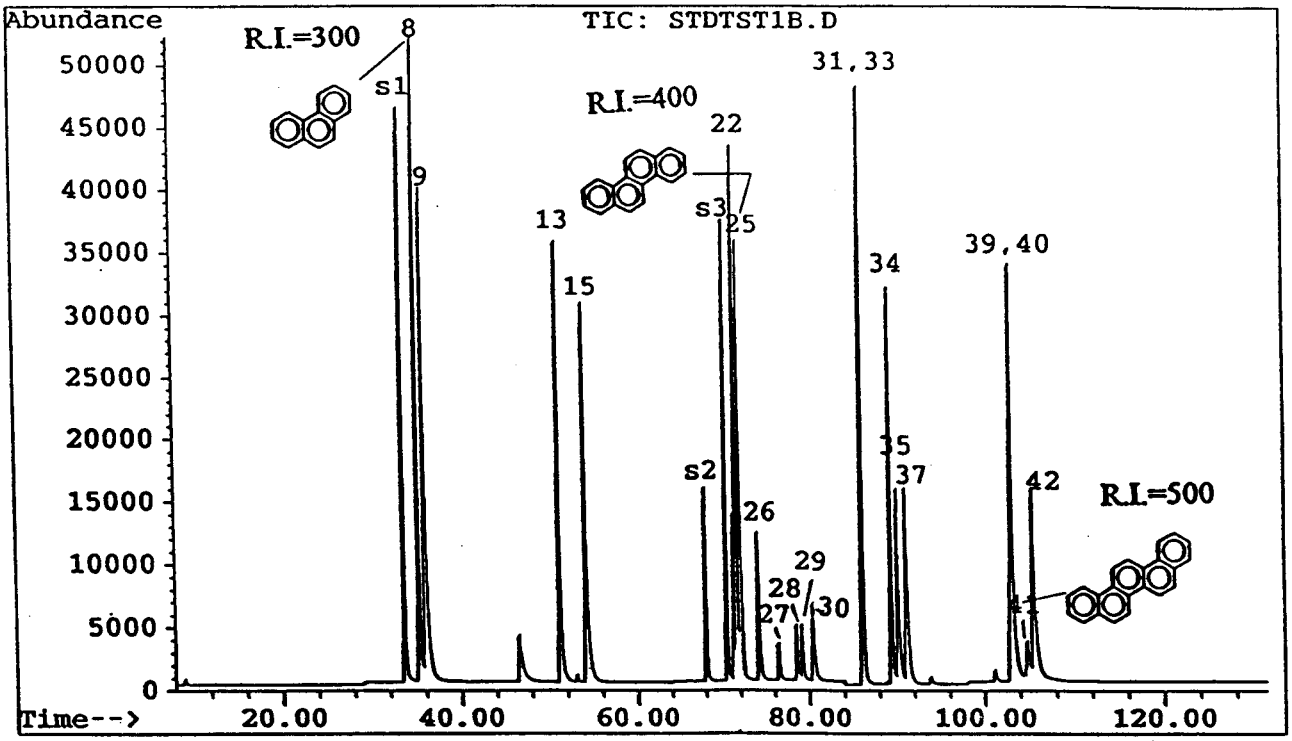
The ratio of retention time differences $(t_x - t_z)\,/(t_{z+1} - t_z)$ in equation 1 is the relative elution position of a compound compared to two standards. Assuming that the nature of the stationary phase, surface activity, instrumental conditions, etc., affect the elution of all polycyclic aromatic compounds proportionally, then this ratio should be a constant on a given stationary phase irrespective of column length or column diameter. Lee *et al.* (50, 51) reported retention index values for a large number of PAC using a home-made glass capillary

Figure 9. GC-MS total ion current chromatograms of two calibration standards.

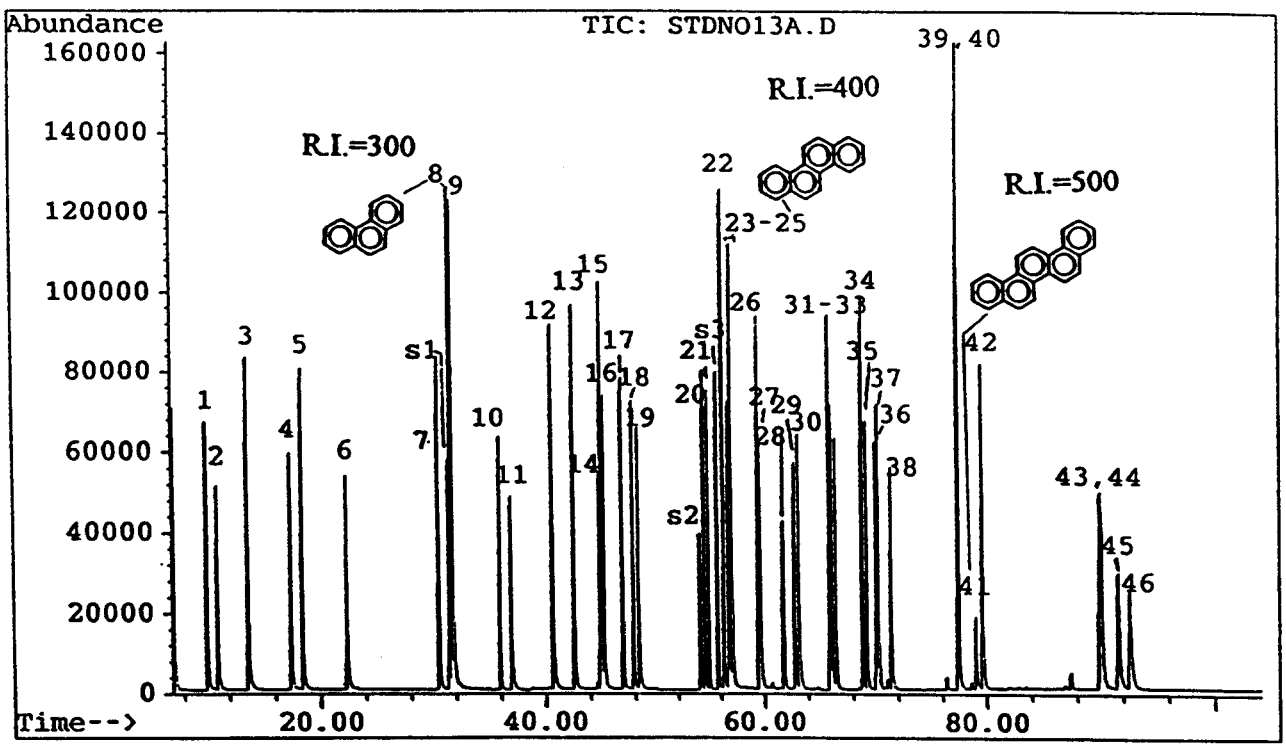
a. A calibration standard containing 23 compounds was analyzed on 60 m DB-5ms column using Method A.
b. A calibration standard containing 46 compounds was analyzed on 30 m DB-17ht column by Method B.
Peak identifications by number are as follows:

Peak No.	Compound Name	Peak No.	Compound Name
1	2-methylnaphthalene	23	Cyclopenta[cd]pyrene
2	1-methylnaphthalene	24	d12Chrysene
3	Biphenyl	25	Chrysene
4	Acenaphthylene	26	Benzanthrone
5	Acenaphthene	27	2-Nitrofluoranthene
6	Fluorene	28	Benz[a]anthracene-7,12-dione
s1	Dibenzothiophene	29	1-Nitropyrene
7	d10Phenanthrene	30	2-Nitropyrene
8	Phenanthrene	31	Benzo[b]fluoranthene
9	Anthracene	32	Benzo[k]fluoranthene
10	o-Terphenyl	33	Benzo[j]fluoranthene
11	1-Methylphenanthrene	34	Benzo[e]pyrene
12	Anthraquinone	35	Benzo[a]pyrene
13	Fluoranthene	36	d12Perylene
14	d10Pyrene	37	Perylene
15	Pyrene	38	3-Methylcholanthrene
16	m-Terphenyl	39	Indeno[1,2,3-cd]pyrene
17	p-Terphenyl	40	Dibenz[a,c]anthracene
18	Benzo[a]fluorene	41	Picene
19	Benzo[b]fluorene	42	Benzo[ghi]perylene
s2	Benzo[b]naphtho[2,1-d]thiophene	43	Coronene
20	Benzo[ghi]fluoranthene	44	Dibenzo[a,e]pyrene
21	Benzo[c]phenanthrene	45	Dibenzo[a,i]pyrene
s3	Benzo[b]naphtho[2,3-d]thiophene	46	Dibenzo[a,h]pyrene
22	Benz[a]anthracene		

a


Abundance
TIC: STDTST1B.D
R.I.=300
R.I.=400
R.I.=500
s1
s2
s3
8
9
13
15
22
25
26
29
28
27
30
31,33
34
35
37
39,40
42
41
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Time-->
20.00
40.00
60.00
80.00
100.00
120.00

b


Abundance
TIC: STDNO13A.D
R.I.=300
R.I.=400
R.I.=500
1
2
3
4
5
6
7
s1
8
9
10
11
12
13
14
15
16
17
18
19
20
21
s2
s3
22
23-25
26
27
28
29
30
31-33
34
35
36
37
38
39,40
41
42
43,44
45
46
160000
140000
120000
100000
80000
60000
40000
20000
0
Time-->
20.00
40.00
60.00
80.00

column coated with SE-52, a silicone polymer with 5% phenyl and 95% methyl substitution; this phase is similar to the commercially available DB-5ms column we have used in this study.

The retention indices of compounds that eluted before naphthalene were calculated from a linear extrapolation of the naphthalene-phenanthrene interval. In the same manner, indices for compounds that had longer retention times than picene were calculated from linear extrapolation extension of the chrysene-picene interval.

In order to apply this linear retention index system and to obtain reproducible retention index values, certain requirements for the temperature program were outlined by Lee *et al.* (50, 51). First, the temperature program should be a linear with a single rate of change of temperature. In the present work two methods used linear temperature program rates of 1.6^{0}C/min. (Method A) and 2.5^{0}C/min. (Method B). Second, the initial temperature must be at least 30 to 40^{0}C less than the elution temperature of the first retention index standard; in addition, all of the standards must elute during the temperature programming period. While naphthalene was the first retention index standard in Lee's work, phenanthrene was chosen to be the first retention index standard in this study and the retention index values of compounds eluting prior to phenanthrene were not calculated (except dibenzothiophene which eluted just before phenanthrene).

The retention index data of selected PAH and PASH were determined on both DB-

5ms and DB-17ht columns. Figure 9 shows the GC-MS total ion current chromatograms of two PAH calibration standards which were analyzed on the two columns, respectively. Three retention index standards are labelled on both chromatograms. The calibration standard injected on DB-5ms column (Method A) had 23 compounds in it, while the calibration standard injected on DB-17ht column (Method B) had 46 compounds. More compounds were included in the Method B standard because the DB-17ht column performed better and because the suite of analytes had increased.

The retention index values were calculated for selected compounds in the two calibration standards and are compared with literature data (50) in Table 5. Because the columns used in this study were different from the one used in the literature, the retention index data were expected to be different from the literature values. Lee and co-workers used a home-made SE-52 coated glass capillary column which had a stationary phase that was similar but not identical to the polysiloxane phase used in a modern DB-5ms column.

From the data listed in Table 5, it was found that benzo[b]fluoranthene and benzo[j]fluoranthene have the same retention index values on both the DB-5ms and SE-52 columns, which means the two compounds coeluted on both columns. However, these compounds were well separated on DB-17ht column.

Table 5. Comparsion of Retention Index Data for Selected Standard Compounds on Three Columns

Peak No.	MW	Compound Name	R.I. on DB-5ms	R.I. on DB-17ht	R.I. on SE-52[a]
s1	184	Dibenzothiophene	295.72±0.07	295.68±0.10	295.37±0.17
8	178	Phenanthrene	**300.00**	**300.00**	**300.00**
9	178	Anthracene	301.81±0.08	300.61±0.15	301.08±0.11
13	202	Fluoranthene	343.14±0.12	343.28±0.08	344.51±0.06
15	202	Pyrene	351.15±0.12	352.89±0.07	351.51±0.15
s2	234	Benzo[b]naphtho[2,1-d] thiophene	389.14±0.17	388.26±0.19	389.09±0.09
s3	234	Benzo[b]naphtho[2,3-d] thiophene	395.75±0.15	394.87±0.16	395.61±0.10
22	228	Benz[a]anthracene	398.72±0.15	396.98±0.18	398.76±0.04
25	228	Chrysene	**400.00**	**400.00**	**400.00**
31	252	Benzo[b]fluoranthene	443.22±0.18	440.29±0.19	443.13±0.11
33	252	Benzo[j]fluoranthene	443.22±0.18	442.80±0.09	443.13±0.11
34	252	Benzo[e]pyrene	452.94±0.18	453.62±0.12	452.29
35	252	Benzo[a]pyrene	455.08±0.23	455.31±0.14	454.02±0.07
37	252	Perylene	458.15±0.22	460.05±0.12	457.17±0.06
39	276	Indeno[1,2,3-cd]pyrene	494.28±0.34	492.28±0.18	493.24±0.09
40	278	Dibenz[a,c]anthracene	494.99±0.24	492.86±0.19	
41	278	Picene	**500.00**	**500.00**	**500.00**
42	276	Benzo[ghi]perylene	502.37±0.29	502.26±0.33	501.32±0.18

[a] R.I. values from *J. Chromatography* (1982) by Lee *et al.*

III. 1. 3 Detection of PAC Using Mass Spectrometry

Coupled with GC, mass spectrometry was used in the characterization and identification of PAC. Both full scan and S.I.M. programs were used in this study. For quantitative analyses, the S.I.M. method was used.

Table 6 lists the important ions for characterization of unsubstituted and substituted PAH and PASH by mass spectrometry. PASH yield a prominent peak corresponding to the loss of sulfur (M-32) and often lose a CHS unit (M-45). As in the mass spectra of PAH, unsubstituted thia-arenes show intense molecular ions (M+) and weak fragment ions corresponding to losses of 2 and 26 mass units, due to elimination of two hydrogen atoms and a C_2H_2 moiety, respectively. The weak (M-2) ion is generally more intense than the (M-1) ion in parent PAH and thia-arenes. Doubly charged ions such as (M^+/2e) and (M-45)/2e and sometimes (M-26)/2e are observed in these spectra. In the spectra of monomethyl-substituted PAH and thia-arenes, there is a dominant peak of (M-1) correspoding to a loss of H atom. As a result, there is a shift by one mass unit for many of the above fragment ions so that (M-26) becomes (M-27), (M-32) becomes (M-33), and (M-45) becomes (M-46). The (M-1) peak is much more intense than the (M-2) peak in these methyl substituted derivatives.

The two S.I.M. programs used in this study (see experimental section **II.5.1**) included the molecular ion and doubly charged molecular ion of PAH and PASH. For

Table 6. Important Ions for Characterization of Unsubstituted and Substituted PAH and PASH

M/Z	PAH	Me-PAH	PASH	Me-PASH
M	+	+	+	+
M - 1		+		+
M - 26	+			
M - 27				+
M - 32			+	
M - 33				+
M - 45			+	
M - 46				+
M/2	+	+	+	+

Ions with larger + signs were incorporated as part of the S.I.M. program for each compound class.

PASH, the M-32 and M-45 ions were also included in the S.I.M. programs. The ions listed in the Table 2 (Method A for the 60 m DB-5ms column) are the molecular ions of major PAH, PASH, monomethyl-substituted PAH and PASH, dimethyl-substituted PAH and PASH, the M-1 ions of methyl-substituted PAH and PASH, the M-32 and M-45 ions of PASH. For example, in the third ion group (between 50.60 and 64.00 min.), 202 amu PAH and 208 amu PASH are the two principal compound classes along with their methyl-substituted derivatives. Thus, the ions monitored in this ion group included m/z 202 and 208, the parent ions of the PAH and PASH, m/z 216 and 230, the parent ions of the mono- and di-methylated 202 amu PAH, m/z 222 and 236, the parent ions of the mono- and di-methylated 208 amu PASH, 215, 229, 221, and 235 are (M-1) of alkylated PAH and PASH, 163 is the (M-45) ion of the 208 amu PASH and 176 is the (M-32) ion of the 208 amu PASH. So, it is not difficult to detect and confirm the identities of the 202 amu PAH, 208 amu PASH and their methyl-substituted derivatives by examining both parent ions and the fragment ions in their mass spectra. Other compound classes are also detected in the same way by GC-MS in this S.I.M. program.

Table 3 lists all the ions in the S.I.M. program used for the 30 m DB-17ht column (Method B). More ions were monitored in this program compared to the one listed in Table 2, primarily because more compounds were monitored and more confirmatory ions were included in this S.I.M. method. This program was used to detect and identify PAH and

Table 7. Detection Limits[a] of Some PAH Standards Using GC-MS in S.I.M. Mode[b]

Compound	MW	Detection Limit (pg)
Dibenzothiophene	184	15
Phenanthrene	178	7
Anthracene	178	17
Fluoranthene	202	13
Pyrene	202	12
Benzo[b]naphtho[2,1-d] thiophene	234	3
Benzo[b]naphtho[2,3-d] thiophene	234	4
Benz[a]anthracene	228	4
Chrysene	228	11
Benzo[e]pyrene	252	12
Benzo[a]pyrene	252	15
Perylene	252	17
Indeno[1,2,3-cd]pyrene	276	22
Benzo[ghi]perylene	276	23
Dibenz[a,c]anthracene	278	17
Picene	278	30

[a] 60 m DB-5ms column, (Detection Limit defined as S/N=2.5:1)
[b] Instrument had been properly tuned and was used in a typical condition when these detection limits were determined. Thus, these values are indicative of detection limits that can be achieved routinely.

PASH in the same way as discussed in the above paragraph.

The detection limits of some PAH standards in GC-MS analysis by using S.I.M. mode were determined and are listed in Table 7. For most of PAH, detection limits were in the range of 3-30 pg. These numbers are typical of values that can be expected in normal use because they were obtained when the instrument was in a clean but unoptimized condition.

III. 2 Separation of Coal Tar by Alumina Chromatography

The flow chart outlining the separation of coal tar by alumina chromatography was shown previously in Figure 7 (Section **II. 4. 1**). This procedure, first described by Later *et al.* (29) and modified by our research group (24, 32, 33) involves the fractionation of an extract into chemical classes by adsorption column chromatography on neutral aluminum oxide. Table 8 lists the solvents used to elute the column, the fractions collected and the yields obtained in two experiments. Total recoveries of organic materials of 53.4% and 53.5% were obtained in two experiments. These recoveries were lower than expected and may reflect the loss of asphaltenes, phenolics and various insoluble tars. It was felt that a higher recovery could be reached if a more polar solvent such as a methanol/water mixture had been used. Overall, this method is a rapid, inexpensive procedure with good capacity. It can easily be scaled up to produce larger quantities which are sometimes required for the

Table 8. **Weight Percentages of Fractions Isolated from Coal Tar by Neutral Alumina Chromatography**

Fraction	Elution Solvent	Compound Type	Fraction wt.% Trial 1	Fraction wt.% Trial 2
A1	Hexane	Low Mass PAH Aliphatics	2.15	2.38
A2	Benzene	Neutral PAC	23.89	25.20
A3	Dichloromethane	High Mass PAH, N-PAH	5.80	5.28
A4	Methanol	N-PAH Polar PAC	21.58	20.67
Total Yield			53.42	53.53

isolation of trace components and for conducting bioassays to determine the mutagenicity or carcinogenicity of fractions.

A series of analyses was performed on the four alumina fractions to identify the compound classes and the compounds in each fraction; these methods included probe mass spectrometry, GC-MS analysis, normal phase and reversed phase liquid chromatography. The results of these analyses will be discussed in detail in the following sections.

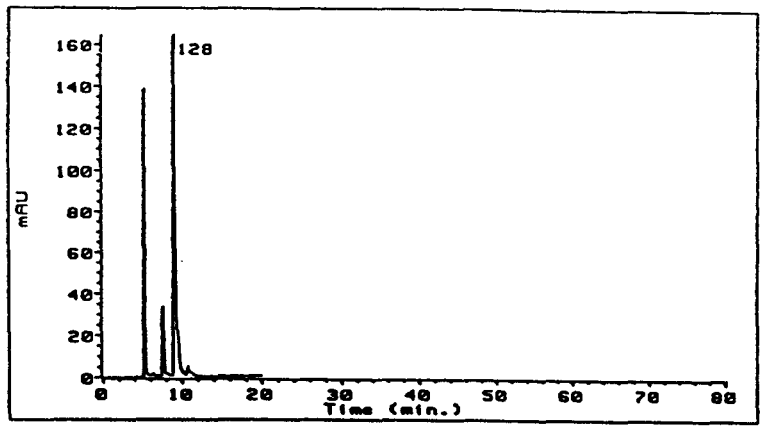
III. 2. 1 Normal Phase HPLC Analyses of Coal Tar Fractions

The normal phase HPLC chromatogram of each of the four fractions is shown in Figure 10. NPLC separates compounds in the order of increasing polarity and thus should be related to the adsorption chromatography elution order from the alumina column. Indeed, the retention time ranges of the components in the four fractions were consistent with the increased polarity of the compounds in the four fractions. Compounds in fraction A1 eluted in less than 10 minutes while compounds in fractions A2 eluted between 4 and 30 minutes. Similarly, compounds in fraction A3 eluted between 22 and 55 minutes. Overall, the elution order from the alumina column was reflected strongly in the NPLC elution order and there was little overlap in the elution of the fractions.

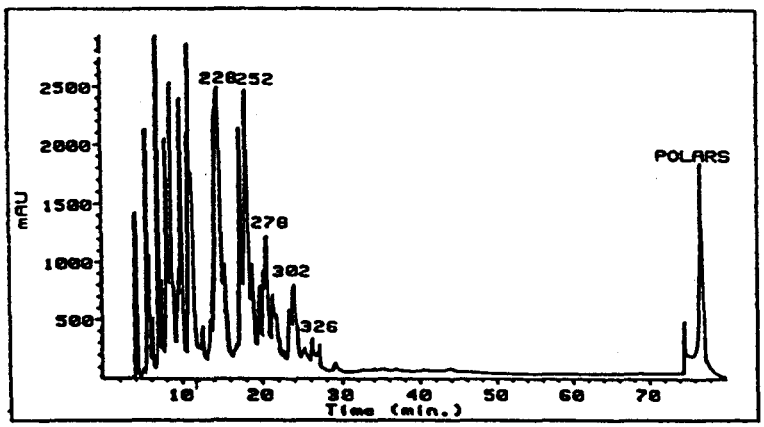
The annotated numbers near peaks in these chromatograms refer to the molecular masses of PAH in those peaks. These values were assigned based on previous work in our

Figure 10. Normal phase HPLC analyses of coal tar fractions A1, A2, A3 and A4

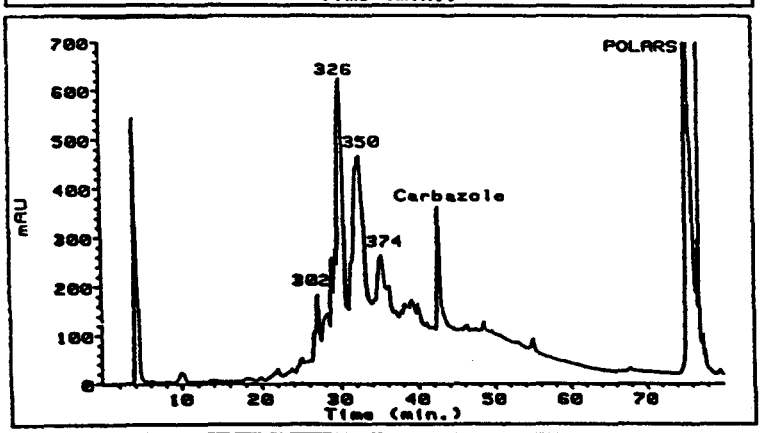
Fraction A1



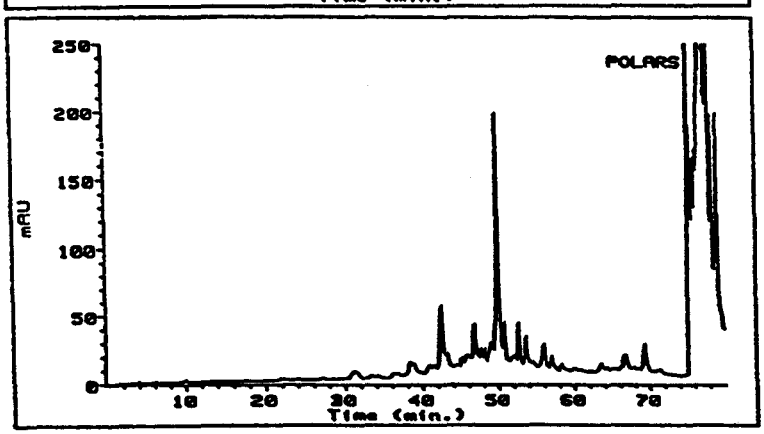
Fraction A2



Fraction A3



Fraction A4

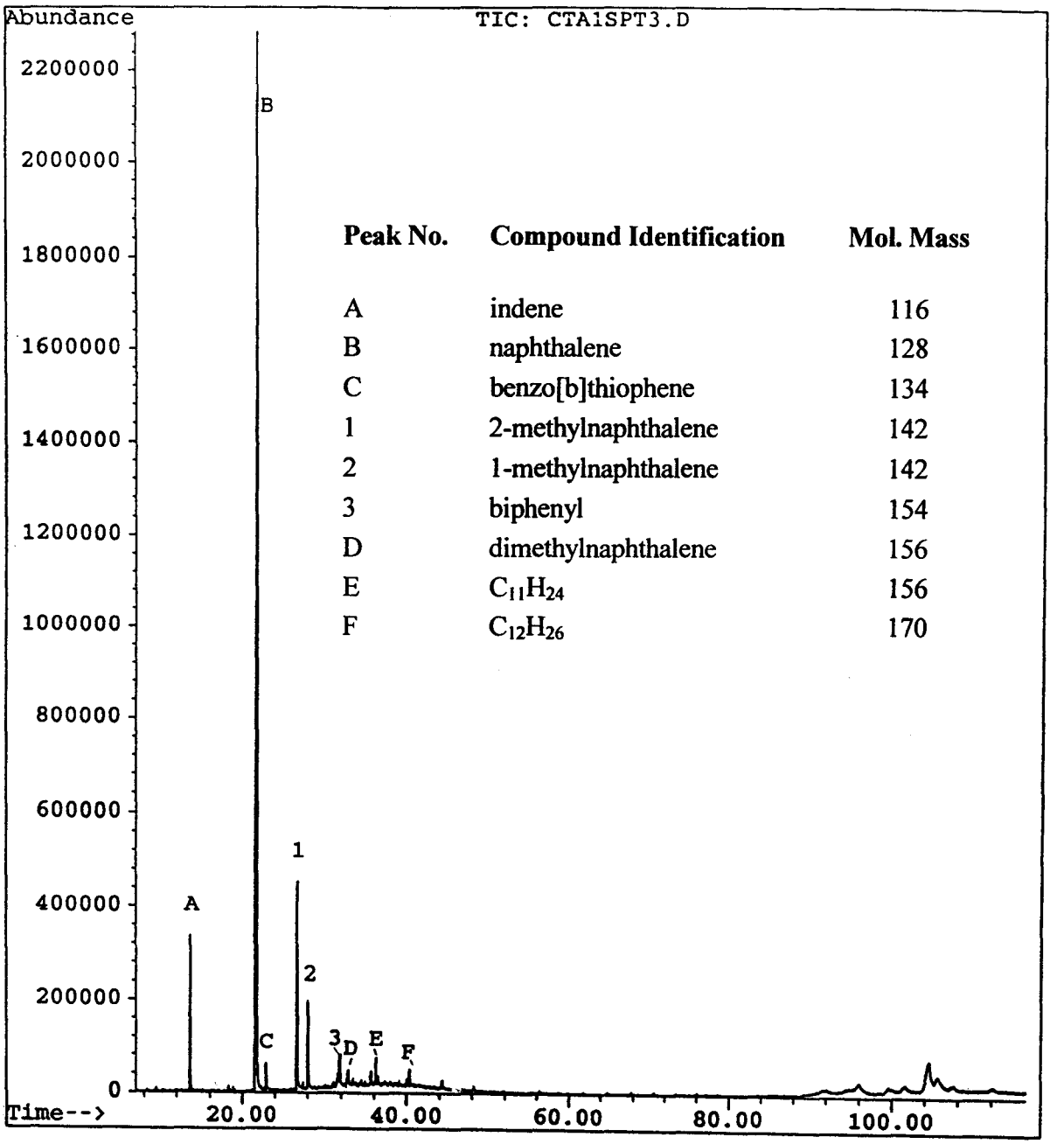


group (for fractions A1 and A2) (23, 24), and on the work discussed below. Based on the NPLC analyses shown in Figure 10, it was decided to analyze the four fractions in different ways. GC-MS analyses were performed on all four fractions. In addition, probe mass spectrometry and LC-MS analyses were carried on fraction A3 due to the high mass compounds in this fraction. Fraction A4 was also analyzed by probe mass spectrometry because it had polar PAC. The results of these analyses are discussed in the following sections.

III. 2. 2 Analyses of Fraction A1

The compounds in fraction A1 had retention times of less than 10 min. in normal phase HPLC (Figure 10) and the major peak at 9.5 min. was assigned to naphthalene based on the comparison of its UV spectrum with library spectra. The GC-MS total ion current (TIC) chromatogram of fraction A1 is shown in Figure 11. Some of compounds in this fraction were identified based upon their retention characteristics together with a comparison of their mass spectra with library spectra. The major peaks in this GC-MS chromatogram were low molecular mass PAH, such as naphthalene, indene and biphenyl along with some aliphatics. No other analytical work was done on this fraction.

Figure 11. GC-MS total ion current chromatogram of fraction A1*.



Peak No.	Compound Identification	Mol. Mass
A	indene	116
B	naphthalene	128
C	benzo[b]thiophene	134
1	2-methylnaphthalene	142
2	1-methylnaphthalene	142
3	biphenyl	154
D	dimethylnaphthalene	156
E	$C_{11}H_{24}$	156
F	$C_{12}H_{26}$	170

*The sample was analyzed on 30 m DB-17ht column by Method B in full scan. Compound identification was based upon comparison of mass spectra with library spectra.

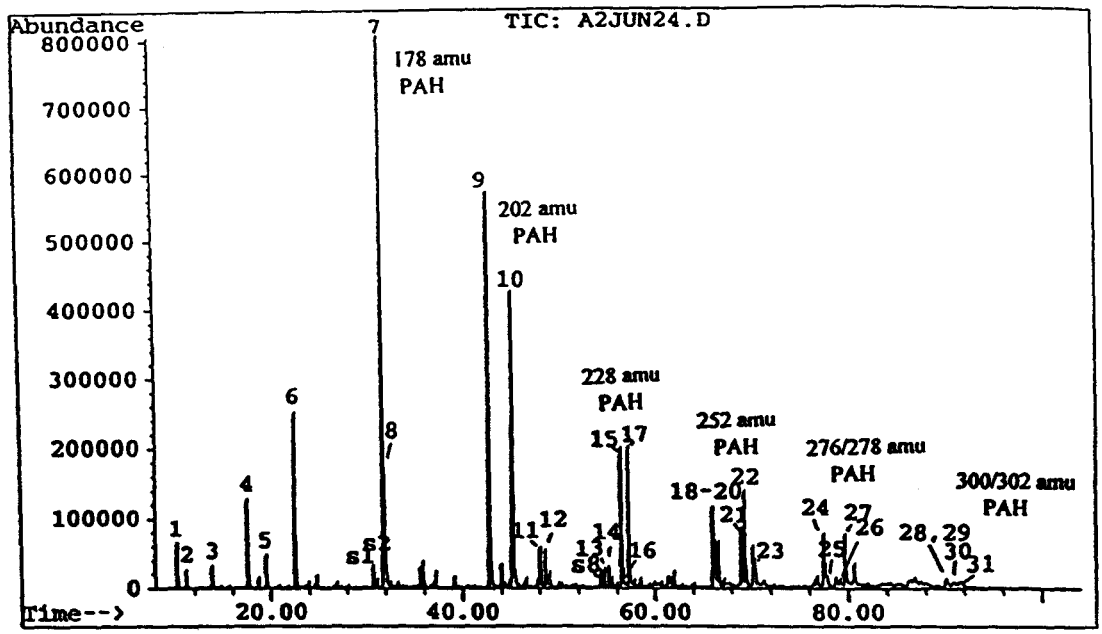
III. 2. 3 Analyses of Fraction A2

Fraction A2 which accounted for about half of the mass recovered from the alumina column contained most of the standard PAH and their alkyl derivatives, together with small amounts of some high molecular mass PAH. The GC-MS total ion current (TIC) chromatogram of this fraction is shown in Figure 12a while Figure 12b shows the superposition of five mass chromatograms of the molecular ions of the major classes of PASH at m/z 184, 208, 234, 258 and 284. The molecular masses of the peaks are labelled on the chromatograms. Compounds identified and quantitated in this fraction are listed in Table 9. Compound identification was based upon comparison of retention times or retention index values and mass spectral data with those of authentic standards. The PASH compounds listed in Table 9 correspond to the major peaks observed in the Figure 12b. Compound s6 was removed from the list because it was not a sulfur-containing PAH. More PASH will be identified when authentic standards are available. Retention index values were determined for all of the compounds listed in Table 9 based on the average of four analyses of this fraction.

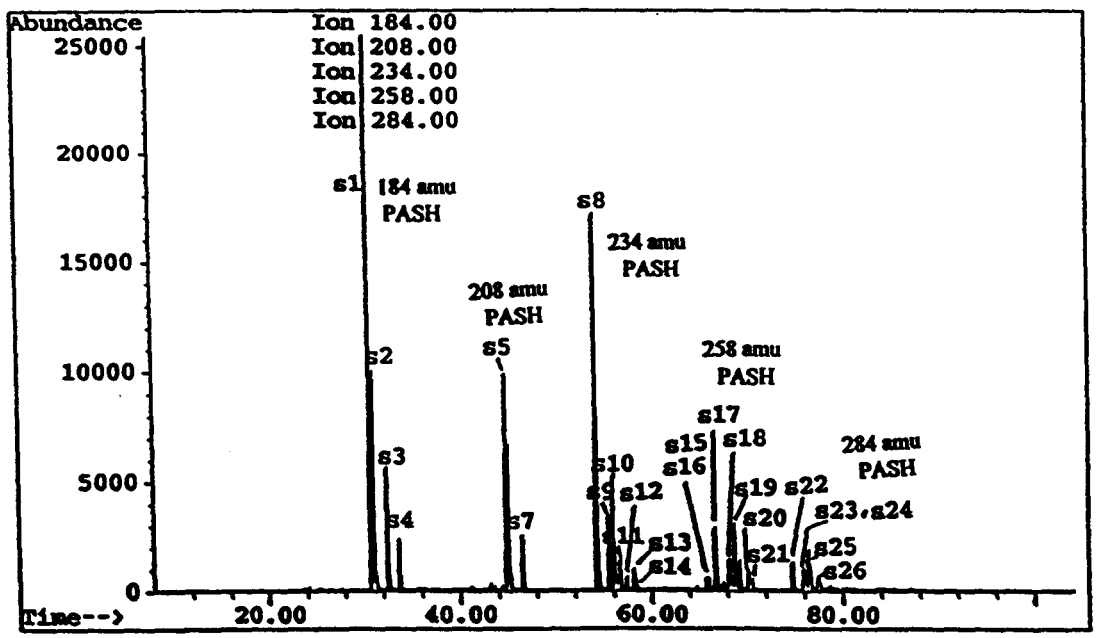
The quantitative analysis of all the major PAH and some PASH in this fraction was done using pyrene-d_{10} and perylene-d_{12} as internal standards. Pyrene-d_{10} was used to quantify the three- and four-ring PAC while perylene-d_{12} was used to quantify five- and six-ring PAC. The total amount of PAH was 158 mg/g coal tar which was about 25 times higher than the

Figure 12. GC-MS analysis of fraction A2*

12a



12b



a. Total ion current chromatogram.

b. Extract ion chromatograms of m/z 184, 208, 234, 258 and 284. Peak number refer to compounds listed in Table 9. s6 was not a PASH as we found later on.

*The sample was analyzed on 30 m DB-17ht column by Method B in S.I.M.

Table 9. Compounds Identified in Fraction A2 by GC-MS Analysis on a 30 m DB-17ht Column (Method B)

Peak No.	Molecular Mass	Compound Name	Retention [a] Index (R.I.)	Conc. μg/g (in Coal Tar)
PAH				
1	142	2-Methylnaphthalene		1400
2	142	1-Methylnaphthalene		572
3	154	Biphenyl		819
4	152	Acenaphthylene		4430
5	154	Acenaphthene		265
6	166	Fluorene		6250
7	178	Phenanthrene	**300.00**[b]	32900
8	178	Anthracene	300.61±0.15	8080
9	202	Fluoranthene	343.28±0.08	28200
10	202	Pyrene	352.89±0.07	21900
11	216	Benzo[a]fluorene	363.95±0.29	1790
12	216	Benzo[b]fluorene	366.03±0.27	1550
13	226	Benzo[ghi]fluoranthene	389.80±0.12	1240
14	228	Benzo[c]phenanthrene	391.57±0.19	687
15	228	Benz[a]anthracene	396.98±0.18	8900
16	226	Cyclopenta[cd]pyrene	398.54±0.15	385
17	228	Chrysene	**400.00**[b]	8430
18	252	Benzo[b]fluoranthene	440.29±0.19	4010
19	252	Benzo[k]fluoranthene	441.31±0.02	2710
20	252	Benzo[j]fluoranthene	442.80±0.09	2340
21	252	Benzo[e]pyrene	453.62±0.12	3160
22	252	Benzo[a]pyrene	455.31±0.14	5750
23	252	Perylene	460.05±0.12	1570
24	276	Indeno[1,2,3-cd]pyrene	492.28±0.18	3660
25	278	Dibenz[a,c]anthracene	492.86±0.19	584
26	278	Picene	**500.00**[b]	428
27	276	Benzo[ghi]perylene	502.26±0.33	3580
28	300	Coronene		602
29	302	Dibenzo[a,e]pyrene		464
30	302	Dibenzo[a,i]pyrene		735
31	302	Dibenzo[a,h]pyrene		759
			Total PAH:	**158000**

PASH				
s1	184	Dibenzothiophene	295.68±0.10	1300
s2	184	Naphtho[1,2-b]thiophene	297.09±0.15	530
s3	184	Naphtho[2,1-b]thiophene	302.59±0.14	337
s4	184	Naphtho[2,3-b]thiophene	307.22±0.35	157
s5	208	Phenanthro[4,5-bcd] thiophene	351.19±0.13	560
s7	208	Phenaleno[6,7-bc]thiophene	357.87±0.27	169
s8	234	Benzo[b]naphtho[2,1-d] thiophene	388.26±0.19	1020
s9	234	Benzo[b]naphtho[1,2-d] thiophene	393.03±0.22	199
s10	234	Benzo[b]naphtho[2,3-d] thiophene	394.87±0.16	379
s11	234	Phenanthro[1,2-b]thiophene	397.51±0.27	96
s12	234	Anthra[2,1-b]thiophene	400.13±0.22	42
s13	234	Phenanthro[2,1-b]thiophene	403.38±0.21	60
s14	234	Phenanthro[2,3-b]thiophene	403.97±0.28	36
s15	258	258 amu PASH	439.05±0.10	24
s16	258	258 amu PASH	439.95±0.21	24
s17	258	Benzo[2,3-phenanthro] [4,5-bcd]thiophene	443.48±0.13	139
s18	258	Triphenyleno[4,5-bcd] thiophene	450.05±0.36	84
s19	258	Chryseno[4,5-bcd] thiophene	452.66±0.25	157
s20	258	258 amu PASH	454.78±0.23	72
s21	258	258 amu PASH	459.08±0.13	48
s22	284	Dinaphtho[1,2-b:1'2'-d] thiophene	485.14±0.34	60
s23	284	Benzo[b]phenanthro [3,4-d]thiophene	485.98±0.32	42
s24	284	Dinaphtho[1,2-b:2',3'-d] thiophene	487.64±0.28	48
s25	284	Benzo[b]phenanthro [3,2-d]thiophene	488.16±0.33	108
s26	284	284 amu PASH	492.96±0.33	36
			Total PASH:	**5730**

[a] Data obtained from four consecutive injections of coal tar fraction A2 in toluene.
[b] Phenanthrene, chrysene and picene are retention index standares.

total amount of PASH (5.73 mg/g coal tar) in this fraction. It is clear that PASH were only minor components in fraction A2 by comparing the level of certain PASH (e.g., s1, s2, s8) to the level of certain PAH (e.g., 7, 8, 15, 17) in Figure 12a. There was no evidence for oxygen-containing or nitrogen-containing PAC in this fraction. The separation of PASH from PAH will be discussed in Section **III. 3**.

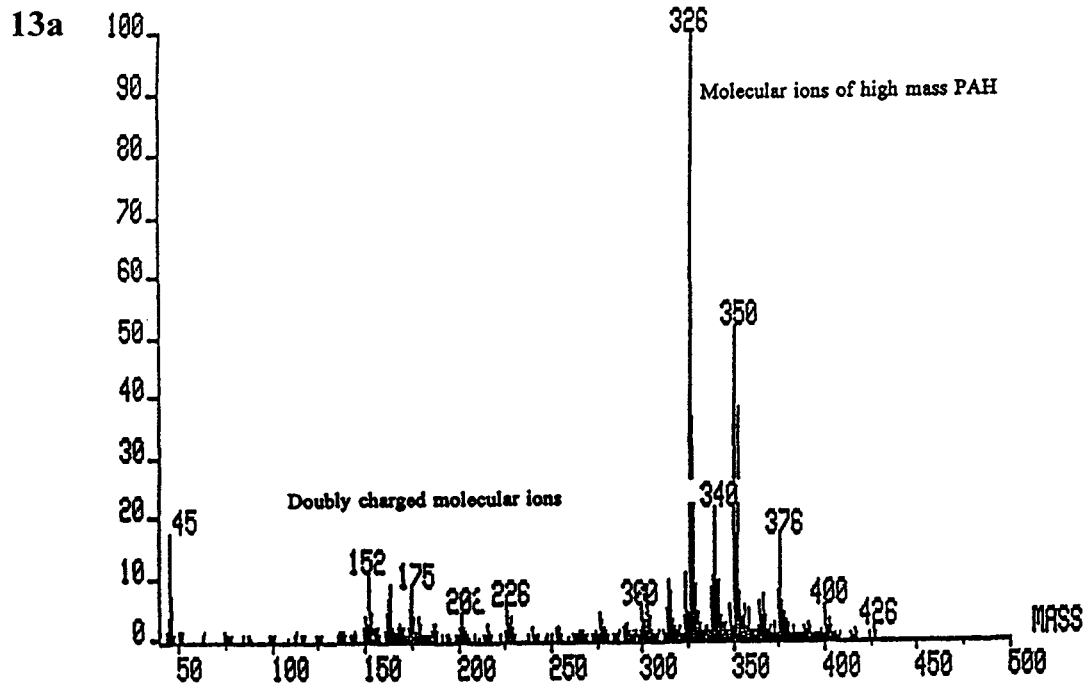
The normal phase HPLC chromatogram of coal tar fraction A2 (Figure 10) showed more peaks than other fractions and all peaks eluted in less than 30 minutes. Normal phase liquid chromatography can separate PAH based on the number of aromatic rings presumably due to the increasing adsorption of the aromatic system with the stationary phase (43). The PAH elute in isomeric groups in order of increasing molecular mass (i.e., increasing number of aromatic pi-electrons) with alkyl-substituted PAH eluting along with the parent PAH. Thus, normal phase chromatography can be used to isolate fractions containing isomeric PAH and related alkyl-substituted isomers. This normal-phase LC procedure has been used as the first step in multi-dimensional chromatographic procedures for the cleanup and isolation of PAH from environmental samples.

III. 2. 4 Analyses of Fraction A3

The normal phase HPLC analysis of fraction A3 (Figure 10) showed that all compounds eluted between 22 and 55 minutes, indicating that more polar species and high

mass PAH were major components of this fraction. Most of compounds in fraction A3 were high molecular mass PAH (labeled on the HPLC chromatogram in Figure 10) and as such were involatile; thus, they would never elute from the GC column. So, GC-MS analysis provided information only about those compounds which could elute from the GC column

Fraction A3 was then analyzed using probe mass spectrometry by DEI and DCI with NH_3 as the reagent gas. The probe mass spectra (shown in Figure 13) provided molecular mass information about the major components in the sample. Figure 13a and 13b are the DEI spectra; 13a shows ions with mass from 50 to 500 while 13b shows the ions in the mass 300 to 420 range. The most intense peak in the DEI spectra (Figures 13a, 13b) arose from 326 molecular mass PAH; the methyl-substituted 326 amu PAH at 340 amu were about 20% of this intensity. Other major ions were associated with 350, 352, 328, 374, 376 and 400 amu PAH (in order of decreasing intensities). Doubly-charged molecular ions corresponding to these high mass PAH were also observed in the spectrum. The ammonia DCI mass spectra (Figures 13c, 13d) show both the PAH and the nitrogen-PAC in the sample. In Figure 13c the volatile, low mass nitrogen-PAC predominate while in Figure 13d the less volatile, high mass PAH predominate. Because all the compounds are protonated under DCI conditions, compounds with even molecular masses in these spectra correspond to the compounds with odd mass value as neutral molecules, e.g., nitrogen-PAC. In the same way, compounds with odd molecular masses in these spectra correspond to the compounds with



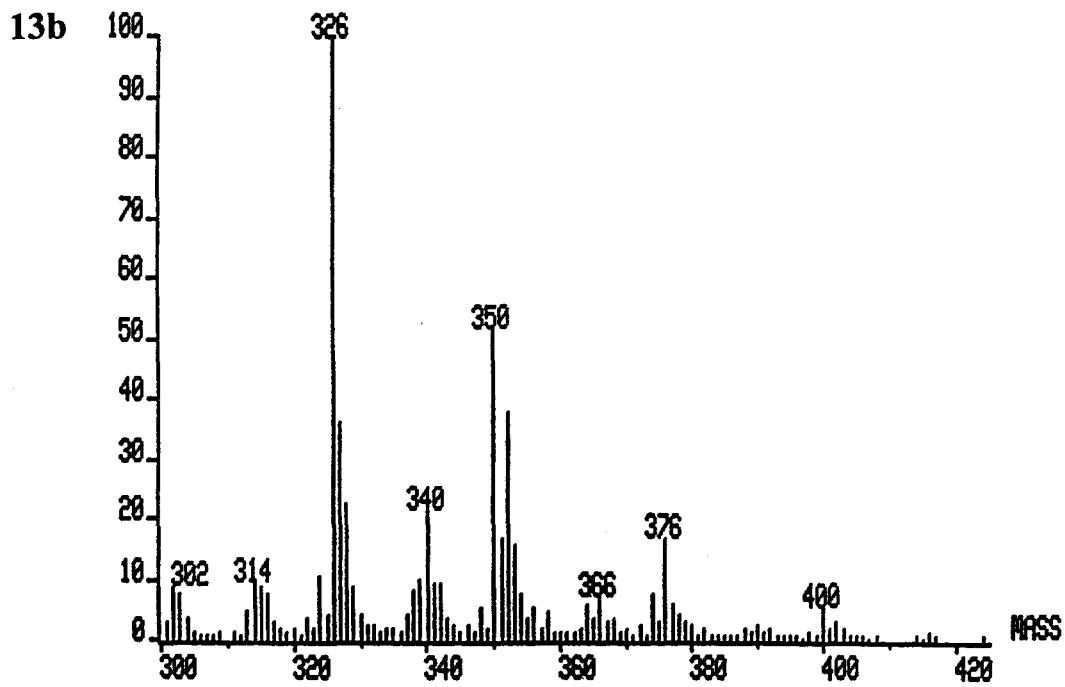
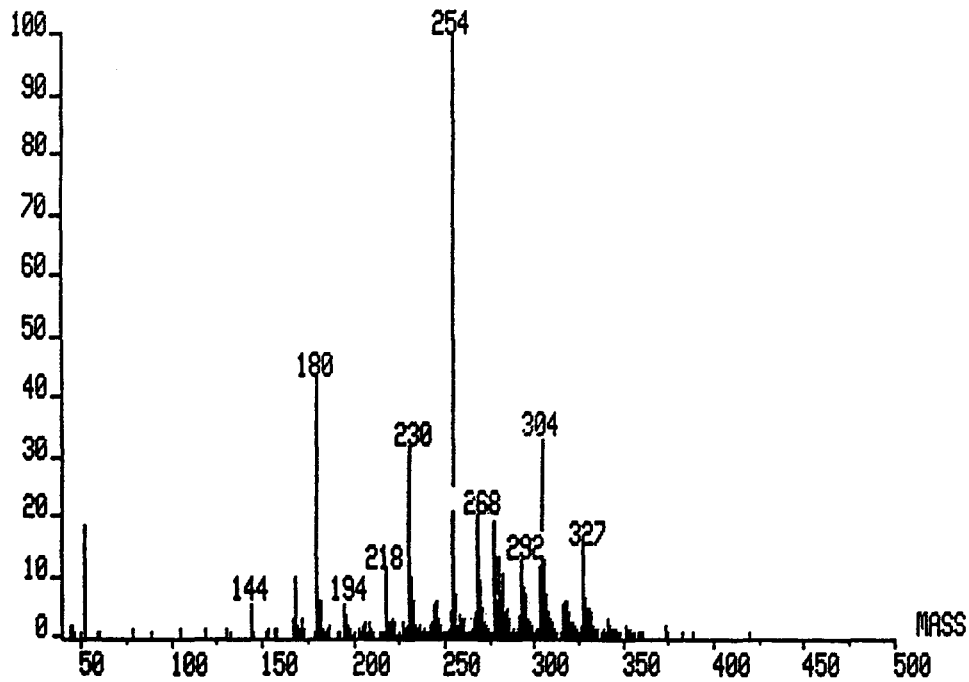


Figure 13. Probe mass spectrometric analyses of fraction A3

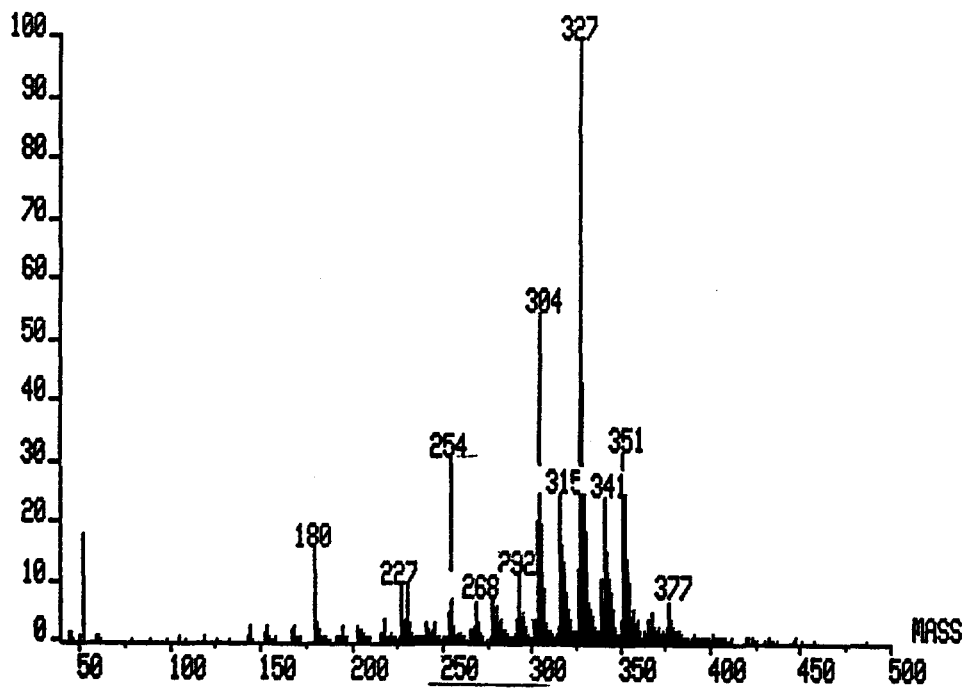
a. DEI probe mass spectrum of coal tar fraction A3. Ions from m/z 50 to 500 are included.

b. DEI probe mass spectrum of coal tar fraction A3. Ions from m/z 300 to 420 are included.

13c



13d



c. Ammonia DCI probe mass spectra of coal tar fraction A3. The volatile and low mass N-PAC were predominate.

d. Ammonia DCI probe mass spectra of coal tar fraction A3. The less volatile, high mass PAH were predominate

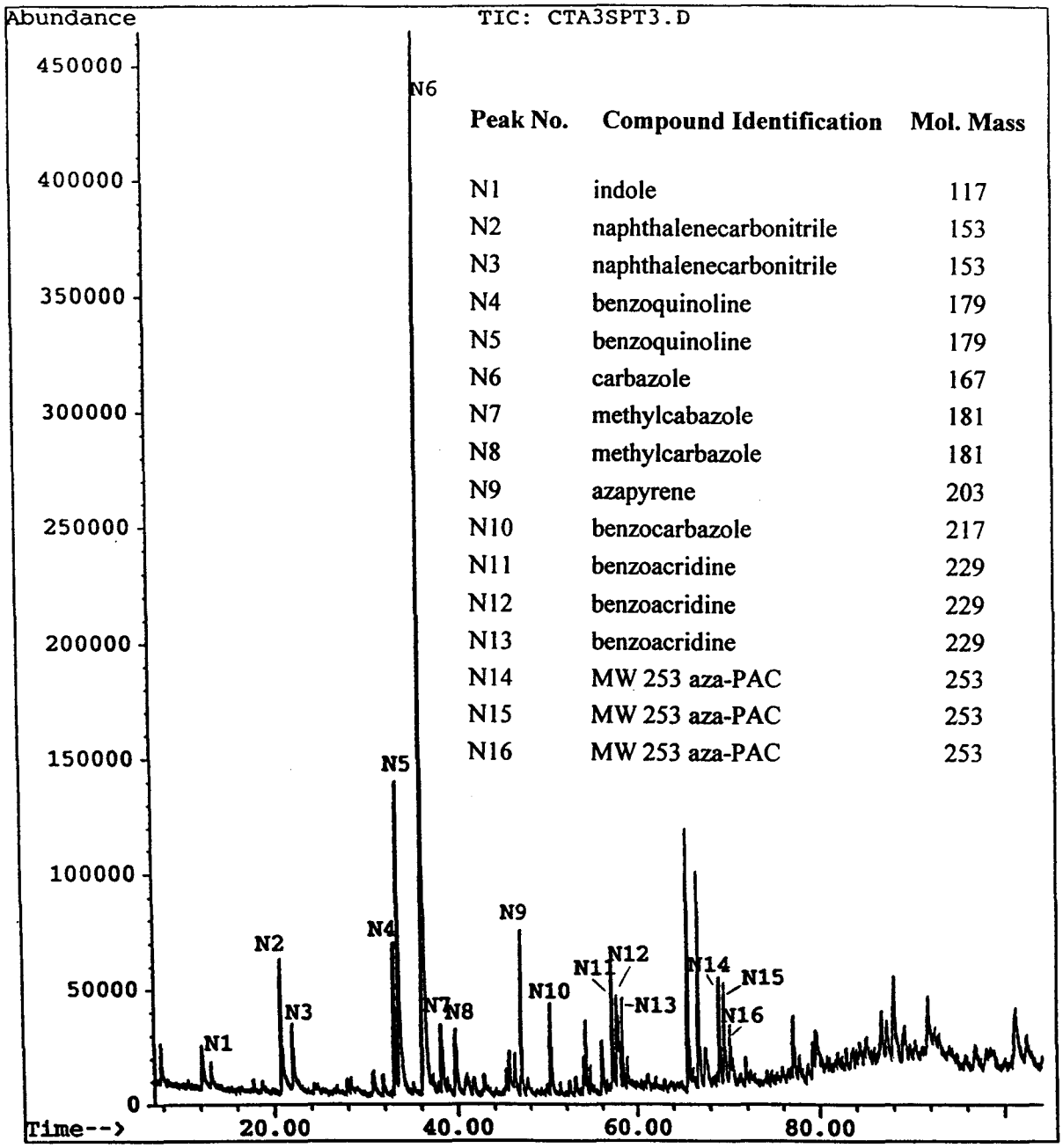
even mass value as neutral molecules. For example, the m/z 254 ion in Figure 13c corresponds to molecular mass 253 aza-aromatics in fraction A3, the peak was arisen from all the 253 amu aza-aromatics in the sample. Similarly, the m/z 327 ion in Figure 13d was produced by all the 326 amu PAH in the sample. From both DEI and ammonia DCI probe mass spectrometric analyses, it was concluded that fraction A3 contained high mass (300, 326, 350, 376, 400 and 426 amu) PAH and nitrogen-PAC (217, 229, 253, 267, 303 amu). Further analyses including GC-MS and LC-MS were performed on this fraction in order to identify nitrogen-PAC and high mass PAH.

Fraction A3 was analyzed using GC-MS in the full scan mode (Figure 14). Most of the peaks in this chromatogram had odd mass values indicating that these peaks were nitrogen-containing compounds. Some of compounds such as indole and carbazole were identified based upon comparison of their mass spectra with library spectra. For most of peaks, the name of possible isomers were listed in Figure 14.

In order to characterize the high mass PAH in fraction A3, LC-MS analyses were performed on the sample. LC-MS ion chromatograms including 374, 350 and 326 amu PAH are shown in Figure 15. Some of high mass compounds were identified in LC-MS analysis by comparing the retention time and mass spectra with the authentic standards. Six compounds were identified in LC-MS analysis due to the unavailability of the standards.

The combination of probe mass spectrometry, GC-MS and LC-MS analyses afforded

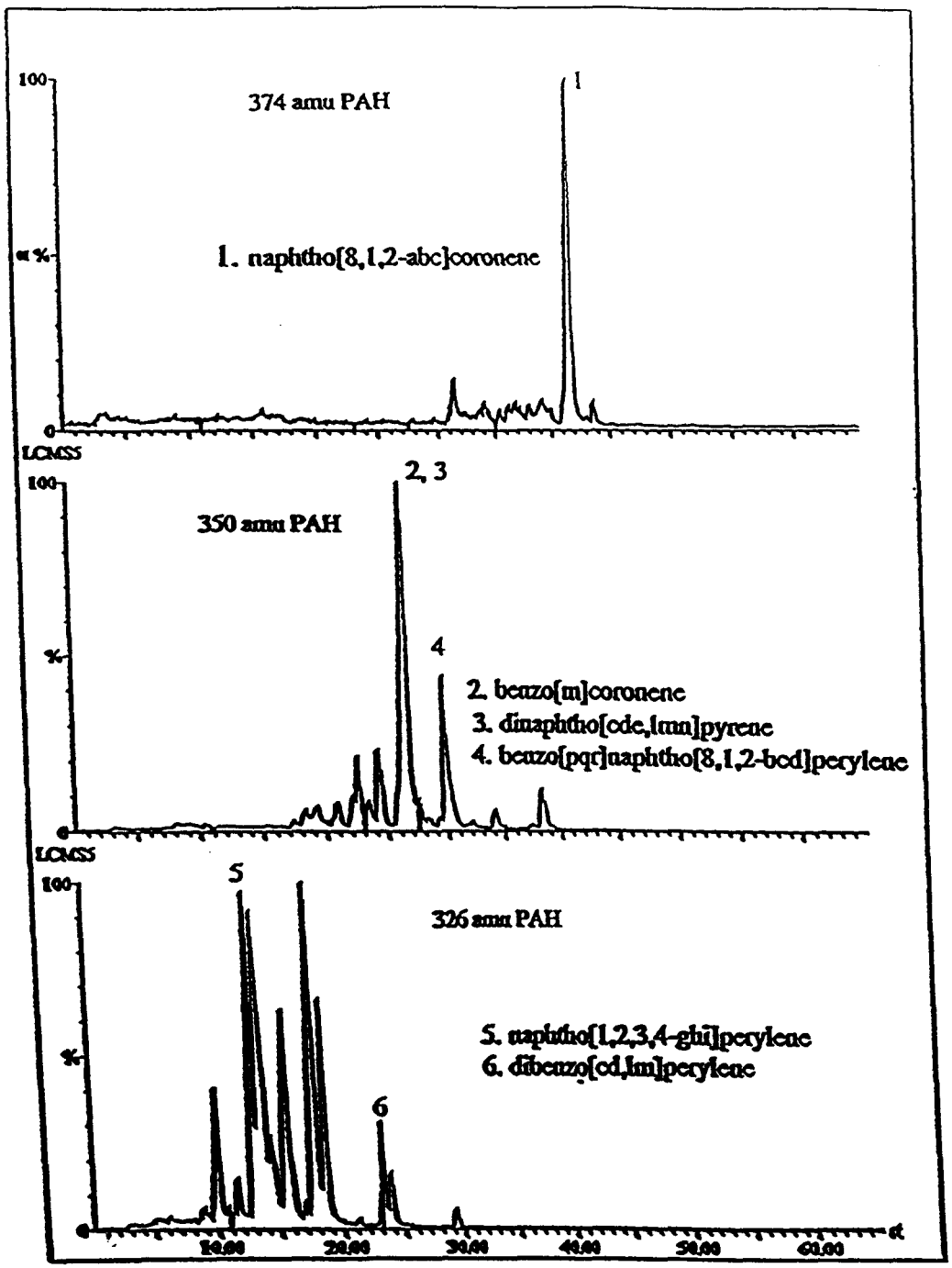
Figure 14. GC-MS total ion current chromatogram of fraction A3*



Peak No.	Compound Identification	Mol. Mass
N1	indole	117
N2	naphthalenecarbonitrile	153
N3	naphthalenecarbonitrile	153
N4	benzoquinoline	179
N5	benzoquinoline	179
N6	carbazole	167
N7	methylcabazole	181
N8	methylcarbazole	181
N9	azapyrene	203
N10	benzocarbazole	217
N11	benzoacridine	229
N12	benzoacridine	229
N13	benzoacridine	229
N14	MW 253 aza-PAC	253
N15	MW 253 aza-PAC	253
N16	MW 253 aza-PAC	253

*The sample was analyzed on 30 m DB-17ht column by Method B in full scan.
(together with the name of possible isomers for various molecular mass compounds).

Figure 15. LC-MS analysis of fraction A3*.



*The ion chromatograms of 374, 350 and 326 amu PAH are included in this figure. Compound identification was based upon comparison of retention time and mass spectra with the authentic standards.

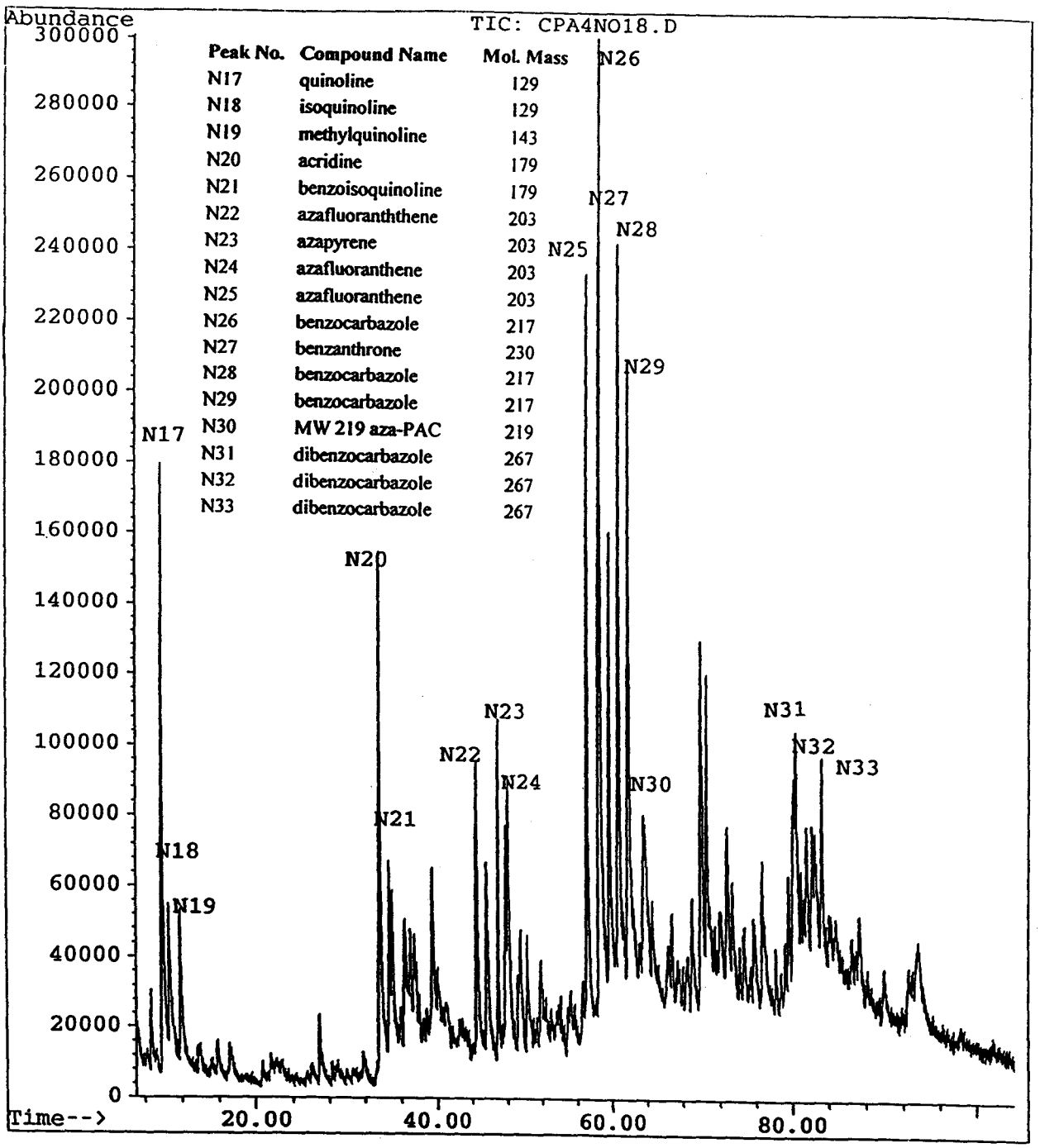
a complete profile of the compounds in fraction A3. Both low molecular mass aza-aromatics and high mass PAH compounds were identified in this fraction. It is clear from these analyses that no single analytical method could provide a representative profile of this sample.

III. 2. 5 Analyses of Fraction A4

Fraction A4 was eluted from the neutral alumina column with methanol and accounted for 21% of the material that eluted from the column. The increased polarity of these components was reflected in the normal phase HPLC analysis (Figure 10) where no compounds eluted before 30 minutes. As with fraction A3, both GC-MS and probe mass spectrometric analyses were performed on this fraction. The GC-MS total ion chromatogram is shown in Figure 16 while the probe mass spectra are shown in Figure 17. From GC-MS analysis (Figure 16), it was found that most compounds eluted from the GC column had odd molecular mass values which corresponded to nitrogen-PAC. The names of the possible isomers are listed in Figure 16.

Figure 17a is the DEI probe mass spectrum of fraction A4; Figure 17b is the ammonia DCI probe mass spectrum. The two spectra show that nitrogen-PAC were the major components in this fraction. Representative structures of some peaks are shown on the DEI probe mass spectrum (Figure 17).

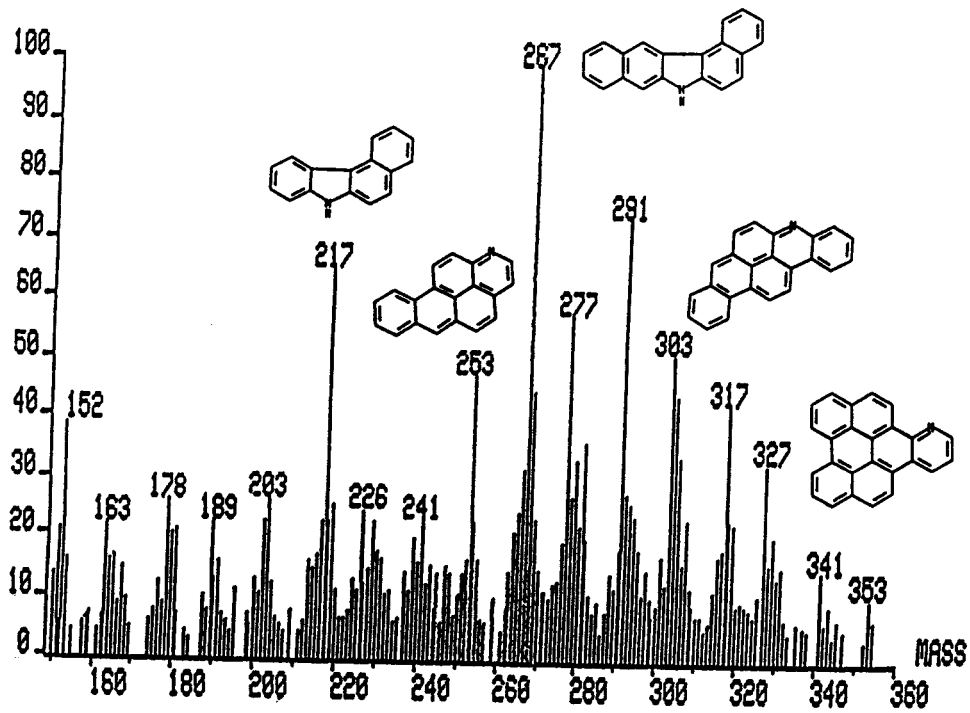
Figure 16. GC-MS total ion chromatogram of fraction A4*



Peak No.	Compound Name	Mol. Mass
N17	quinoline	129
N18	isoquinoline	129
N19	methylquinoline	143
N20	acridine	179
N21	benzoisoquinoline	179
N22	azafluoranththene	203
N23	azapyrene	203
N24	azafluoranthene	203
N25	azafluoranthene	203
N26	benzocarbazole	217
N27	benzanthrone	230
N28	benzocarbazole	217
N29	benzocarbazole	217
N30	MW 219 aza-PAC	219
N31	dibenzocarbazole	267
N32	dibenzocarbazole	267
N33	dibenzocarbazole	267

*The sample was analyzed on 30 m DB-17ht column by Method B in full scan.
Compound identification was based upon comparison of mass spectra with library spectra.

17a



17b

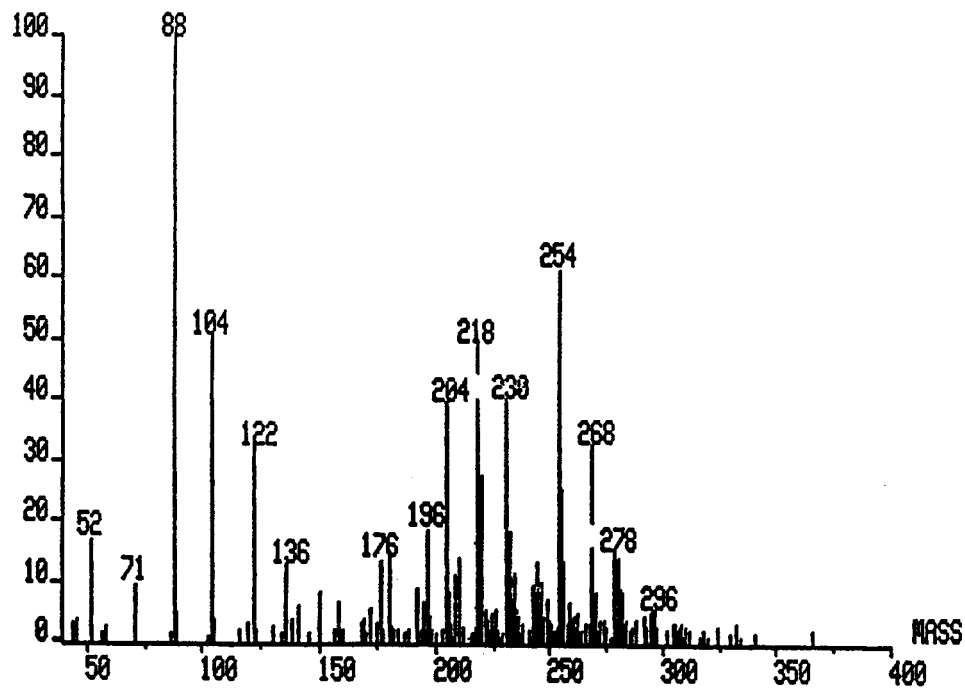


Figure 17. Probe mass spectrometric analyses of fraction A4.

a. DEI probe mass spectrum of coal tar fraction A4 (together with representative structures of compounds for various molecular masses).

b. Ammonia DCI probe mass spectra of coal tar fraction A4.

It was found in this study that the compound classes in fractions A3 and A4 were different from what was reported by Later (29). In the original method, Later *et al.* (29) reported the nitrogen-PAC in fraction A3 and hydroxyl polycyclic aromatic hydrocarbons in fraction A4. Our results showed the high molecular mass PAH was the major component in fraction A3 besides the nitrogen-PAC. The difference was that we analyzed the sample using not only GC-MS, which was the only method used by Later, we also used HPLC and probe mass spectrometric techniques which were crucial for the characterization of high molecular mass PAH. In fraction A4, instead of hydroxyl polycyclic aromatic hydrocarbons, most of compounds we found were nitrogen-PAC. The possible reason is that the solvent we used (methanol) for elution of fraction A4 was not as effective as the solvent used in the original method (tetrahydrofuran/ethanol).

III. 3 Separation of Thia-arenes from PAH

III. 3. 1 Separation Using Oxidation/Reduction Procedure

In the two methods used for separation of PASH from PAH, we chose to use the oxidation/reduction procedure first because it could be scaled up easily. Oxidation/reduction method makes use of the fact that thia-arenes can be converted into polar sulfones by oxidation. The sulfones can then be readily separated from polycyclic aromatic hydrocarbons by column chromatography on silica according to their greater polarity. Reduction of the sulfones back to PASH with lithium aluminum hydride is reported to give a solution containing only the sulfur aromatics of the sample.

The oxidation of coal tar fraction A2 with H_2O_2 (and the following reduction with $LiAlH_4$) were performed according to Lee's procedure (35). A sample of 1g of fraction A2 was taken through the whole sequence of reactions and chromatographic separations (oxidation-silica gel chromatography-reduction-silica gel chromatography), only 1 mg (4.4%) of PASH-enriched fraction was obtained. This low recovery made the oxidation/reduction procedure almost useless in the separation of PASH from PAH. By analyzing the intermediates from each reaction and separation step with GC-MS in full scan, we found problems which cause the inefficiency of the procedure; and our results were similar to Andersson's (19). First, refluxing the fraction A2 together with hydrogen peroxide in acetic acid oxidized not only the sulfur atom, it also oxidized the aromatic rings. So, both PASH

and PAH were oxidized under the reaction conditions and PASH were also oxidized to other products. Compounds containing the anthracene ring system were oxidized to quinones which were further oxidized to carboxylic acids easily. In fact, there appeared (M-44) ions which corresponded to the loss of carboxylic group in the GC-MS analysis of the oxidation product. Second, $LiAlH_4$ not only reduced the sulfones back to PASH, it also hydrogenation of the 2,3-double bond in sulfones to give new compounds.

All of the side reactions lead to losses of many analytes and possibly to the formation of new PASH. Andersson(19) concluded that the oxidation/reduction procedure should be avoided both in the study of PAH and of PASH. Then it was decided to use another separation procedure, ligand-exchange chromatography, as shown below.

III. 3. 2 Separation of Thia-arenes from PAH by Ligand-exchange Chromatography

The ligand exchange chromatographic procedure reported by Nishioka *et al.* (34) involved chromatography on silica gel containing 5% palladium chloride. This methodology was evaluated and modified in this study. The basis for the separation of PASH on $PdCl_2$-silica gel lies in the thiophilic nature of heavy transition metal salts. Thus, thiophenic compounds should be retained preferentially on $PdCl_2$ column; washing should remove unretained compounds and a more powerful solvent should remove the retained thiophenes.

III. 3. 2. 1 Development of a Thia-arene Separation Method

It was reported in the original procedure (34) that PASH were eluted from the $PdCl_2$-silica column in the form of $PdCl_2$/PASH complexes. These complexes were destroyed prior to GC analysis by the addition of diethylamine prior to direct injection onto a GC column (34). If this treatment is left out and if hydrogen is used as the carrier gas, some compounds are desulfurized in the injection port or during the GC analysis. Although the carrier gas was helium and not hydrogen, which meant that desulfurization should not occur, it was deemed wise from a chromatography perspective to remove any Pd salts prior to GC analysis of the sample.

Nishioka's method used diethylamine to destroy any Pd-thiophene complexes in solution; the Pd diethylamine mixture was injected directly onto the GC column. We chose to use an alternative method involving an extraction step to remove the Pd, thereby avoiding the injection of Pd onto the GC column. We selected an aqueous cyanide treatment to remove Pd species from the organic eluent. The formation of $Pd(CN)_4^{2-}$ complex resulted the release of PASH from the $PdCl_2$/PASH complexes. This $Pd(CN)_4^{2-}$ complex was removed from the organic phase by partitioning into the aqueous layer. While this procedure entailed a mini-extraction and a subsequent wash with water, it was felt that this method would not result in any strong base or Pd being injected onto the GC column. Thus, the life of the GC column should not be shortened as it would be if it were subjected routinely to

injection of diethylamine and Pd salts.

In our hands, Nishioka's procedure did not give us the results as reported, even though we followed the $PdCl_2$-silica preparation procedures accurately according to the published method. Thus modifications to Nishioka's procedure seemed appropriate and necessary. We proposed to:

1. Prepare an adsorbent using the same $PdCl_2$-silica preparation procedure as outlined in the literature but to use different solvents to elute the PAH and PASH.

2. Prepare a new Pd-silica column material by using a soluble palladium complex ($Pd(benzonitrile)_2Cl_2$) to prepare the adsorbent.

Both proposals were tested and are discussed below.

III. 3. 2. 2 Development of a Solvent System for $PdCl_2$-silica Column Chromatography

Our goal was to elute most if not all PAH from the column with a suitable solvent before using a more polar solvent to elute the PASH. The ideal procedure would separate PAH from PASH cleanly in a few fractions. Thus, the selection of proper elution conditions was critical to the success of this method. Good solvents for PAH and PASH, such as chloroform, were not suitable for the separation of PAH and PASH because they resulted in the coelution of the two classes of compounds. When ether solvents such as diethyl ether and tetrahydrofuran were used, significant amounts of $PdCl_2$ were eluted from the column.

A series of four experiments was carried out to develop a solvent system based on hexane and dichloromethane; the same amount of coal tar fraction A2 (100 mg) was used in each experiment together with the same amount of $PdCl_2$-silica (5 g).

At the outset we decided to evaluate the $PdCl_2$-silica method using coal tar, a complex mixture, rather than standards for two reasons. First, we would need to do this work on coal tar in any event. Second, model compounds are just that; experiments which may work or fail on model compounds may not reflect conditions needed to separate compounds in a complex mixture or with a wider range of properties than those of the standards. In addition, the high efficiency of GC-MS meant that analyses of coal tar fractions would yield a wealth of information about the PAH and PASH in each fraction.

Each experiment was performed in an identical fashion. After adsorbing 100 mg of coal tar fraction A2 onto a small amount of $PdCl_2$-silica (0.5 g) by evaporation at reduced pressure, the silica material was loaded on top of 5 g of active $PdCl_2$-silica gel prepared according to Nishioka *et al.* The volumes and compositions of solvents used to elute PAH and PASH are provided in Figures 18-21.

Each fraction was analyzed by GC-MS using Method A, however, the method of analysis was somewhat unusual. From the GC-MS analyses of each fraction, the peak areas under five different molecular mass classes of PAH (178, 202, 228, 252 and 276 amu) and five different molecular mass classes of PASH (184, 208, 234, 258 and 284 amu) were

determined. For each molecular mass class, the peak areas of all isomers were summed to give a single value for that class. For example, the peak area of the 178 molecular mass PAH was the sum of the areas of phenanthrene and anthracene. The peak areas of all molecular mass classes of PAH and PASH should afford the same total value; thus, each fraction could be normalized by such a procedure. This procedure was used in all four experiments discussed below and the results are summarized in Figures 18-21.

The results of the first experiment are shown in Figure 18. More than 99% of the PAH and 73% of the PASH were eluted by dichloromethane/hexane (1:1) in the first three fractions (total volume: 30 mL), indicating that this solvent was too powerful since both PAH and PASH eluted from the column. However, 27% of the PASH were still retained on the column, mainly in the last two fractions which meant that 1:1 dichloromethane/hexane was not strong enough to get all of the PASH off the column. The PASH that were retained on the column were 234, 258 and 284 amu PASH (Figure 18). It should be remembered that the absolute area under the PAH peaks is about 25 times greater than under the PASH peaks; in Figure 18 and subsequent figures all PAH and PASH classes are normalized to 100%.

A second experiment used larger volumes of solvents in different solvent steps. A total of five fractions were collected from the $PdCl_2$-silica column and were analyzed by GC-MS; the result of this experiment is plotted in Figure 19. The first two fractions contained 84% of the PAH but only 19% of the PASH; the remaining PAH and most of the

Figure 18. Elution profiles of PAH/PASH in the eight fractions collected from Pd-SiO_2 experiment # 1

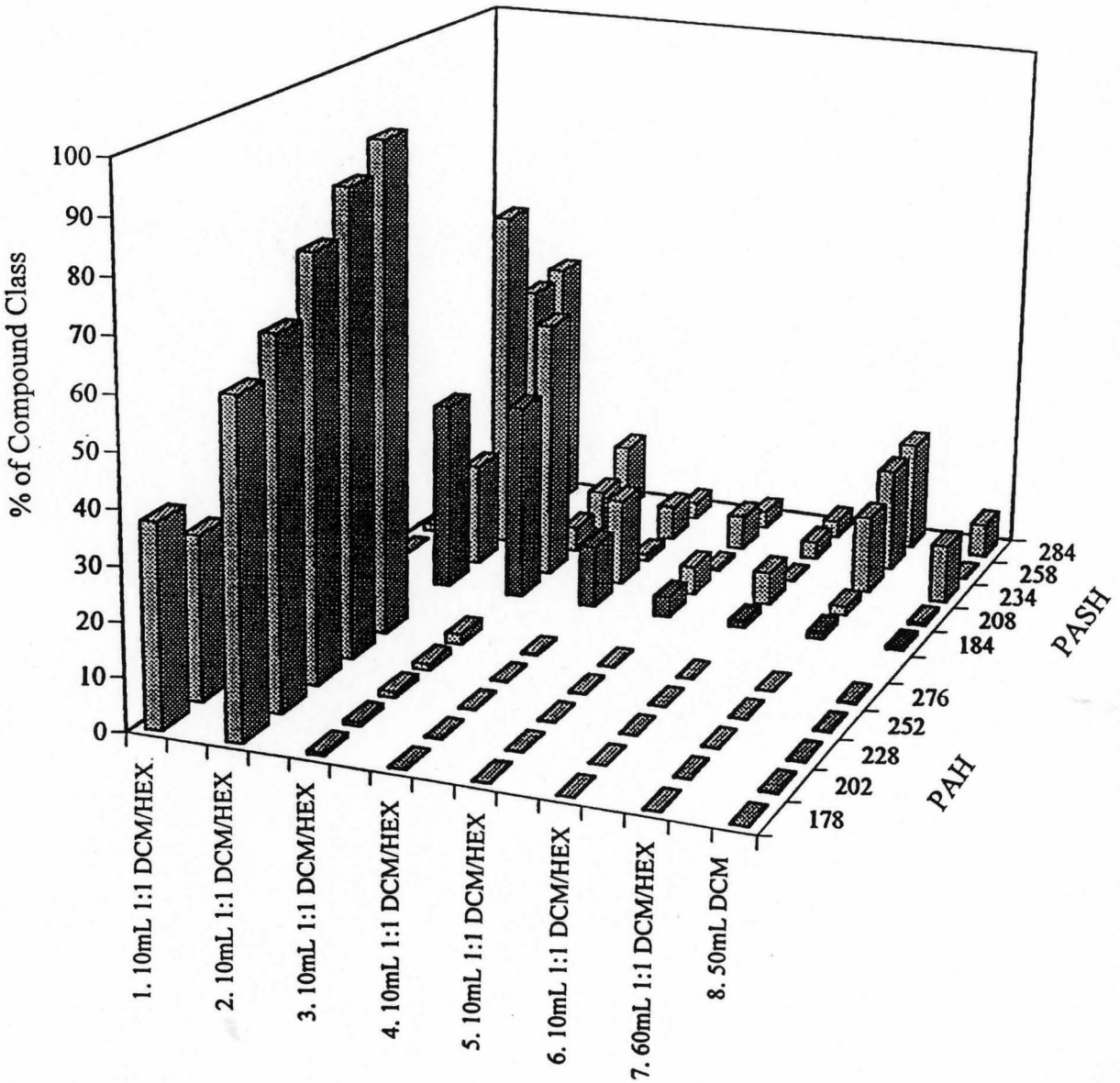
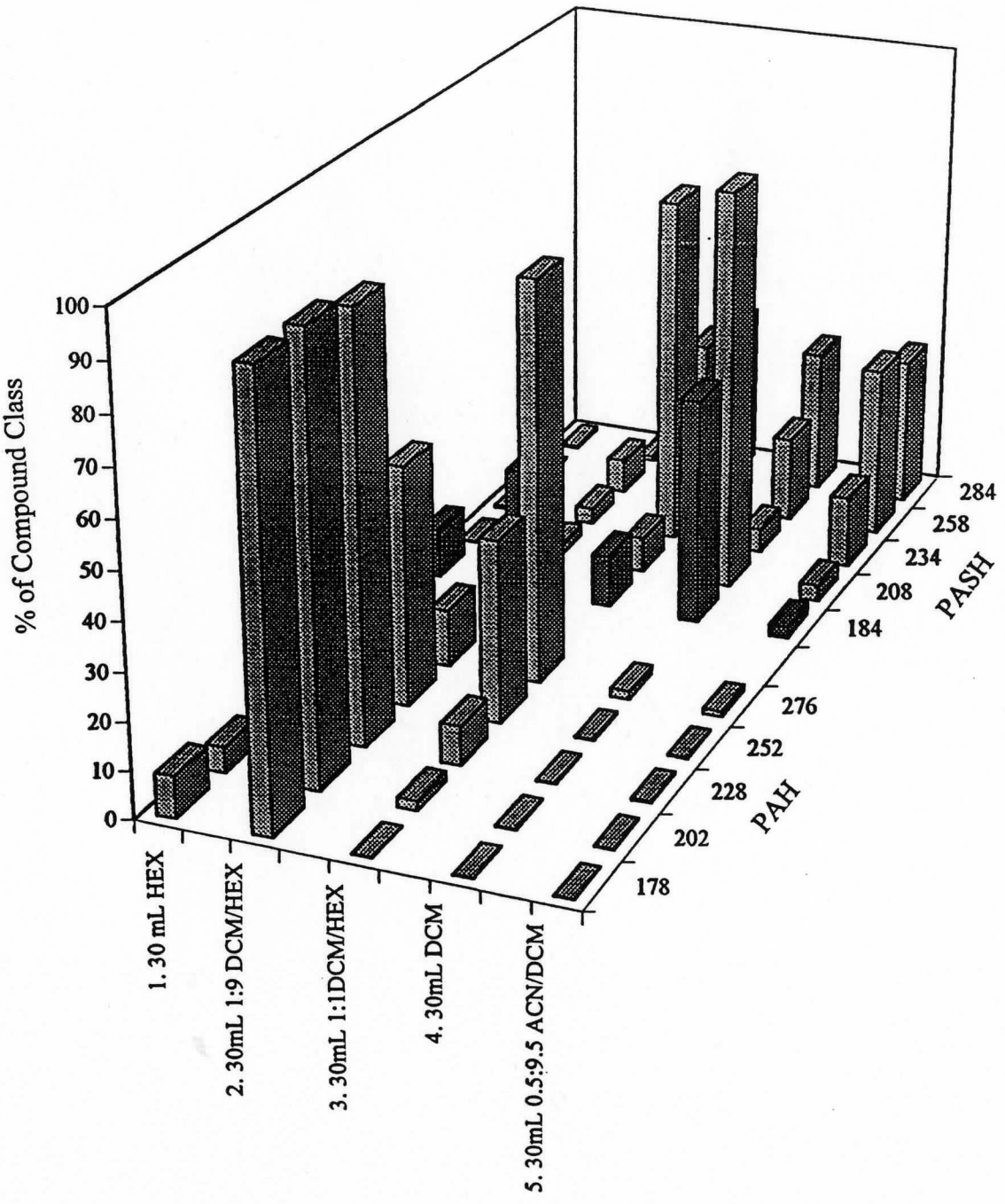


Figure 19. Elution profiles of PAH/PASH in the five fractions collected from Pd-SiO_2 experiment # 2



PASH were eluted from the column in the last three fractions. The second experiment was much more encouraging than the first and clearly pointed in the right direction. The second fraction which was eluted with 30 mL of hexane:dichloromethane (9:1) afforded a high yield of PAH but little PASH.

In the third experiment, the column was eluted with a total of 90 mL of dichloromethane/hexane (1:9, 3 x 30 mL) before it was eluted with 30 mL of dichloromethane/acetonitrile (95:5). The results are shown in Figure 20. More than 95% of the PAH were eluted in the first two fractions (60 mL) while more than 70% of the PASH were eluted in the latter two fractions. If the four fractions were taken as two parts, the first two fractions were highly enriched in PAH while the latter two were enriched in PASH. In the fourth experiment, the solvent system was further fine- tuned and simplified; the results are summarized in Figure 21. More than 80% of all PAH eluted in the first fraction while more than 70% of all PASH were retained. In the second fraction the remainder of the PAH and PASH on the column were eluted. Less than 1% of either the PAH or the PASH were found in the third fraction. Thus, we had optimized the $PdCl_2$-silica gel column separation as best as we could.

The column elution protocol of the fourth experiment removed almost all of the 178, 202 and 228 PAH from the column while most of the PASH were retained. However, a modest proportion of the PASH eluted with the first solvent and there seems little that can

Figure 20. Elution profiles of PAH/PASH in the four fractions collected from Pd-SiO_2 experiment # 3

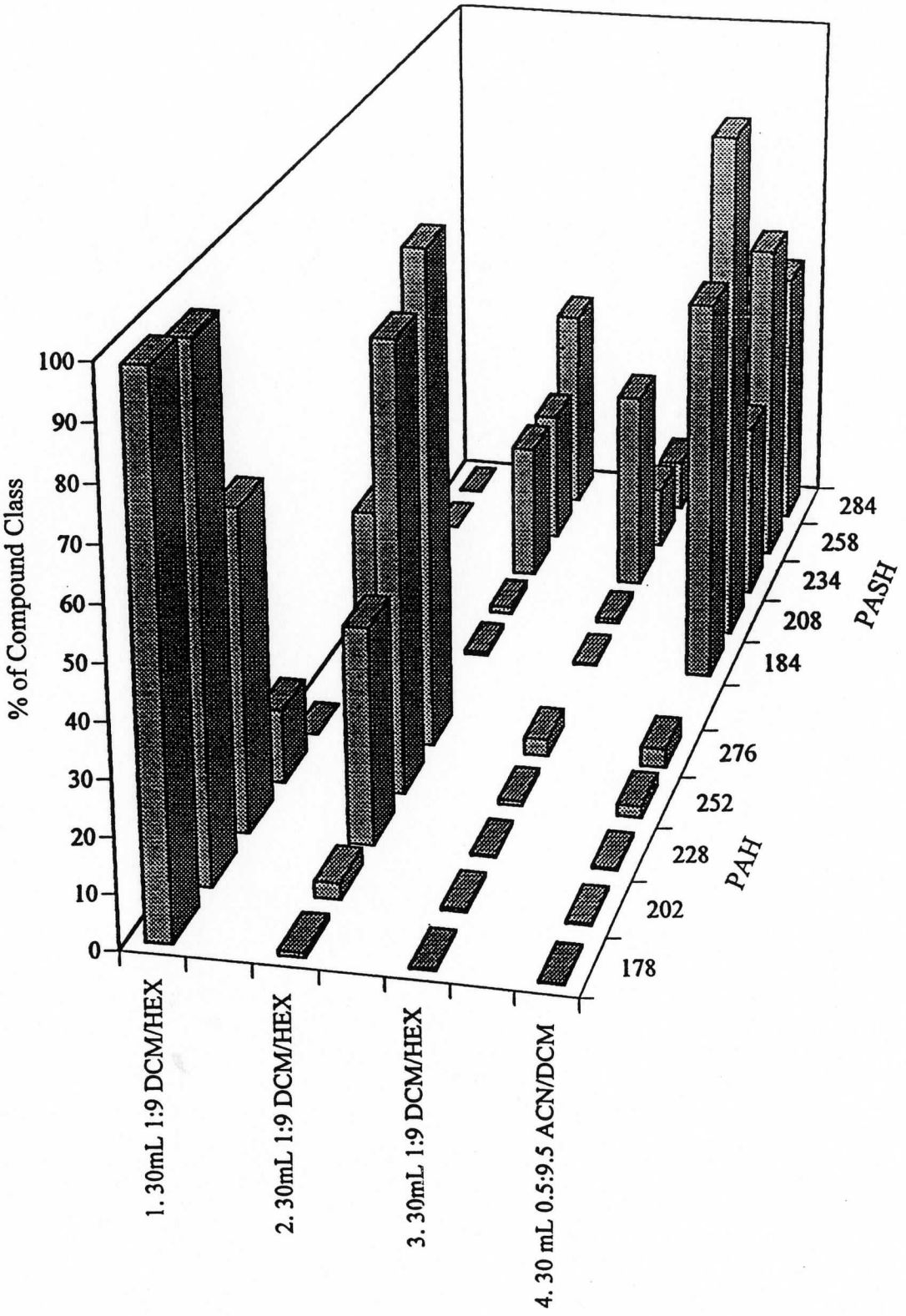
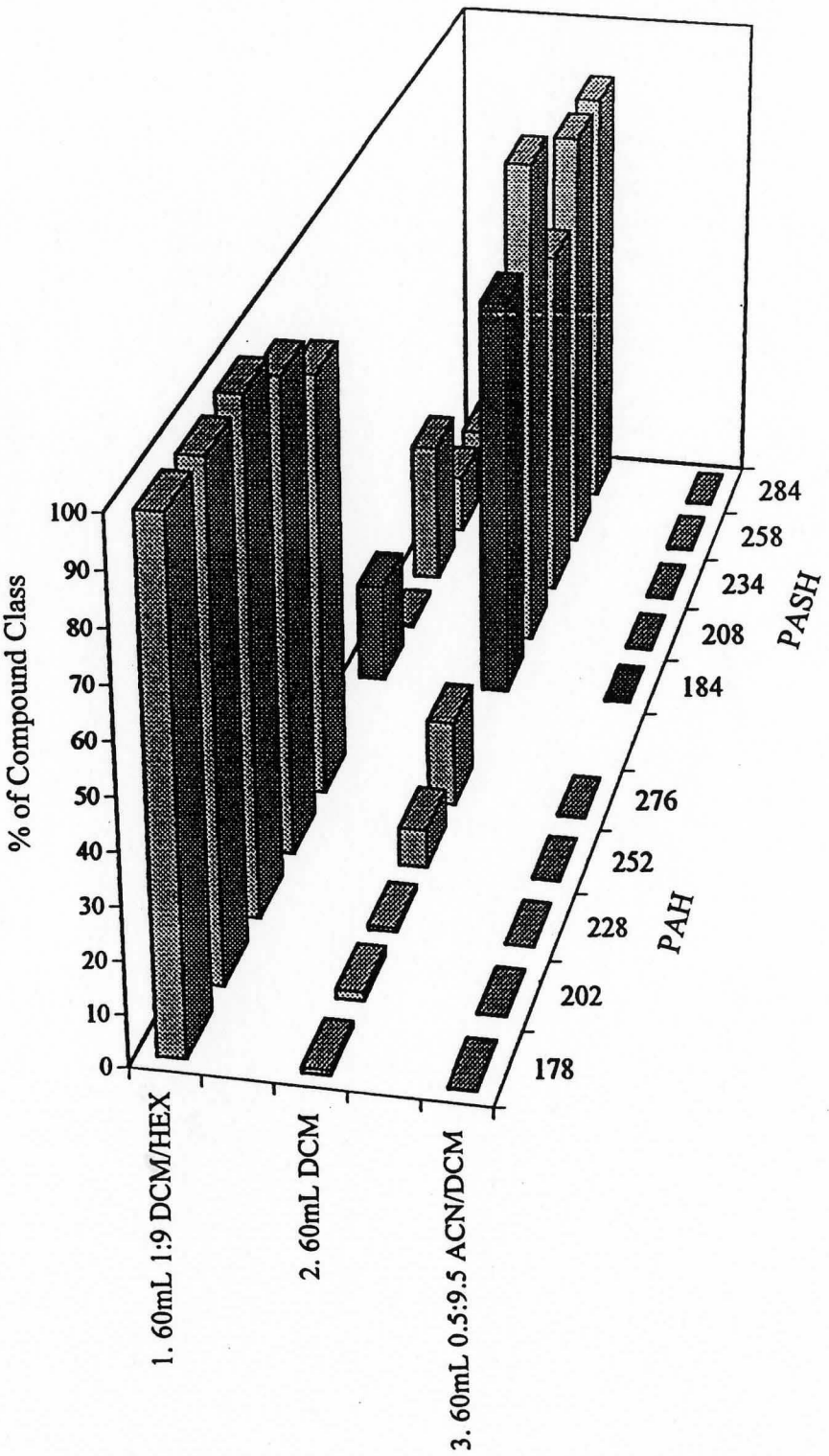


Figure 21. Elution profiles of PAH/PASH in the three fractions collected from Pd-SiO_2 experiment # 4



be done to change that. Interestingly, the PAH that were retained by the column and eluted with dichloromethane along with the PASH were the 252 amu and 276 amu classes of PAH. This retention may be due to the adsorption of these large PAH to the silica surface. Higher mass PAH have more unsaturated rings than the lower mass PAH and thus would have a greater tendency to be adsorbed to the silica gel. In a typical separation, 100 mg coal tar fraction A2 was separated on 5 g of $PdCl_2$-silica; the PAH-rich fraction (called fraction A2-P1) contained 72.5 mg or 72.5% of the A2 fraction or 17.8% of the original coal tar. The thia-arene-rich fraction (called fraction A2-P2) contained 15.6 mg, or 15.6% of the fraction A2 or 3.8% of the coal tar.

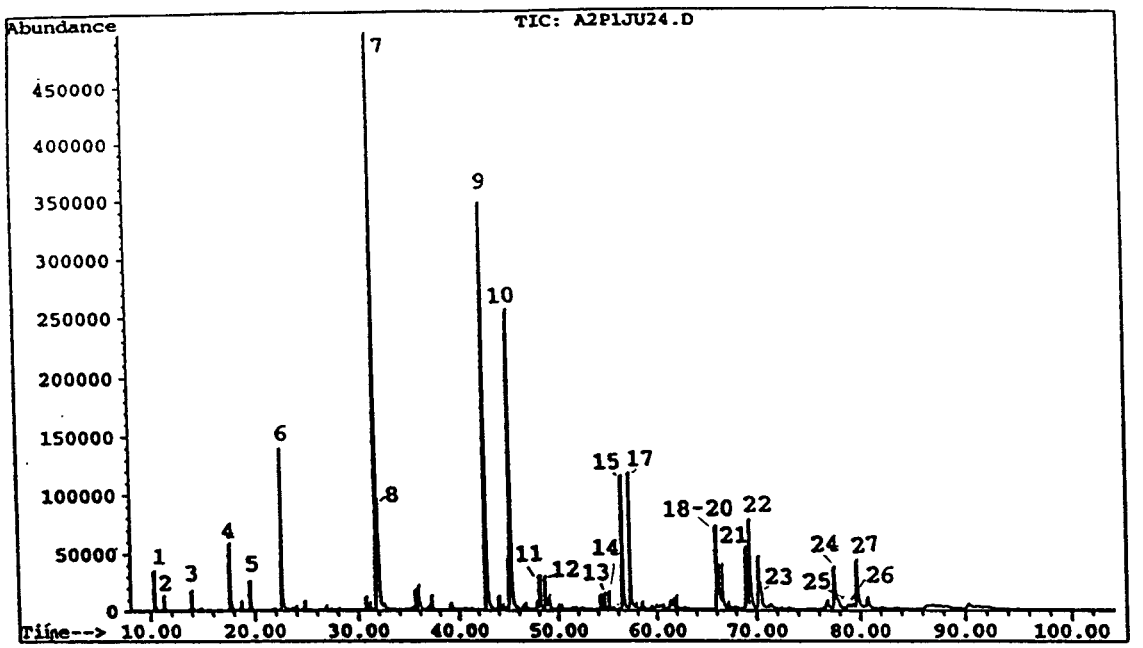
III. 3. 2. 3 Analyses of Fractions Separated by $PdCl_2$-silica Column

III. 3. 2. 3. a GC-MS analyses of fractions separated by $PdCl_2$-silica column

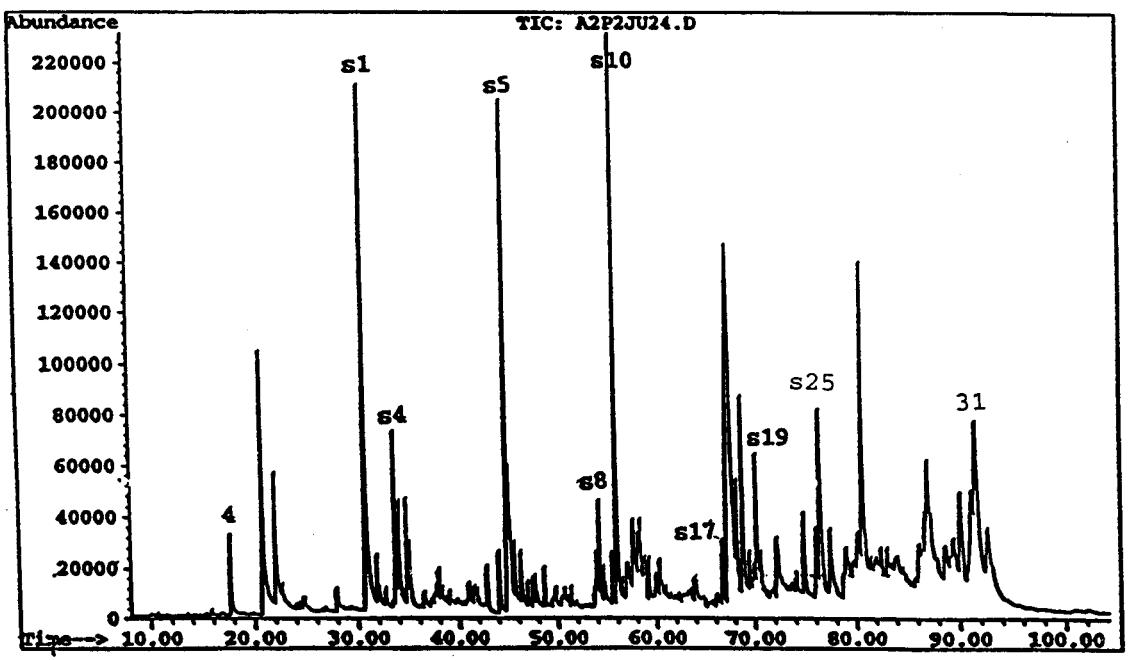
The two fractions collected from the optimized $PdCl_2$-silica column (experiment 4) were analyzed by GC-MS and normal phase LC; this section deals with the GC-MS analyses while the next section deals with the NPLC analyses. Figures 22a and 22b show the GC-MS total ion current chromatograms of fractions A2-P1 and A2-P2, respectively. The GC-MS chromatogram of fraction A2-P1 is almost identical to the GC-MS chromatogram of fraction A2 (Figure 9a) because of the high abundance of PAH in both fractions. However, the GC-MS chromatogram of A2-P2 fraction is very different because all the major peaks are sulfur-

Figure 22. GC-MS analyses of fractions A2-P1 and A2-P2

22a



22b



a. GC-MS total ion current chromatogram of fraction A2-P1.

b. GC-MS total ion current chromatogram of fraction A2-P2.
The samples were analyzed on 30 m DB-17ht column by Method B in S.I.M.
Peak number refer to compounds listed in Table 9.

containing compounds.

Quantitative analyses were performed on these two fractions and these data are compiled and compared with similar data from coal tar fraction A2 in Table 10. The recoveries of each compound in both fractions are listed in Table 10 along with total recoveries. The overall recoveries of PAH were quite good (>80%); while most of PAH were found in fraction A2-P1, higher molecular mass PAH such as picene (278 amu), dibenzo[a,e]pyrene, dibenzo[a,i]pyrene and dibenzo[a,h]pyrene (302 amu) had modest recoveries in fraction A2-P2 (1-25%).

The elution profile of PASH was different from the PAH profile because some PASH were retained by the $PdCl_2$-silica column and eluted in fraction A2-P2 while others co-eluted with the PAH in fraction A2-P1. Although the total recoveries were quite good for most PASH (70%), the retention of individual PASH on $PdCl_2$-silica gel varied from compound to compound. Of the 25 PASH quantified in Table 10, 12 eluted primarily (>90%) if not exclusively with the PAH in fraction A2-P1; these PASH are noted in Table 10 with the designation "P1". Of the remaining 13 PASH, 8 eluted primarily (>90%) or exclusively in fraction A2-P2; these PASH are designated "P2" in Table 10. The five remaining PASH eluted in both fractions and these are designated "P1/P2" in Table 10. Thus, 13 of the 25 PASH identified in coal tar eluted partially or completely in the PASH-rich fraction and are thus potentially amenable to isolation chromatographically. The structures of the 20

Table 10. Quantitation[a, b] of PAH and PASH in Coal Tar Fractions A2, A2-P1 and A2-P2.

Peak No.	Mol. Wt.	Compound Name	Conc. A2 µg/g	Conc. A2-P1 µg/g	Conc. A2-P2 µg/g	PercentageRecovery in A2-P1 %	Percentage Recovery in A2-P2 %	Percentag Recovery After Pd/SiO2 Column	Fraction in Which Compounds Eluted
PAH									
1	142	2-Methylnaphthalene	1400	1210	1.5	86.5	0.11	86.6	P1
2	142	1-Methylnaphthalene	574	496	0.5	86.3	0.095	86.4	P1
3	154	Biphenyl	820	707	0.5	86.2	0.05	86.3	P1
4	152	Acenaphthylene	4430	3230	36.8	72.8	0.83	73.6	P1
5	154	Acenaphthene	267	238	N.D.	89	N.D.	89	P1
6	166	Fluorene	6240	5580	4.9	89.3	0.079	89.4	P1
7	178	Phenanthrene	32900	32200	24.5	97.9	0.07	98	P1
8	178	Anthracene	8080	7900	17.2	97.8	0.21	98	P1
9	202	Fluoranthene	28200	26400	27	93.5	0.095	93.6	P1
10	202	Pyrene	21900	19700	19.6	89.7	0.089	89.8	P1
11	216	Benzo[a]fluorene	1790	1570	1.8	87.8	0.1	87.9	P1
12	216	Benzo[b]fluorene	1550	1340	3.1	86.7	0.2	86.9	P1
13	226	Benzo[ghi]fluoranthene	1240	1100	6.1	86.9	0.5	87.4	P1
14	228	Benzo[c]phenanthrene	687	614	2.8	89	0.4	89.4	P1
15	228	Benz[a]anthracene	8890	7550	14.7	84.9	0.16	85.1	P1
16	226	Cyclopenta[cd]pyrene	385	15	24.5	3.8	6.4	10.2	P1
17	228	Chrysene	8430	7220	14.7	85.6	0.17	85.8	P1
18	252	Benzo[b]fluoranthene	4010	3610	4.9	90	0.12	90.1	P1
19	252	Benzo[k]fluoranthene	2710	2510	4.9	92.5	0.18	92.7	P1

Peak No.	Mol. Wt.	Compound Name	Conc. A2 µg/g	Conc. A2-P1 µg/g	Conc. A2-P2 µg/g		PercentageRecovery in A2-P1 %	Percentage Recovery in A2-P2 %	Percentag Recovery After Pd/SiO2 Column	Fraction in Which Compounds Eluted
20	252	Benzo[j]fluoranthene	2340	2180	4.9		93.9	0.2	94.1	P1
21	252	Benzo[e]pyrene	3160	3000	1.3		93.7	0.077	93.8	P1
22	252	Benzo[a]pyrene	5750	5120	13.4		89	0.21	89.2	P1
23	252	Perylene	1570	1360	5.4		86.6	0.31	86.9	P1
24	276	Indeno[1,2,3-cd]pyrene	3660	3350	7.4		91	0.2	91.2	P1
25	278	Dibenz[a,c]anthracene	587	638	N.D.		108	N.D.	108	P1
26	278	Picene	429	334	4.9		77.7	1.1	78.8	P1
27	276	Benzo[ghi]perylene	3580	3270	7.4		91.4	0.2	91.6	P1
28	300	Coronene	601	223	N.D.		37.1	N.D.	37.1	P1
29	302	Dibenzo[a,e]pyrene	466	294	63.8		63	13.7	76.7	P1/P2
30	302	Dibenzo[a,i]pyrene	734	378	81		51.5	11	62.5	P1/P2
31	302	Dibenzo[a,h]pyrene	761	533	191		70	25	95	P1/P2
		Total	**158400**	**144000**	**590.5**	**Avg.**	**82.2**	**2.2**	**84.2**	
PASH						**STD.**	**20.0**	**5.6**	**18.4**	
s1	184	Dibenzothiophene	1300	753	344		58	27	84.6	P1/P2
s2	184	Naphtho[1,2-b]thiophene	530	474	52.3		89	9.9	98.9	P1
s3	184	Naphtho[2,1-b]thiophene	337	299	2.6		89	0.76	89.8	P1
s4	184	Naphtho[2,3-b]thiophene	157	3	106		2	66	68	P2
s5	208	Phenanthro[4,5-bcd]thiophene	560	160	368		28	65.5	93.5	P1/P2
s7	208	Phenaleno[6,7-bc]thiophene	169	N.D.	1.2			0.74	0.7	P2
s8	234	Benzo[b]naphtho[2,1-]thiophene	1020	856	49.1		84	4.82	88.8	P1
s9	234	Benzo[b]naphtho[1,2-d]thiophene	199	140	24.5		75	12.2	87.2	P1

Peak No.	Mol. Wt.	Compound Name	Conc. A2 µg/g	Conc. A2-P1 µg/g	Conc. A2-P2 µg/g		PercentageRecovery in A2-P1 %	Percentage Recovery in A2-P2 %	Percentag Recovery After Pd/SiO2 Column	Fraction in Which Compounds Eluted
s10	234	Benzo[b]naphtho[2,3-d]thiophene	379	N.D.	295			77.4	77.4	P2
s11	234	Phenanthro[1,2-b]thiophene	96	69	N.D.		82.6		82.6	P1
s12	234	Anthra[2,1-b]thiophene	42	29	N.D.		72.7		72.7	P1
s13	234	Phenanthro[2,1-b]thiophene	60	N.D.	22.1			37	37	P2
s14	234	Phenanthro[2,3-b]thiophene	36	N.D.	34.4			90.9	90.9	P2
s15	258	258 amu PASH	24	15	0.8		68.2	3.52	71.7	P1
s16	258	258 amu PASH	24	17	0.7		77.8	3.11	80.9	P1
s17	258	Benzo[2,3-phenanthro [4,5-bcd]]thiophene	139	83	29.4		59.5	21	80.6	P1/P2
s18	258	Triphenyleno[4,5-bcd]thiophene	84	N.D.	47.1			56.6	56.6	P2
s19	258	Chryseno[4,5-bcd]thiophene	157	12	100		7.8	63.8	71.7	P2
s20	258	258 amu PASH	72	39	N.D.		54.8		54.8	P1
s21	258	258 amu PASH	48	49	N.D.		103		103	P1
s22	284	Dinaphto[1,2-b:1'2'-d]thiophene	60	27	1.1		44.8	1.79	46.6	P1
s23	284	Benzo[b]phenanthro[3,4-d]thiophene	42	20	N.D.		48.7		48.7	P1
s24	284	Dinaphto[1,2-b:2',3'-d]thiophene	48	7	30.9		16	67.4	83.4	P1/P2
s25	284	Benzo[b]phenanthro [3,2-d]thiophene	108	20	127		18.2	117	135	P1/P2
s26	284	284 amu PASH	36	N.D.	35.8			102	102	P2
		Total	**5727**	**3072**	**1672**	**Avg.**	**56.8**	**41.4**	**76.3**	
						STD	**30.2**	**37.9**	**26.1**	

N.D.: below the detection limit (0.17µg/g).

[a] Samples were analyzed on DB-17ht column using GC-MS Method B.

[b] Concentrations are expressed in µg/g of coal tar sample

identified PASH are shown in Appendix I along with the fractions they eluted.

The recoveries of PASH were good but not as high or as consistent as recoveries of PAH. The recovery of one thia-arene, phenaleno[6,7-bc]thiophene (s7), was very low (0.7%) and the recoveries of five others (s13, s18, s20, s22 and s23) were in the 37-57% range. Of these six, three (s7, s13 and s18) eluted in the A2-P2 fraction; the obvious explanation for these poor recoveries would be strong chelation to the Pd species on the column. The other three PASH eluated exclusively in the A2-P1 fraction along with the PAH. There is no obvious explanation for the poor recoveries of these compounds given the high recoveries for many compounds which eluated in fraction A2-P1. Overall those PASH which eluted in fraction A2-P2 had lower recoveries than other PASH; presumably reflecting incomplete decomplexation from the $PdCl_2$-silica column.

Is there any correlation between the structure and retention for PASH? Andersson (52) studied the retention behaviour of a lesser number of PASH. He determined the retention capacity factors k' of various sulfur-containing aromatics by using a liquid chromatographic column filled with $PdCl_2$-silica gel. Using a small number of standards he found that PASH with terminal thiophene rings eluted considerably faster than PASH whose heterocyclic ring was in an internal position. He concluded that the $PdCl_2$-silica gel column can not separate thiophene-terminal PASH from PAH. In our case, we saw the elution of both terminal PASH and internal PASH in fraction A2-P1 (s11 and s10); we also

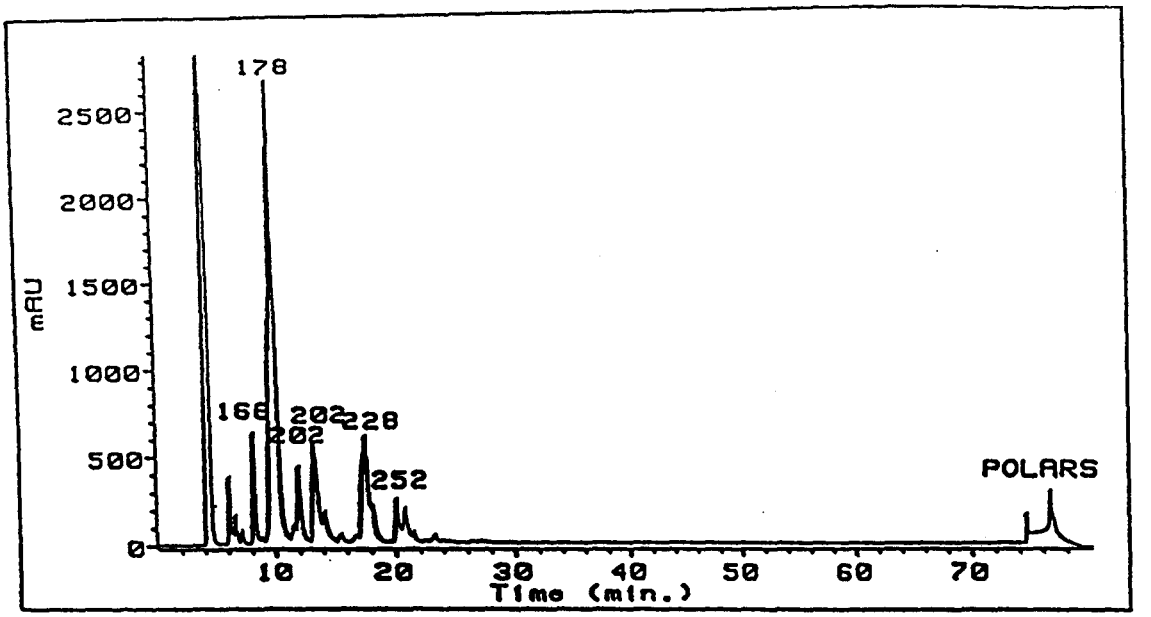
found the 302 amu PAH eluted partially in fraction A2-P2. Our results showed that the $PdCl_2$-silica gel column approach can separate some but not all PASH from PAH.

III. 3. 2. 3. b Normal phase HPLC analyses of fractions separated by $PdCl_2$-silica column

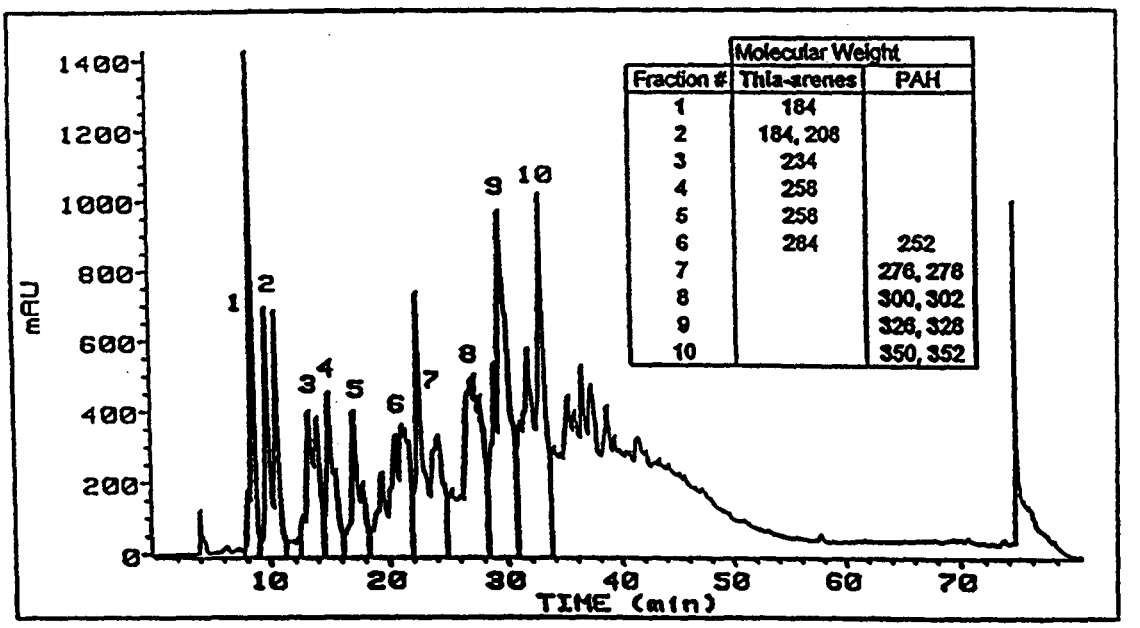
The normal phase liquid chromatograms of fractions A2-P1 and A2-P2 are very different and are shown in Figure 23. Fraction A2-P1 contained PAH with molecular mass values less than 276 amu; all compounds eluted prior to 24 min. The molecular masses of these PAH are annotated on Figure 23a. The normal phase liquid chromatogram of fraction A2-P2 was much more complex than that of A2-P1 fraction; ultraviolet-absorbing peaks eluted between 8 and 55 minutes (Figure 23b). Besides PASH, this fraction also contained PAH with molecular masses equal to and exceeding 252 amu. These larger PAH were adsorbed strongly to the silica gel and were eluted by dichloromethane used to elute the thiophenic compounds. While PAH with molecular masses of 252, 276, 278, 300 and 302 amu can be analyzed easily by GC-MS, it is difficult to analyze PAH with molecular masses exceeding 302 amu by GC-MS. However, high mass PAH are easily analyzed using HPLC methods.

In order to determine the molecular mass distribution of PASH in fraction A2-P2, a sample was analyzed by normal phase liquid chromatography and ten fractions were

23a



23b



Fraction #	Molecular Weight: Thia-arenes	PAH
1	184	
2	184, 208	
3	234	
4	258	
5	258	
6	284	252
7		276, 278
8		300, 302
9		326, 328
10		350, 352

Figure 23 a. Normal phase HPLC chromatogram of coal tar fraction A2-P1.

b. Normal phase HPLC chromatogram of coal tar fraction A2-P2. Inset shows molecular masses of principal PASH and PAH on the numbered fractions collected during this HPLC analysis; these data were derived from GC-MS analyses of fractions 1-8 (Figure 24).

collected as shown in Figure 23b. Fractions numbered 1-8 were analyzed by GC-MS; fractions 9 and 10 were analyzed by probe MS. The total ion current chromatograms of the GC-MS analyses of the first eight fractions are shown in Figure 24. By the combination of NPLC separation and GC-MS analysis, it was found that all the PASH with molecular masses between 184 and 258 had eluted in the first five fractions (before 22 min.) in the normal phase chromatogram. Fractions 6, 7 and 8 contained mixtures of PAH and PASH; the 284 amu PASH was coeluted with 252 amu PAH. Probe MS analyses of fractions 9 and 10 showed that they consisted of PAH with molecular masses exceeding 300 amu.

It is worth noting that the 252, 276 and 278 amu PAH found in the A2-P2 fraction account for only a very small percentage of each of these PAH classes; values range from only 0.077% to 1.1% of the PAH in coal tar. The 302 amu PAH have abundances between 11 and 25 % of the PAH in coal tar. These low relative percentages of PAH attest to the very low levels of PASH in these coal tar fractions.

The combination of $PdCl_2$-silica column chromatography followed by normal phase liquid chromatography afforded a multi-dimensional chromatographic methodology for the separation and analysis of 13 of the 25 PASH identified in coal tar. Of the various mass classes of the 13 PASH in this fraction, all could be isolated in pure form except for the three 284 amu class PASH. Unfortunately, this class along with the 258 amu class have the greatest probability for exerting genotoxic effects based on the structures and activities of the

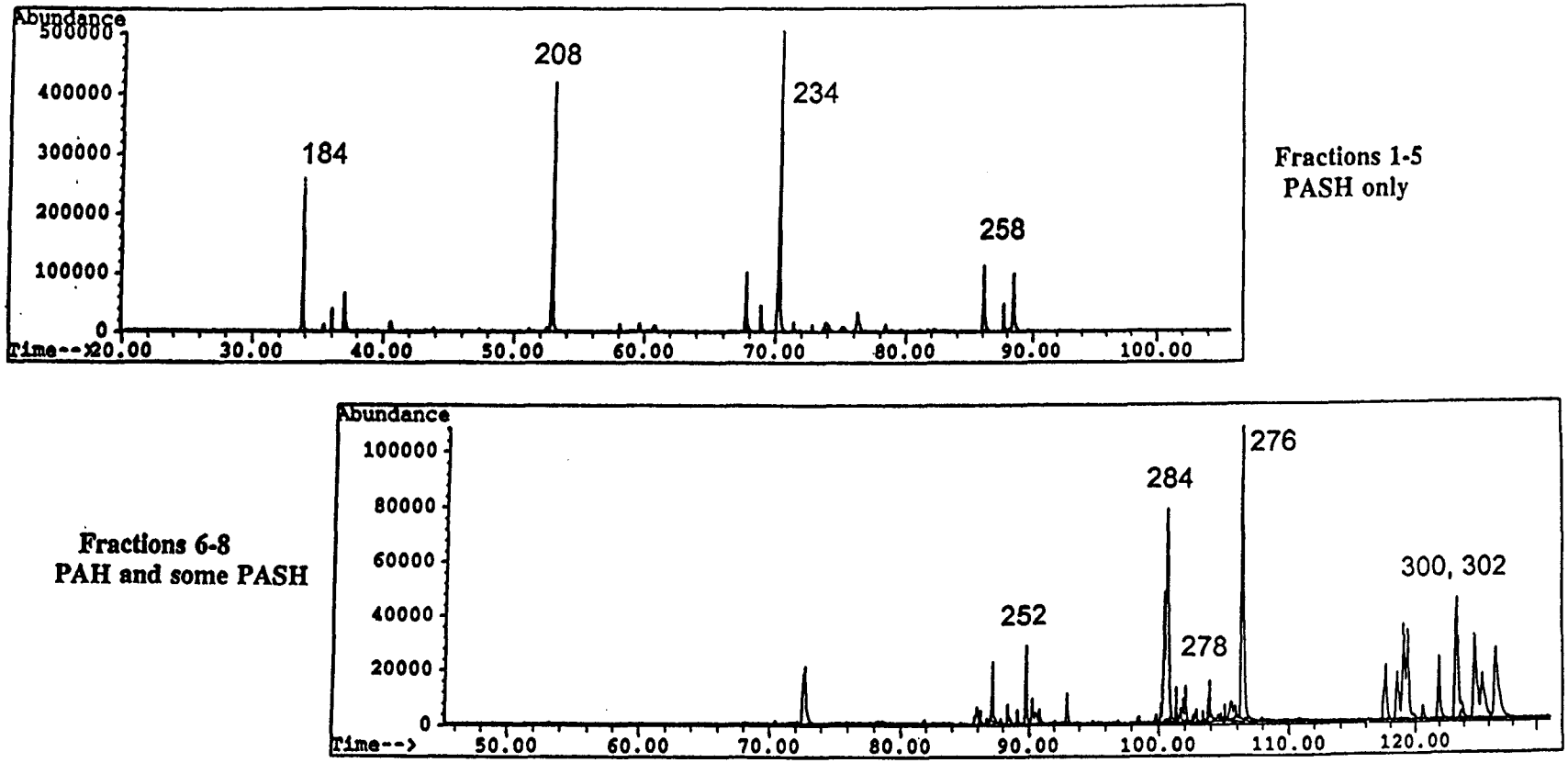


Figure 24. Superposition of total ion current chromatograms from GC-MS analyses of fractions collected during normal phase HPLC analysis of fraction A2-P2. Top panel: HPLC fraction 1-5; bottom panel: HPLC fraction 6-8. The molecular masses of the major peaks are shown above the peaks and are summarized in the inset in Figure 23b.

252, 276 and 278 amu classes of PAH.

III. 3. 3 Separation of Thia-arenes from PAH by $PdCl_2$-Silica Column Prepared Using bis(benzonitrile) Palladium Chloride

We considered a modification for the preparation of $PdCl_2$-silica to overcome a potential problem with the Nishioka procedure. The main disadvantage of $PdCl_2$ is that it is insoluble in water; it is hard to get it uniformly distributed on the surface of the silica gel. The particles of $PdCl_2$ are macroscopic so very little of the Pd in a particle is available to be used in binding thia-arenes. By using a soluble palladium compound it was hoped that the Pd would coat the silica in thin layers, thus utilizing the Pd more efficiently. An organic solvent soluble form of $PdCl_2$ (bisbenzonitrile palladium chloride) was prepared by reacting palladium chloride with benzonitrile in a water bath (48). The bis(benzonitrile) palladium chloride was dissolved in dichloromethane and was adsorbed uniformly onto silica gel by removing the solvent under reduced pressure. This "$Pd(PhCN)_2Cl_2$-silica" was divided into two parts. One half was used directly to make a column while the other half was dried and activated at 170^0C for more than 24h before use. It was hoped that the latter procedure in particular would afford $PdCl_2$ adsorbed to silica gel once the benzonitrile had been driven off during the drying.

The same amount of coal tar was loaded onto the two columns. The solvent system

Table 11. The Ratios of PAH to PASH in Coal Tar Fractions

Sample	PAH/PASH Area Ratios 178/184 (3-ring)	202/208 (4-ring)	228/234 (4-ring)	252/258 (5-ring)	276/284 (6-ring)
Coal Tar Fraction A2	23.3	46.5	7.9	34.2	31.3
A2-P1/$PdCl_2$	61.3	406.7	10.5	67.6	20.05
A2-P2/$PdCl_2$	0.1	0.2	0.2	2.2	5.2
A2-P1/$Pd(PhCN)_2Cl_2$*	21.9	54.0	8.9	35.5	35.5
A2-P1/$Pd(PhCN)_2Cl_2$**	21.9	45.5	6.1	34.5	19

* Not activiated before use

** Activated at 170^0C for 24h before use

developed in the previous section was used and two fractions were collected from each column giving a total of four fractions which were then worked up and analyzed by GC-MS. No PASH peaks were observed in either of the A2-P2 fractions. The ratio between the abundances of PAH and PASH with the same number of rings in several samples was calculated (see Table 11) for these experiments as well as for the previous $PdCl_2$-silica experiment.

It was clear that both columns prepared using $Pd(PhCN)_2Cl_2$ had not retained PASH as well as the standard $PdCl_2$-silica experiment. For example, the ratio of the area under the 178 amu PAH was compared to the area under the 184 amu PASH and found to be 23.3 times greater. That is, the 178 amu PAH were about 23 times more abundant than the corresponding PASH in coal tar or, in other words, the 184 amu PASH were only 4.3% as abundant as 178 amu PAH. After $PdCl_2$-silica chromatography the A2-P2 fraction (entry A2-P2/$PdCl_2$) showed a ratio of 0.1, that is , there was now 10 times more 184 amu PASH in this fraction relative to 178 amu PAH. Not all of the 184 amu PASH, however, were in fraction A2-P2, but what PASH were there were relatively free of PAH contamination. Thus, fraction A2-P1 contained essentially all of the 178 amu PAH; moreover, this fraction had a ratio of 61.3 compared to 23.3 in coal tar, an increase of almost three-fold. This means that fraction A2-P1 had been depleted about three-fold of its 184 amu PASH content relative to coal tar fraction A2. In summary, for the 184 amu PASH, the $PdCl_2$-silica procedure

separated about two-thirds of these PASH from the bulk of 178 amu PAH.

In contrast, the bisbenzonitrile $PdCl_2$-silica columns had 178 amu PAH to 184 amu PASH ratio of 21.9, values essentially unchanged from the value for coal tar fraction A2. Clearly, these latter columns had not sequestered removed any 184 amu PASH. The ratios for the other PAH/PASH pairs were similar to the ratios in coal tar fraction A2, indicating that these latter $PdCl_2$ columns were completely ineffective.

III. 4. Isolation of High Molecular Mass PAH from Fraction A3

III. 4. 1 Preparation of 326 amu PAH Fraction by Semi-preparative NPLC

It has already been shown that fraction A2 contained the majority of the PAH and PASH compounds in coal tar while fraction A3 contained high molecular mass PAH and some aza-aromatics. Published work from our group (23, 24) had shown that the 302, 326 and 350 amu classes of PAH derived from coal tar-contaminated sediments in Sydney Harbour, Nova Scotia, exhibited mutagenic responses when tested in Ames microbiological reversion assay. These fractions accounted for about 25% of the total dose responses of the sample; the 302 amu fraction accounted for 15%, the 326 amu fraction, for 9% and the 350 amu fraction, for 1%. Furthermore, mutation chromatographic analyses using reversed phase HPLC (Figure 6) had identified regions of the chromatogram which contained the compounds responsible for this bioassay response.

The focus of the current work was the 326 amu PAH fraction and the development of an approach to achieve the isolation and identification (if possible) of individual PAH from this fraction that may be responsible for this bioassay response. Ultimately, bioassay analyses would need to be done to determine which compounds were mutagens and which were not. The first task, however, was the fractionation and analysis of fraction A3 by normal phase HPLC together with probe mass spectrometry to identify the molecular masses of the PAH in each fraction.

A Whatman PAC semi-preparative column was used and three repetitive injections were made. Gradient B (described in the experimental section) was used for the first and the second injections while Gradient A (which removed the accumulated polar components) was used for the third injection. Figure 25 shows the normal phase liquid chromatogram of coal tar fraction A3 and the fractions collected from it. The molecular masses of the compounds in each fraction were obtained by electron impact probe mass spectrometric analyses. Figure 26 shows the probe mass spectra of three of these fractions while the spectra of all 13 fractions are compiled in Appendix II.

These probe mass spectra showed that the NPLC method was surprisingly efficient in the separation of PAH into peaks containing individual high molecular mass classes. The mass spectra were very simple spectra. In general, these spectra exhibited an ion corresponding the molecular mass of the PAH class and its doubly charged counterpart; some

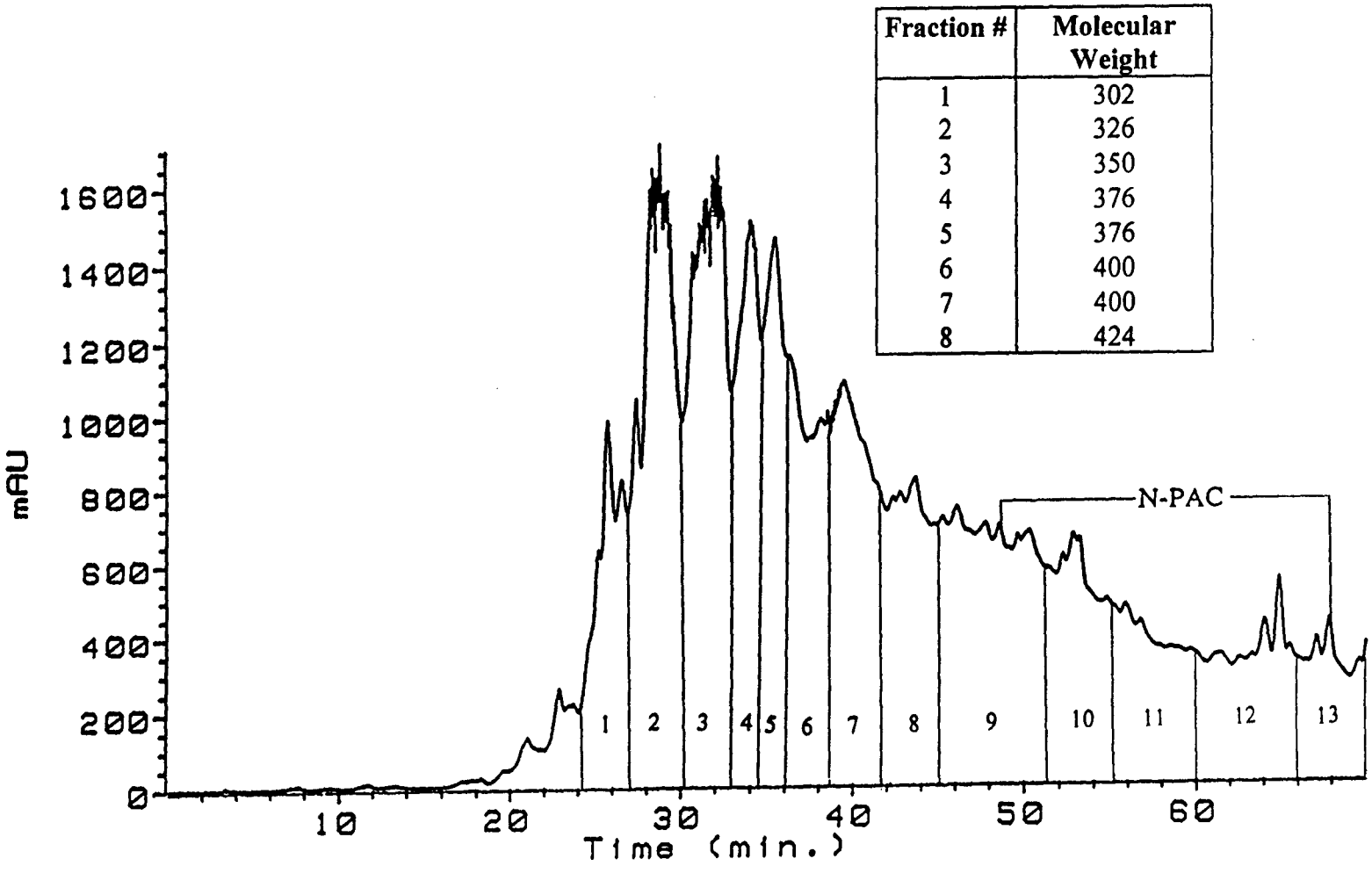
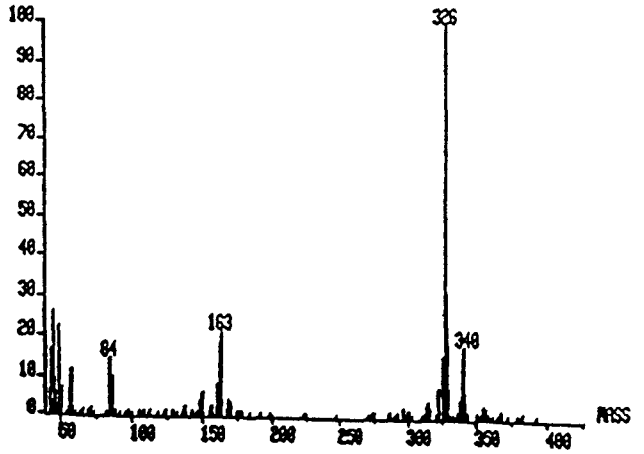
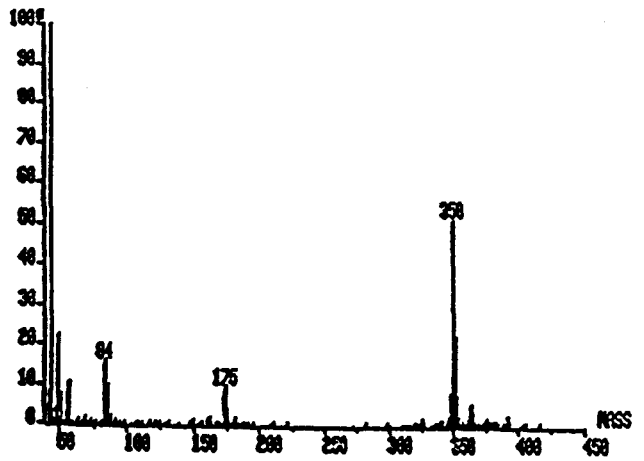


Figure 25. Normal phase HPLC separation of fraction A3. Inset shows molecular masses of fractions 1 to 8 collected during this HPLC analysis as determined by DEI probe mass spectrometric analyses.

Fraction 2



Fraction 3



Fraction 5

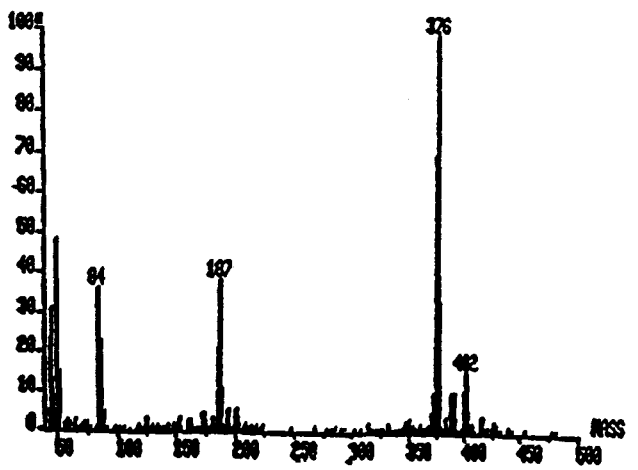


Figure 26. DEI probe mass spectra of three PAH fractions collected from NPLC analysis of fraction A3.

fractions also contained monomethylated derivatives as evidenced by the peak 14 mass unit greater than the molecular ion.

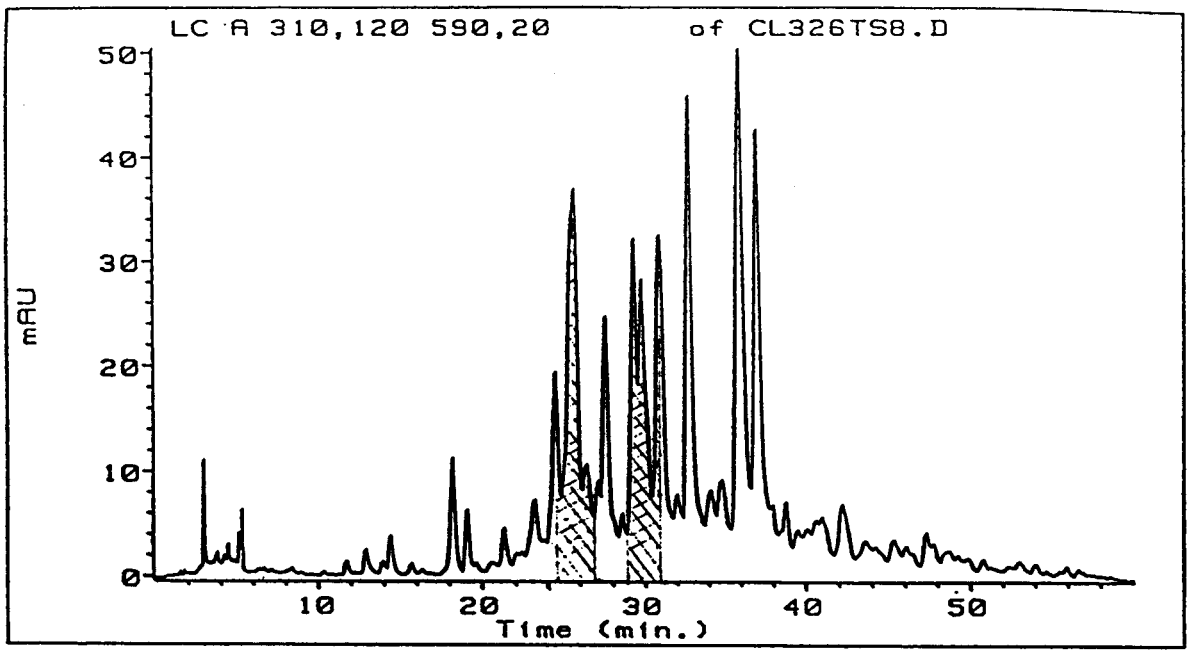
This NPLC method allowed us to isolate fractions containing high molecular mass PAH classes that were surprisingly devoid of their neighbouring mass classes. Since focus of this work was the separation of the 326 amu class of PAH, other fractions were put aside for analysis at a later time.

III. 4. 2 Separation of the 326 amu PAH Fraction from Coal Tar

The 326 amu PAH fraction prepared by semi-preparative NPLC was subjected to RPLC analysis and the resulting chromatogram is shown in Figure 27a. The shaded areas in this chromatogram corresponds to the regions in Figure 6 which showed the greatest responses in the Ames mutation bioassay. The isolation and characterization of individual compounds in these regions was one of our goals. This RPLC analysis (Figure 27a) revealed that the 326 amu PAH fraction was very complex indeed. Judging by the complexity of the UV/Vis spectra across several peaks, it was likely many peaks contained more than one component. For example, the broad peak at a retention time of about 25 minutes contained at least three components as determined by inspection of UV spectra across this peak.

In order to separate the components of this mixture, this fraction was subjected to a second NPLC separation using a shallower solvent gradient. Using program C, the 326

27a



27b

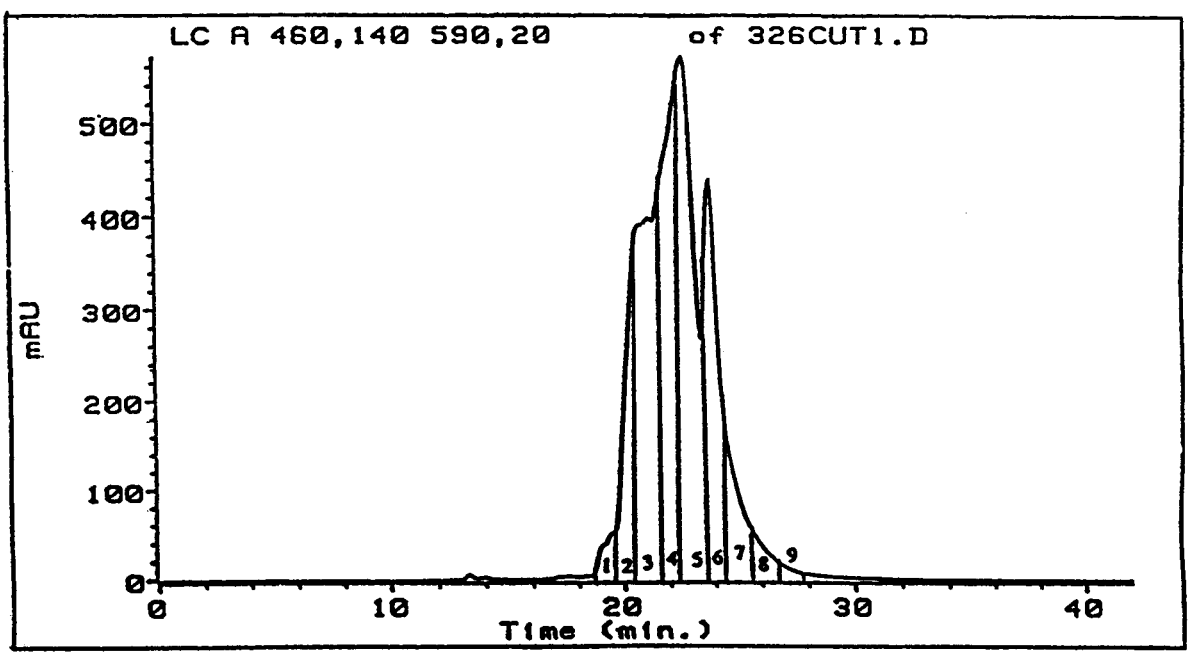


Figure 27 a. Reversed phase HPLC analysis of 326 amu PAH fraction

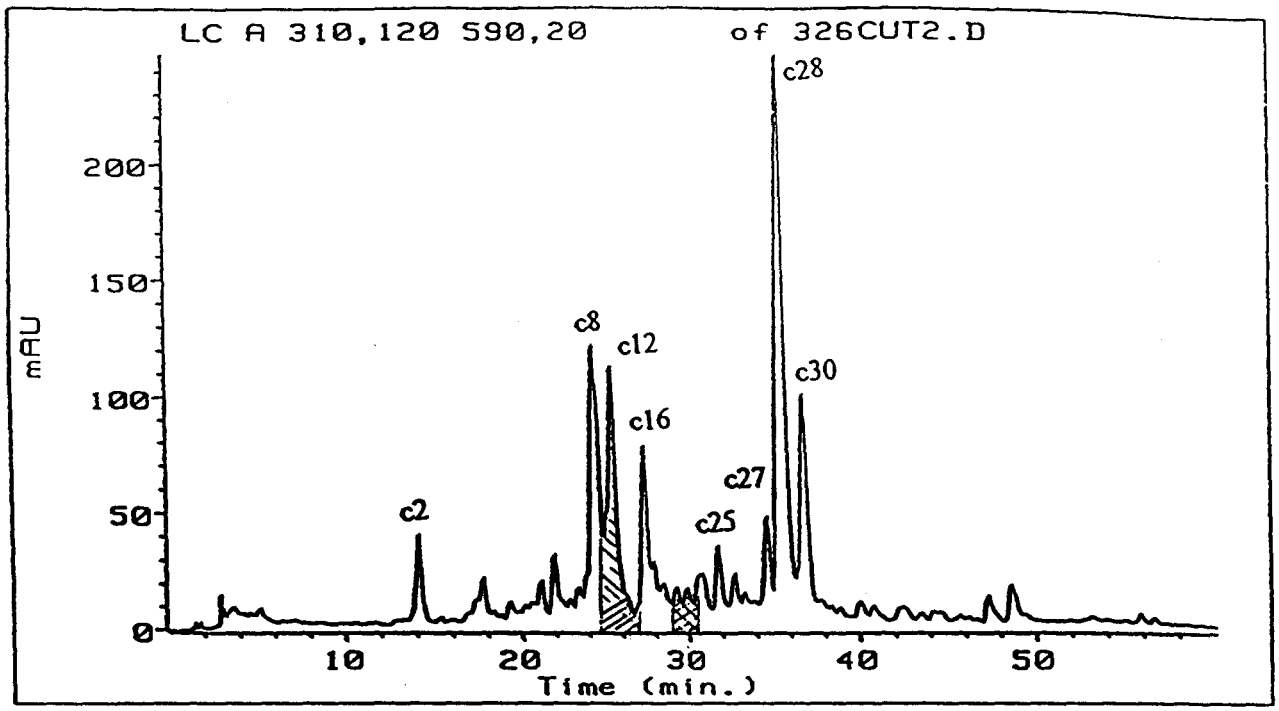
b. Normal phase HPLC separation of 326 amu PAH fraction

amu PAH fraction was reinjected onto the normal phase column, and nine 60-second subfractions were collected (Figure 27b). Of these nine subfractions, the middle seven were analyzed by reversed phase liquid chromatography. Subfractions 1 and 9 were not used because of the relatively low abundances of the components in them.

The reversed phase liquid chromatograms of the seven subfractions are shown in Figures 28a to 28g; the regions containing the mutagenic response are shaded in each chromatogram. Each of the chromatograms of the seven subfractions were different from each other and there were often more than ten major peaks in each fraction. The complexity of this 326 amu PAH fraction was clearly revealed by these reversed phase chromatograms. Thirty-five peaks were collected and are labelled on the seven chromatograms (Figures 28a-28g) as c1, c2,...c35 in the order of increasing elution by RPLC. Table 12 lists the retention times of the 35 peaks along with the subfractions they were collected from. Eight of the 35 peaks are in the region of the mutagenic activity; these are peaks c11 to c15 and peaks c20 to c22. Future biological tests on these 8 peaks will hopefully determine which ones are the active mutagens.

Probe mass spectrometric analyses were performed on all 35 peaks and fluorescence excitation and emission spectra were obtained for some of the peaks. The results of the probe mass spectrometric analyses are listed in Table 12 and the mass spectra are collected in Appendix III. From the probe mass spectrometric analyses, it was found that most but not

28a



28b

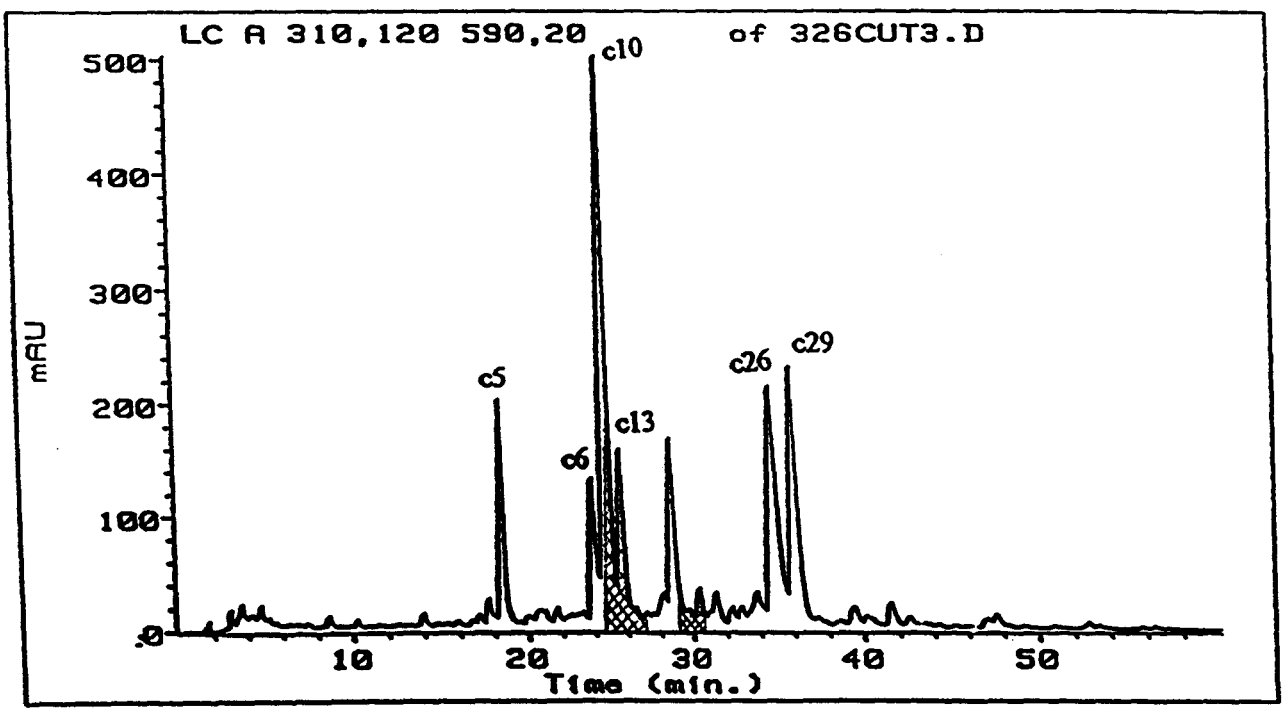
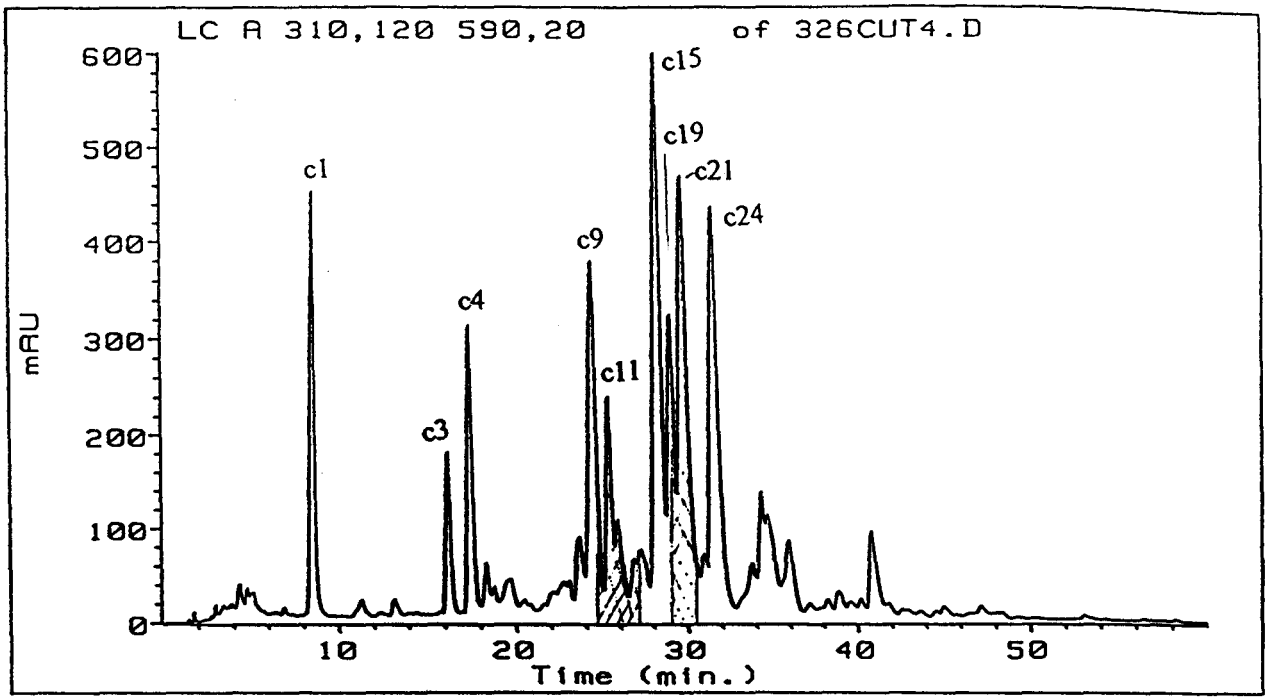


Figure 28 a. Reversed phase HPLC separation of subfraction 2

b. Reversed phase HPLC separation of subfraction 3

28c



28d

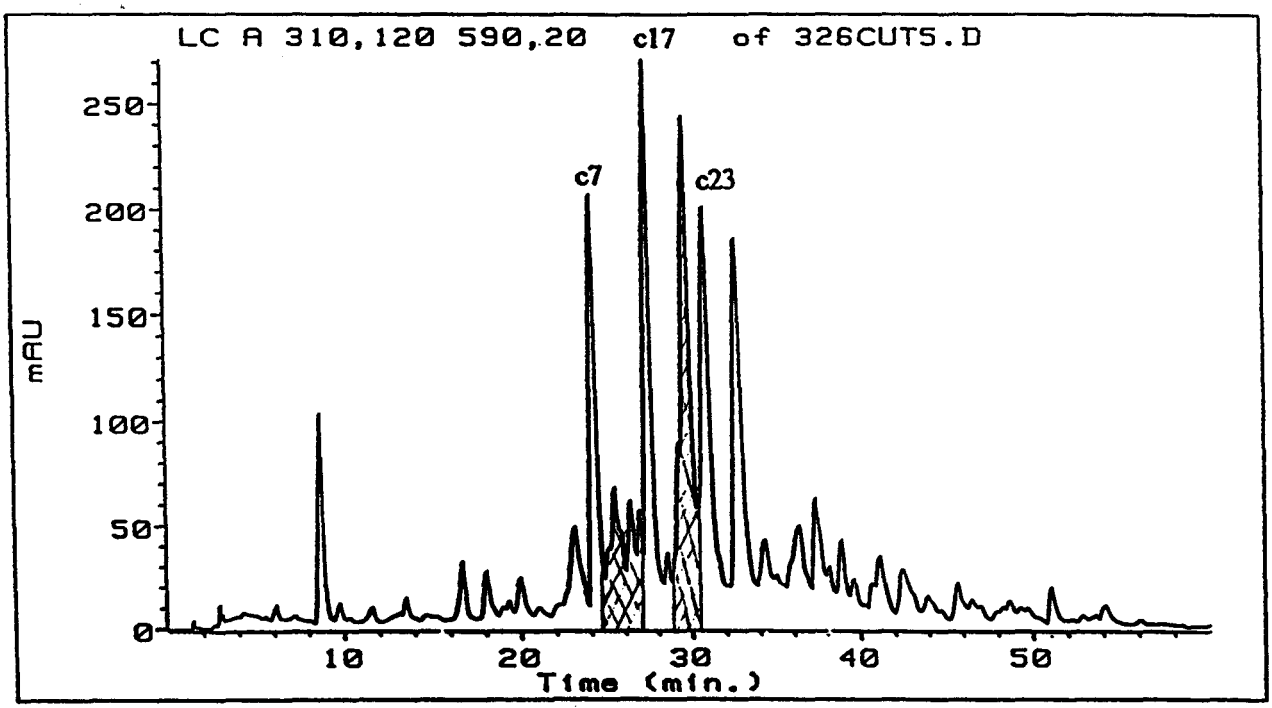
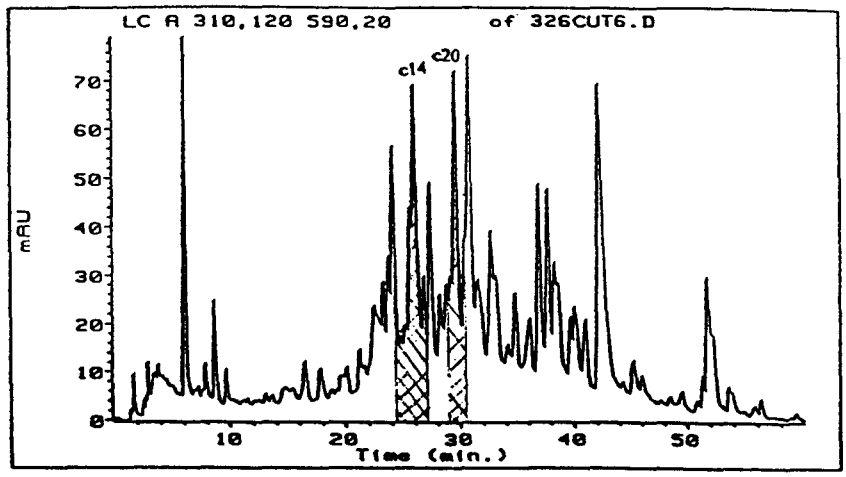


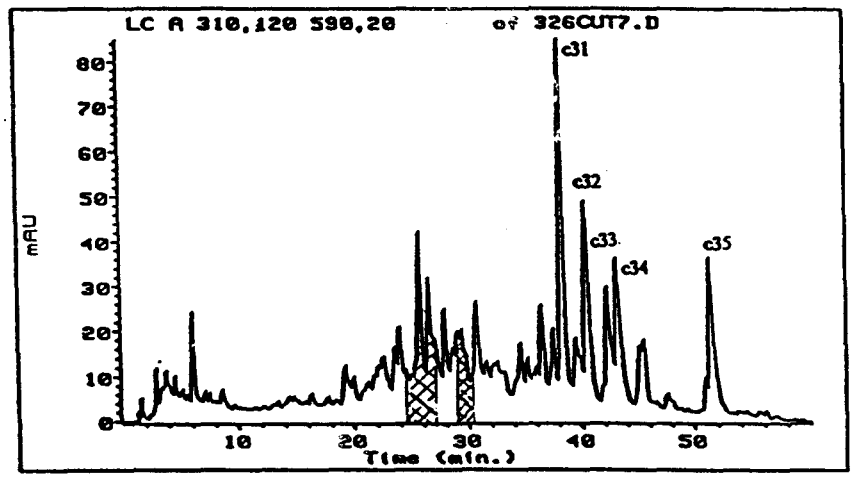
Figure 28 c. Reversed phase HPLC separation of subfraction 4

d. Reversed phase HPLC separation of subfraction 5

28e



28f



28g

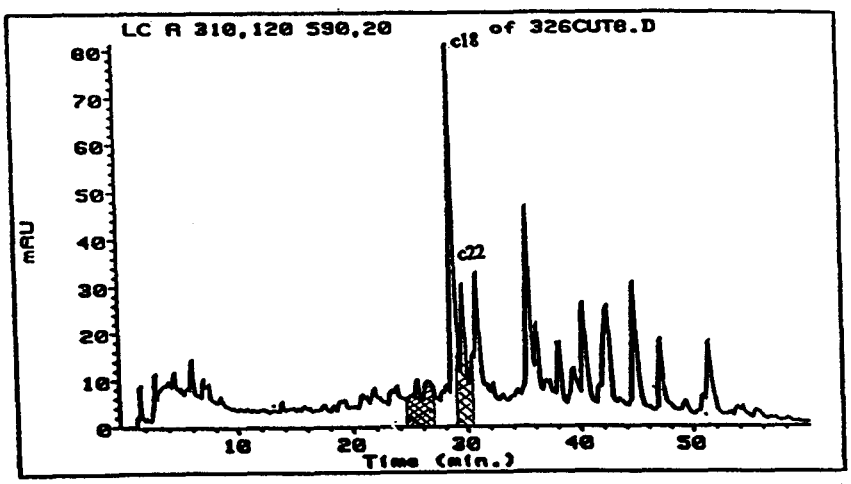


Figure 28 e. Reversed phase HPLC separation of subfraction 6
f. Reversed phase HPLC separation of subfraction 7
g. Reversed phase HPLC separation of subfraction 8

Table 12. RPLC and probe mass spectrometry data for 326 amu PAH isomers from coal tar

Peak No[c]	Isolated from NPLC Subfraction	RPLC Retention Time	Mol. Mass[a]	Number of Components[b]
c1	4	8.56	326	1
c2	2	14.14	326/342	2
c3	4	16.17	326/328	2
c4	4	17.36	328	1
c5	3	18.45	328	2
c6	3	23.87	326	1
c7	5	24.13	326	1
c8	2	24.21	326	2
c9	4	24.31	326	3
c10	3	24.56	326/328	2
*c11	4	25.28	326	1
*c12	2	25.35	328	2
*c13	3	25.56	326	1
*c14	6	25.95	326/340	2
*c15	4	26.13	326	1
c16	2	27.32	326	2
c17	5	27.42	326	1
c18	8	28.78	352	2
c19	4	28.96	326	1
*c20	6	29.57	326/340	2
*c21	4	29.63	326	2
*c22	8	29.72	352	2
c23	5	30.85	326	2

c24	4	31.42	326	2
c25	2	31.77	326/340	2
c26	3	34.64	326	2
c27	2	34.65	326	2
c28	2	35.29	326	1
c29	3	35.86	326	1
c30	2	36.73	326	2
c31	7	38.29	326/352	3
c32	7	40.39	326/352	2
c33	7	42.27	326/352	2
c34	7	43.13	326/352	2
c35	7	51.37	326/350	2

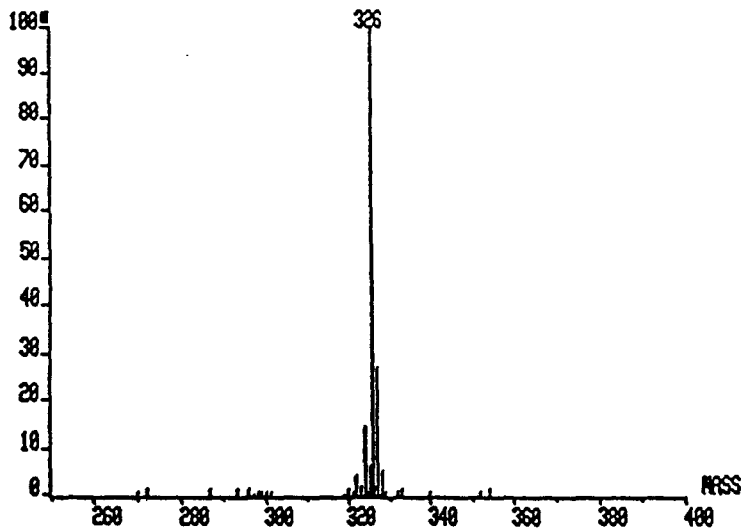
[a] The mass of the most abundant peak in the mass probe spectrum of that fraction.
[b] Determined by comparing the UV spectra at the leading edge, apex and trailing edge of the peak.
[c] Asterisks denote peaks which elute within the zones containing mutagenic responses.

all of the peaks were 326 amu PAH; some fractions contained 328, 350 and 352 amu PAH while a couple of fractions contains monomethylated 326 amu PAH derivatives (m/z 340). Depending on the level of impurities, the molecular weights of the 35 peaks were assigned in Table 12 as 326 amu PAH, 328 amu PAH, or 326/328, 326/340 mixtures, etc. Figure 29 shows representative probe mass spectra of two RPLC peaks (c10, c19). One spectrum (Figure 29a, peak c19) has a base peak at m/z 326 while the other (Figure 29b, peak c10) has strong peaks at m/z 326 and m/z 328, indicating that at least two components with two molecular masses were present.

Because probe mass spectrometric analysis can not tell the difference between isomers with the same molecular mass, another technique was needed to determine the purity of the 35 peaks. By using the "peak purity" program of the HP ChemStation software, the "peak purity" of each peak was determined. This program compares the UV spectra of the peak at the leading edge, at the apex and at the trailing edge. If the three UV spectra are identical, then the peak is said to be "a pure compound". Twelve of the 35 peaks listed in Table 12 were found to contain one component. Of the 12 peaks, 10 were 326 amu PAH while the other two peaks were a 302 and a 328 amu PAH. This multi-dimensional chromatography approach was successful in isolating a number of pure compounds from the very complex 326 amu PAH fraction.

Fluorescence spectroscopic analysis was performed on the ten "pure" 326 amu PAH

29a



29b

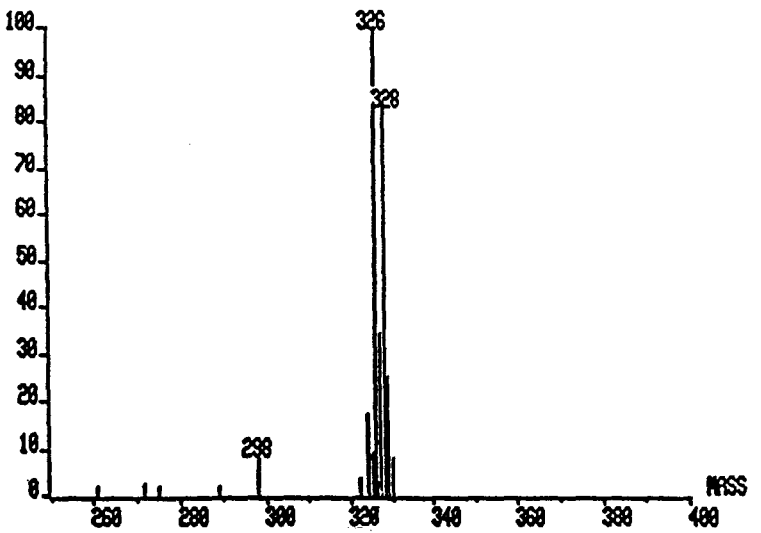


Figure 29 a. DEI probe mass spectrum of peak c19

b. DEI probe mass spectrum of peak c10

peaks. The fluorescence excitation and emission spectra of six peaks are shown in Appendix IV. The fluorescence excitation and emission spectra can be used as unique fingerprints of these compounds. The identities of these compounds may be determined if the fluorescence spectra of standard samples become available.

IV. CONCLUSIONS

A coal tar sample was separated into four fractions by alumina chromatography and these fractions were characterized using a range of chromatographic and mass spectral techniques. The overall recovery of organic compounds from coal tar using this alumina separation was 53.4% and 53.5% in two experiments. Fraction A1 (2.2 %) contained low mass PAH and some aliphatics while fraction A2 (25%) contained polycyclic aromatic hydrocarbons and thia-arenes up to mass 302 amu. Fraction A3 (5.5 %) contained high molecular mass PAH and some aza-arenes while fraction A4 (21 %) contained aza-arenes primarily. All fraction were analyzed by GC-MS and many compounds were quantitated using deuterated internal standards.

In fraction A2 PAH and sulfur-containing PAH (thia-arenes) accounted for 15.8% and 0.57% of the coal tar respectively. The attempted separation of thia-arenes from PAH using an oxidation-chromatography-reduction approach was a failure; while some PASH were recovered, the overall yield was only 0.1% of fraction A2. The separation of thia-arenes from PAH using a ligand exchange chromatographic method based on $PdCl_2$-silica gel as the absorbent was more successful. A solvent elution protocol was optimized which resulted in separation of some PASH from PAH. Of the 25 thia-arenes identified in this coal tar sample 12 were not retained by the Pd-silica column and eluted with PAH; the remaining 13 were either partially (5) or fully (8) retained by the column and were eluted subsequently to afford

a sulfur-rich fraction. The PAH which co-eluted with the PASH were higher mass PAH (252, 276, 278 and 302 amu). Analysis of this sulfur-rich fraction using normal phase HPLC resulted in the separation of 10 of the 13 PASH from higher mass PAH. Unfortunately, the three 284 amu PASH in this fraction coeluted with 252 amu PAH and could not be isolated without PAH contamination. Overall, the thia-arene separation methods were only partially succussful in isolating thia-arenes from PAH.

Fraction A3 was separated by semi-preparative normal phase HPLC into several fractions which were shown by mass spectrometry to contain high molecular mass PAH, ranging from 300/302 amu to 474 amu and aza-arenes from 179 to 327 amu. The 326 amu PAH fraction was further separated and analyzed by a combination of normal phase and reversed phase chromatographic techniques. Individual components collected from this 326 amu fraction were analyzed by probe mass spectrometry and fluorescence spectroscopy. Eight peaks were found eluted in the regions which had shown a strong genotoxic responses in the Ames assay in previous work done in our group; these eight compounds will be evaluated to determine which are active mutagenic active.

Overall, the work in this thesis had demonstrated the power of multi-dimensional chromatographic approaches to the separation and isolation of minor components from complex mixtures.

REFERENCES

1. H.H. Lowry, *Chemistry of Coal Utilization*, Volume II, John Wiley & Son, New York, 1945.

2. H.H. Lowry, *Chemistry of Coal Utilization*, Supplementary Volume, John Wiley & Son, New York, 1963.

3. G.J. Pitt and G.R. Millward, *Coal and Modern Coal Processing: An Introduction,* Academic Press, 1979.

4. M.J. Chadwick and N. Lindman., *Environmental Implications of Expanded Coal Utilization,* Pergamon Press, 1982.

5. L. Noel, M. Authier-Martin, G. Patry, A. Marcoux, P. Jean, *Polycyclic Aromatic Compounds,* 9, 373-380 (1996).

6.S.A. Wise, B.A. Benner, G.D. Byrd, S.N. Chesler, R.E. Rebbert and M.M. Schantz, *Anal. Chem.,* 60, 887-894 (1988).

7. M. Cooke, A.J. Dennis and G.L. Fisher, *Polynuclear Aromatic Hydrocarbons*, Battelle Press, 1982.

8. I. Hieger, *Biochemical J.*, 24, 505-511 (1930).

9. J.W. Cook, C.J. Hewett and I. Hieger, *J. Chem. Soc.*, 395-405 (1933).

10. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Volumn 35, Polynuclear Aromatic Compounds, Part 4, p.84, 1985.

11. D.J. Poulton, *J. Great Lakes Res.,* 13 (2), 193-201 (1987).

12. T. Mayer, E. Nagy, *Water Poll Res. J. Can.,* 27 (4), 807-831 (1992).

13. T.P. Murphy, H. Brouwer, M.E. Fox, E. Nagy, L. McArdle and A. Moller, *Coal Tar Contamination Near Randle Reef, Hamilton Harbour,* N.W.R.I. Contribution No. 90-17

14. C.H. Marvin, *"A Multi-Media Bioassay-Directed Investigation of the Hamilton Harbour Area of Western Lake Ontario"*, Ph.D. Thesis, Department of Chemistry, McMaster University, 1994.

15. C.H. Marvin, L. Allan, B.E. McCarry, D.W. Bryant, *Inter. J. Environ. Anal. Chem.*, 49, 221-230 (1992).

16. J.Jacob, *Sulfur Analogues of Polycyclic Aromatic Hydrocarbons (Thiaarenes)*, Cambridge University Press, Cambridge, 1990.

17. C.J. Thompson, *Organic Sulfur Chemistry*, Pergamon Press, Oxford, 1981. p.184.

18. S.A. Wise, B.A. Benner, S.N. Chesler, L.R. Hilpert, C.R. Vogt and W.E. May, *Anal. Chem.*, 58, 3067-3077 (1986).

19. J. T. Anderson, *Intern. J. Environ. Anal. Chem.*, 48, 1-15 (1992).

20. M. Nishioka, J.S. Bradshaw, M.L. Lee, Y. Tominaga, M. Tedjamulia and R.N. Castle, *Anal.Chem.*, 57, 309 (1985).

21. T.G. Albro, P. A. Dreifuss and R.F. Wormsbecher, *J. High Resolut. Chromatogr.*, 16, 13-17 (1993).

22. G. Grimmer, H. Brune, G. Dettbarn, J. Jacob, J. Misfeld, U. Mohr, K.W. Naujack, J. Timm, and R. Wenzel-Hartung, *Fres. J. Anal. Chem.*, 339, 792-795 (1991).

23. C.H. Marvin, J.A. Lundrigan, B.E. McCarry and D.W. Bryant, *Environ. Toxicol. Chem.*, 14, 2059-2066 (1995).

24. C.H. Marvin, L. Allan, B.E. McCarry, and D.W. Bryant, *Env. Molec. Mutagen.*, 22, 61-70 (1993).

25. A.E. Legzdins, B.E. McCarry, C.H. Marvin, and D.W. Bryant, *Int. J. Environ. Anal. Chem.*, 60, 79-94 (1995).

26. H.S. Hertz, J.M. Brown, S.N. Chesler, F.R. Guenther, L.R. Hilpert, W.E. May, R.M. Parris and S.A. Wise, *Anal. Chem.*, 52, 1650-1657 (1980).

27. B.W. Wilson, M.R. Petersen, R.A. Pelrøy and J.T. Cresto, *Fuel,* 60, 289-290 (1981).

28. R.E. Royer, C.E. Mitchell, R.L. Hanson, J.S. Dutcher, W.E. Bechtold, *Environ. Res.,* 31, 460-471 (1983).

29. D.W. Later, M.L. Lee, K.D. Bartle, R.C. Kong and D.L.Vassilaros, *Anal. Chem.,* 53, 1612-1620 (1981).

30. A.A. Herod and B.J. Stokes, *Fuel Processing Technology,* 24, 45-51 (1990).

31. B.W. Wilson, R.A. Pelroy, D.D. Mahlum, M.E. Frazier, D.W. Later and C.W. Wright, *Fuel, 63,* 46-55 (1984).

32. C.H. Marvin, L. Allan, B.E. McCarry, D.W. Bryant, *Int. J. Environ. Anal. Chem.,* 49, 221-230 (1992).

33. C.H. Marvin, B.E. McCarry, D.W. Bryant, *J. Great Lakes Res.,* 20, 523-530 (1994).

34. M. Nishioka, R.C. Campbell, M.L.Lee, R.N. Castle, *Fuel,* 65, 270-273 (1986).

35. C. Willey, M. Iwao, R.N. Castle and M.L. Lee, *Anal. Chem.,* 53, 400-407 (1981).

36. T. Kaimai and A. Matsunaga, *Anal. Chem.,* 50, 268-270 (1978).

37. J.W. Vogh and J.E. Dooley, *Anal. Chem.,* 47, 816-821 (1975).

38. W.L. Orr, *Anal. Chem.,* 39, 1163-1164 (1967).

39. W.F. Joyce and P.C. Uden, *Anal. Chem.,* 55, 540-543 (1983)

40. J. T. Andersson, B. Schmid, *J. Chromatogr. A,* 693, 325-338 (1995).

41. M. Moini, D. Chace and F.P. Abramson, *J. Am. Soc. Mass. Spectrom.,* 2, 250-255 (1991).

42. M. Nishioka, P.A. Smith, G.M. Booth, M.L. Lee, H. Kudo, D.R. Muchiri, R.N. Castle, *Fuel,* 65, 711-14 (1986).

43. S.A. Wise, L.C. Sander and W.E. May, *J. Chromatogr.*, 642, 329-349 (1993).

44. M.A. Quilliam and P.G. Sim, *J. Chromatogr. Sci.*, 26, 160-167 (1988).

45. C.M. Pace, and L.D. Betowski, *J. Am. Soc. Mass Spectrom.*, 6, 597-607 (1995).

46. J.F. Anacleto, L. Ramaley, F.M. Benoit, R.K. Boyd, amd M.A. Quilliam, *Anal. Chem.*, 67, 1445-1454 (1995).

47. C.H. Marvin, R.W. Smith, D.W. Bryant and B.E. McCarry, *Analysis of high molecular weight PAH in Environmental samples using atmospheric pressure chemical ionization LC/MS*, EnviroAnalysis'96 Conference, Ottawa, Ontario, May, 13-16, 1996.

48. M.S. Kharasch, R.C. Seyler and F.R. May, *J. Am. Chem. Soc.*, 60, 882-884 (1938).

49. D.K. Skoog, *Principles of Instrumental Analysis*, Third Edition, Saunders College Publishing, 1985.

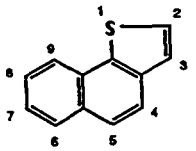
50. D.L. Vassilaros, R.C. Kong, D.W. Later, M.L. Lee, *J. Chromatogr.*, 252, 1-20 (1982).

51. C. Willey, M. Iwao, R.N. Castle, M.L. Lee, *Anal. Chem.*, 53, 400-407 (1981).

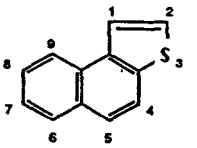
52. J.T. Andersson, *Anal. Chem.*, 59, 2209-2211 (1987).

Appendix I

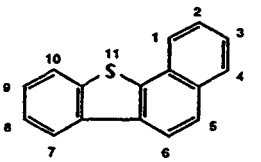
The structures of 20 PASH compounds identified in fractions A2-P1 and A2-P2



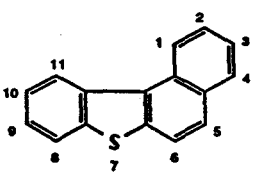
s2, P1



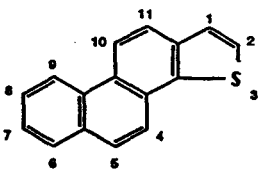
s3, P1



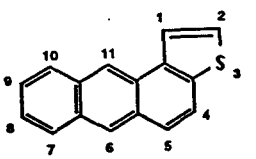
s8, P1



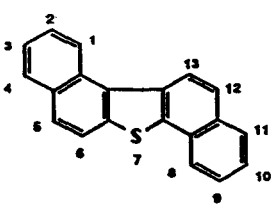
s9, P1



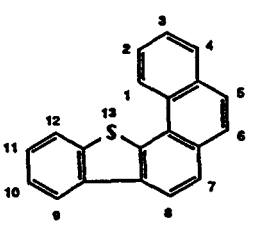
s11, P1



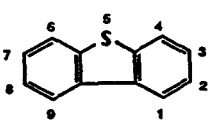
s12, P1



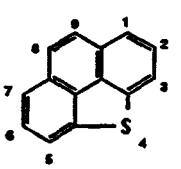
s22, P1



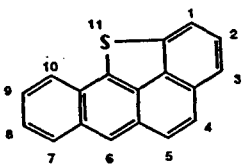
s23, P1



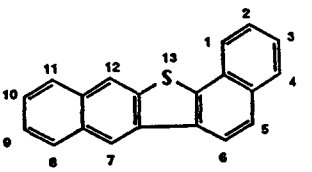
s1, P1/P2



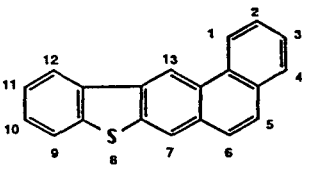
s5, P1/P2



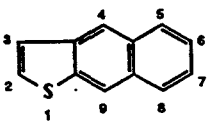
s17, P1/P2



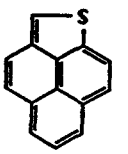
s24, P1/P2



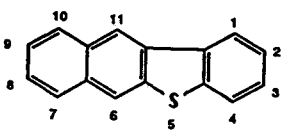
s25, P1/P2



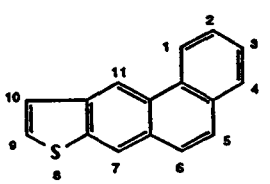
s4, P2



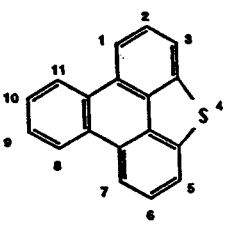
s7, P2



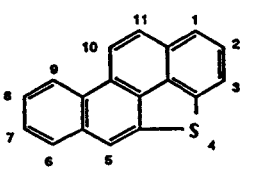
s10, P2



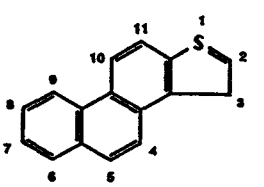
s14, P2



s18, P2

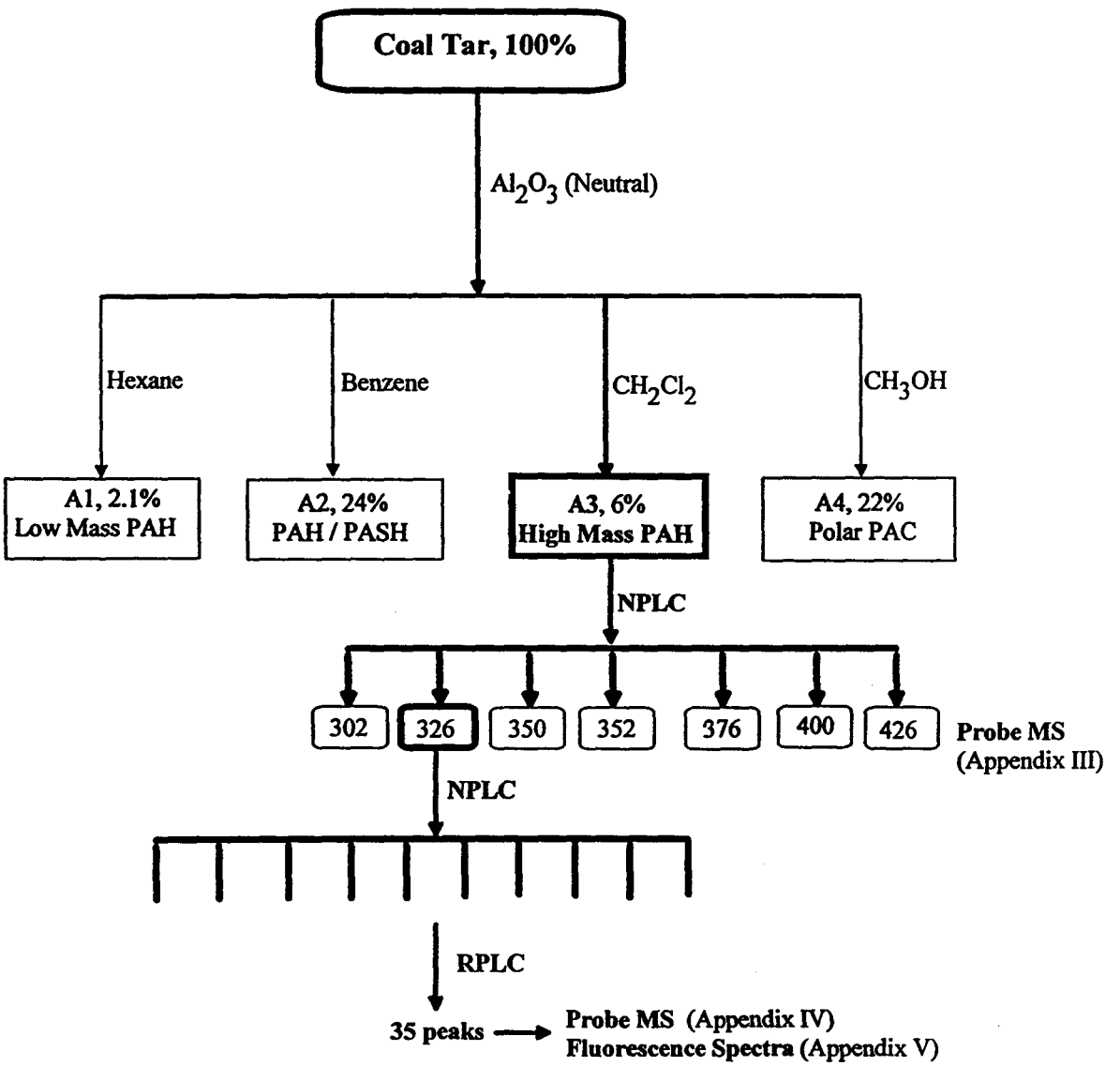


s19, P2



s13, P2

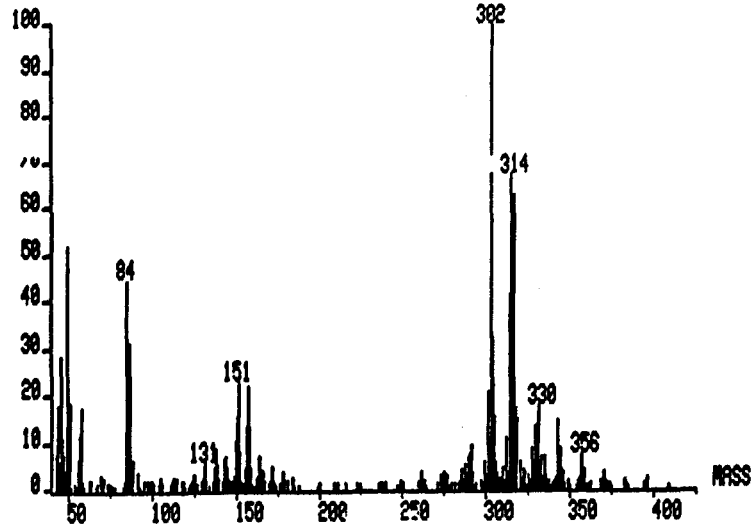
Appendix II. Separation and Analyses of 326 amu PAH



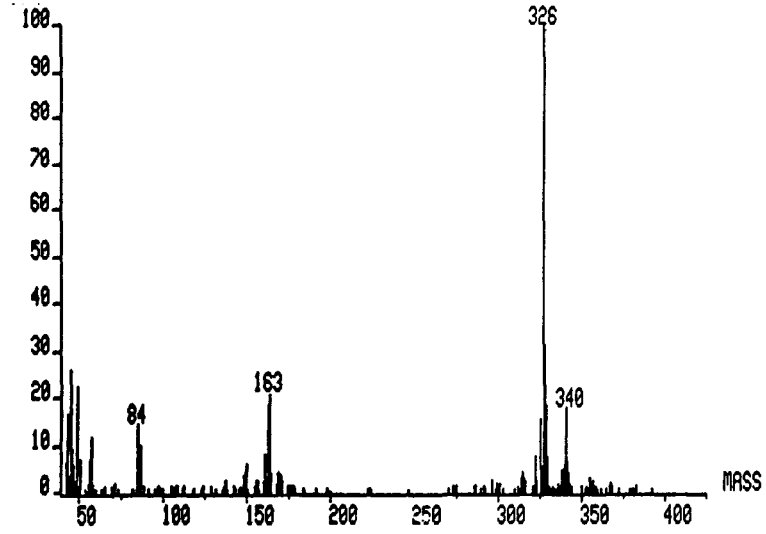
Appendix III

DEI Probe Mass Spectra of 13 Fractions Prepared by NPLC Analyses of Fraction A3

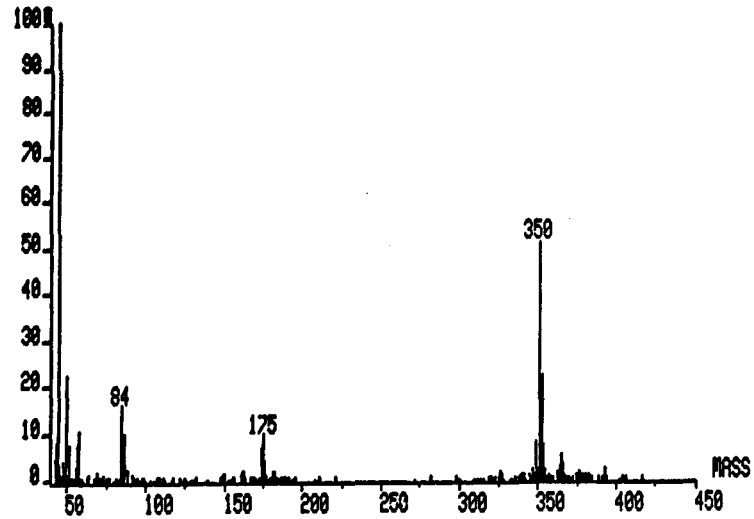
Fraction 1



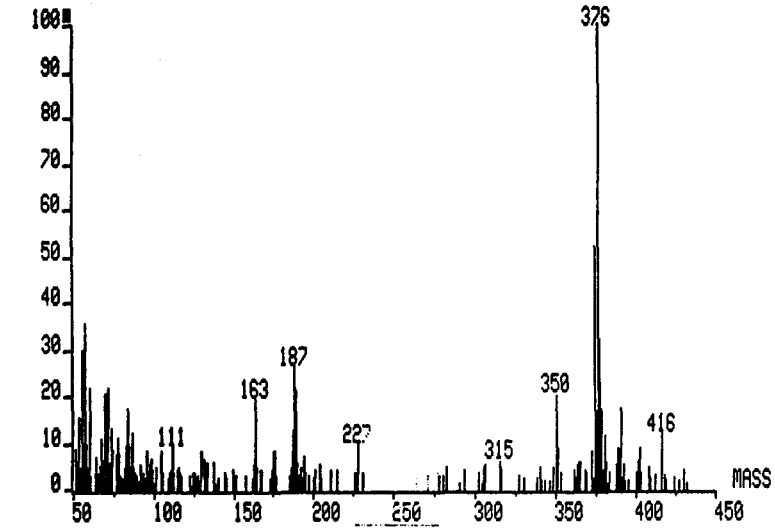
Fraction 2



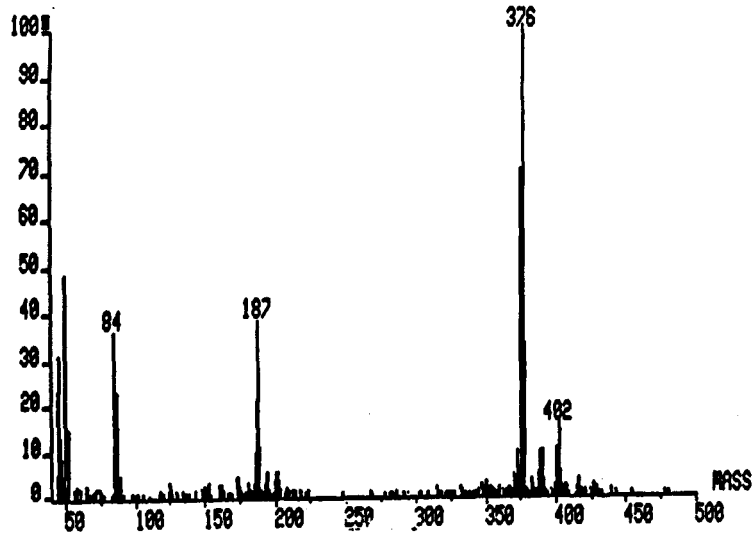
Fraction 3



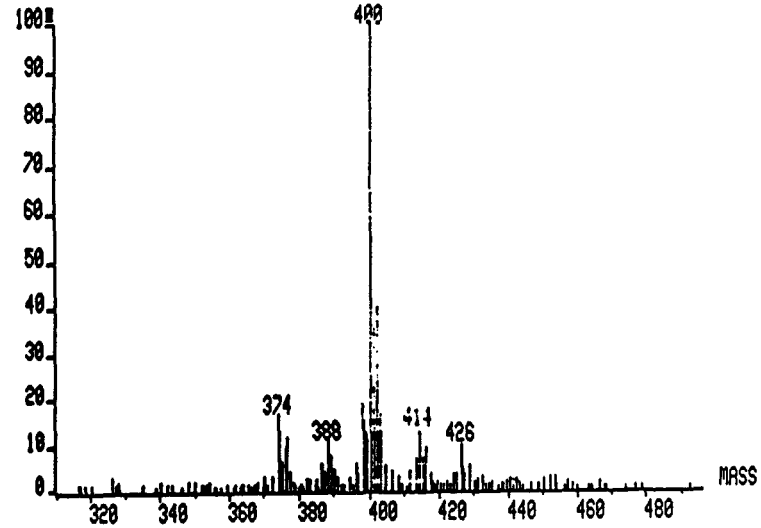
Fraction 4



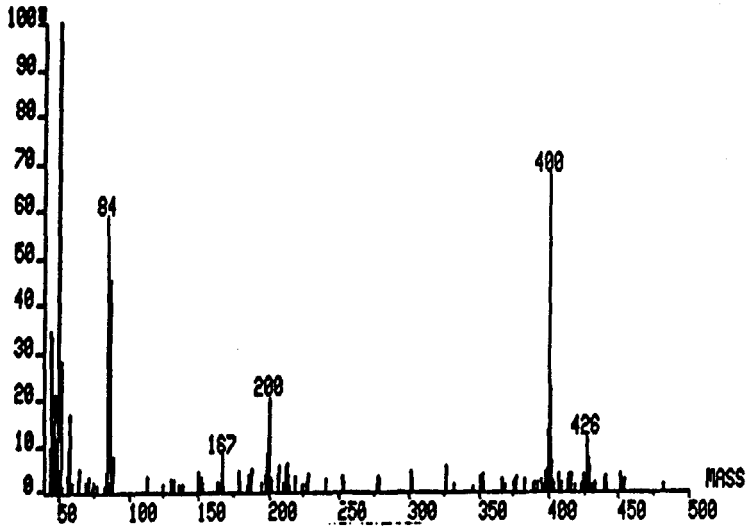
Fraction 5



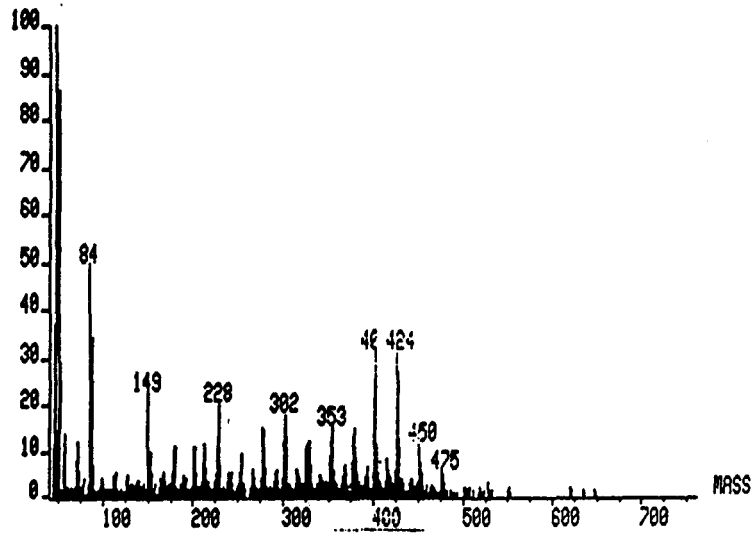
Fraction 6



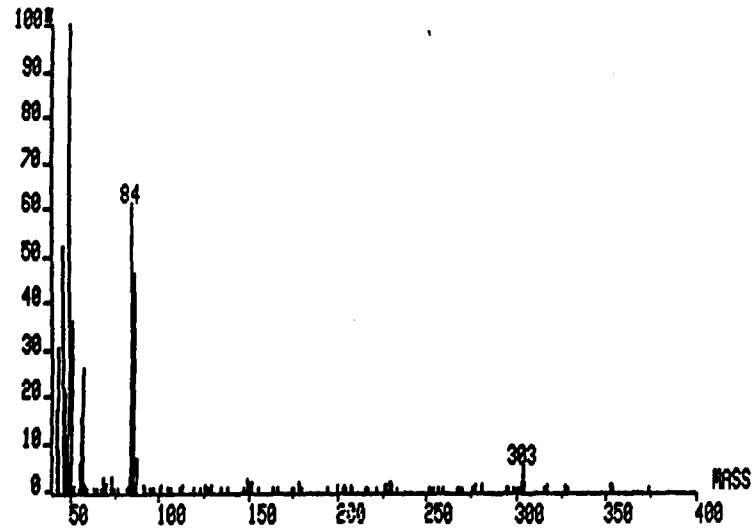
Fraction 7



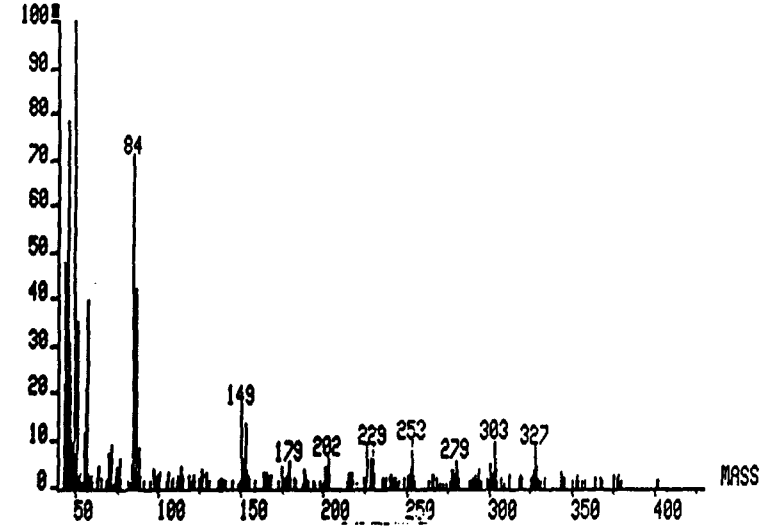
Fraction 8



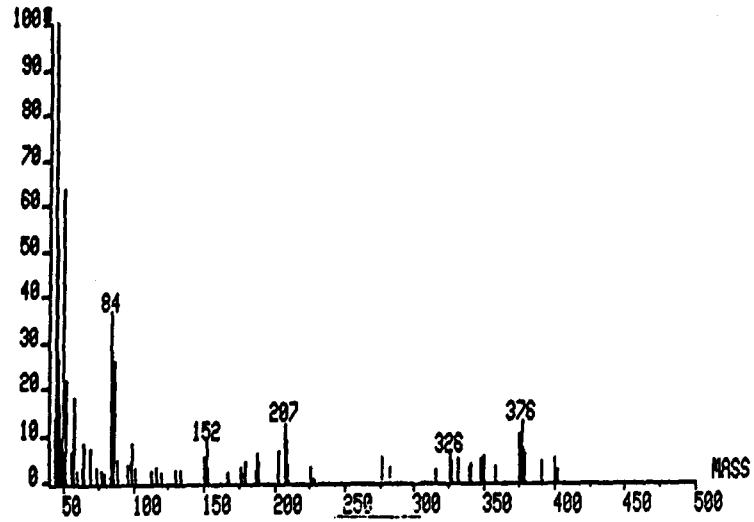
Fraction 9



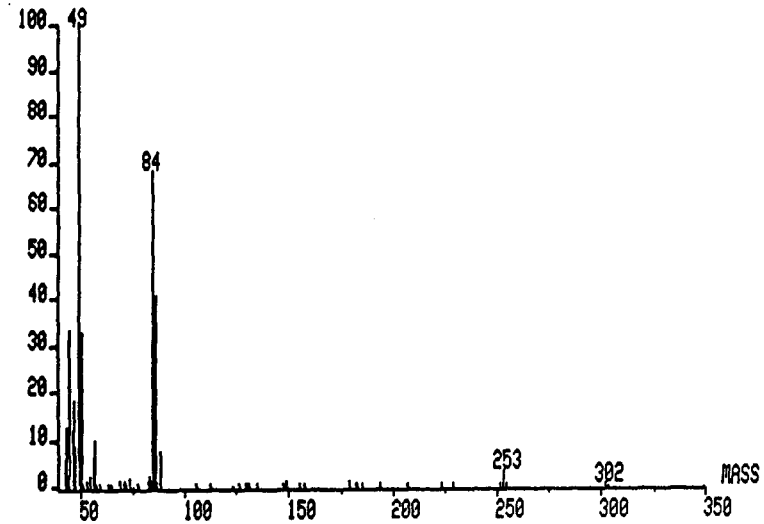
Fraction 10



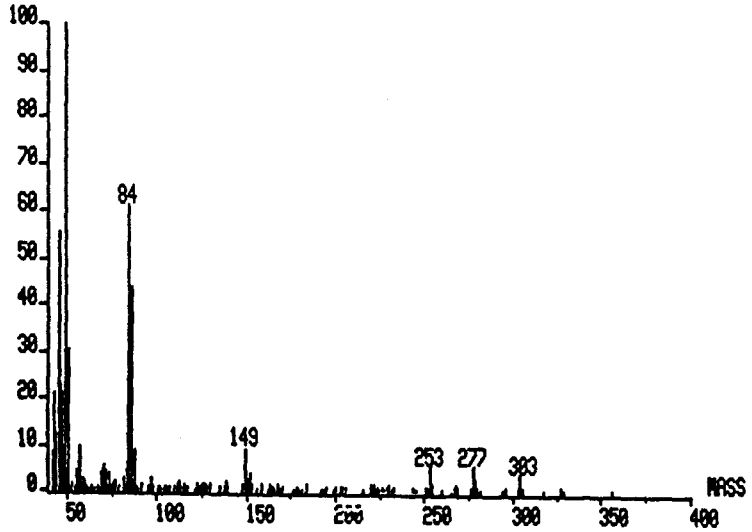
Fraction 11



Fraction 12

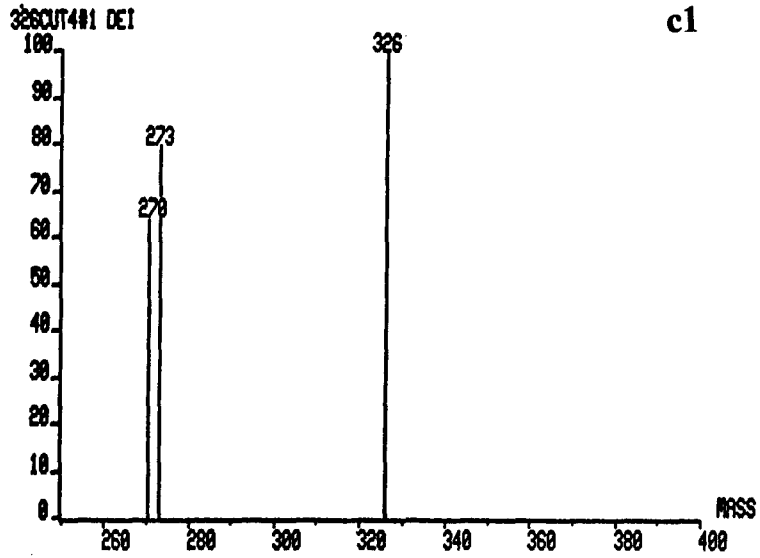


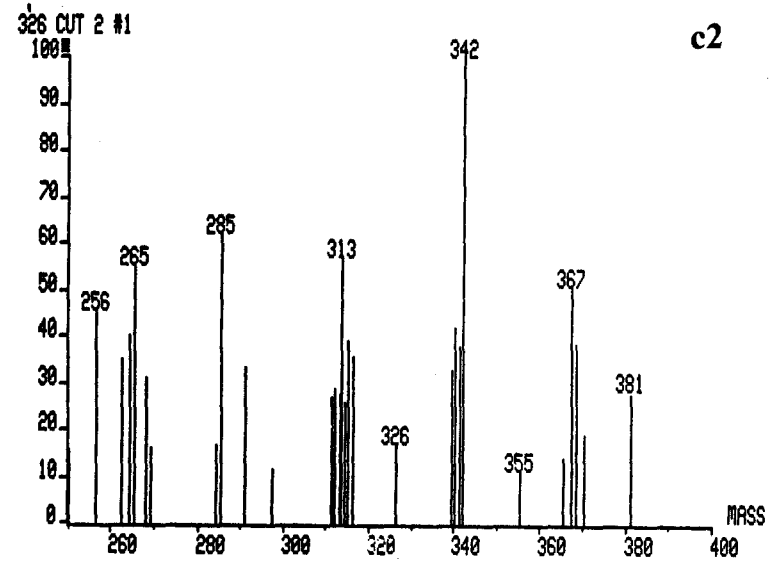
Fraction 13

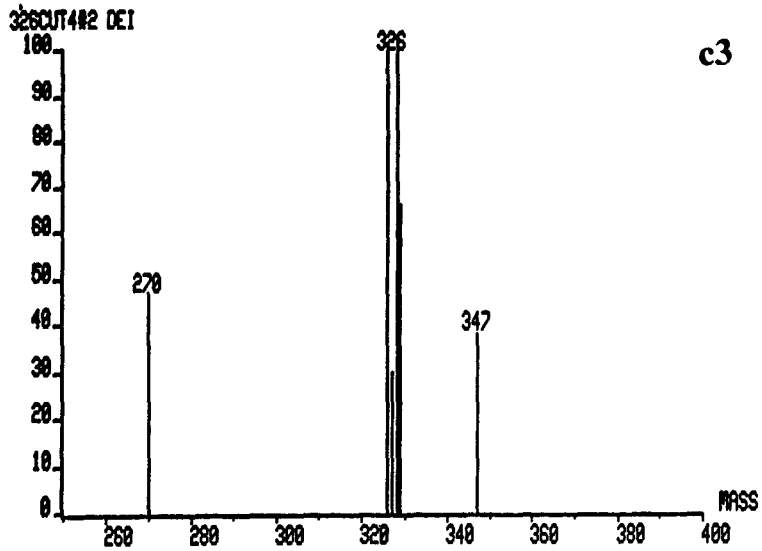

100
90
80
70
60
50
40
30
20
10
0
84
149
253
277
303
MASS
50
100
150
200
250
300
350
400

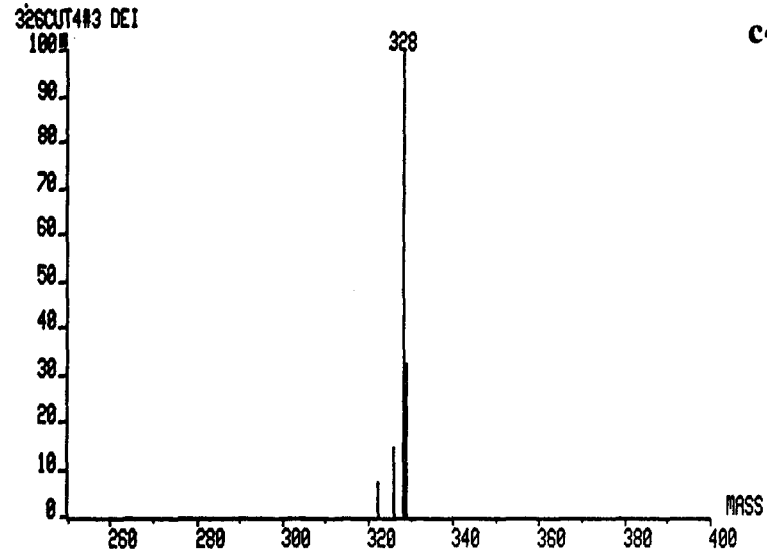
Appendix IV

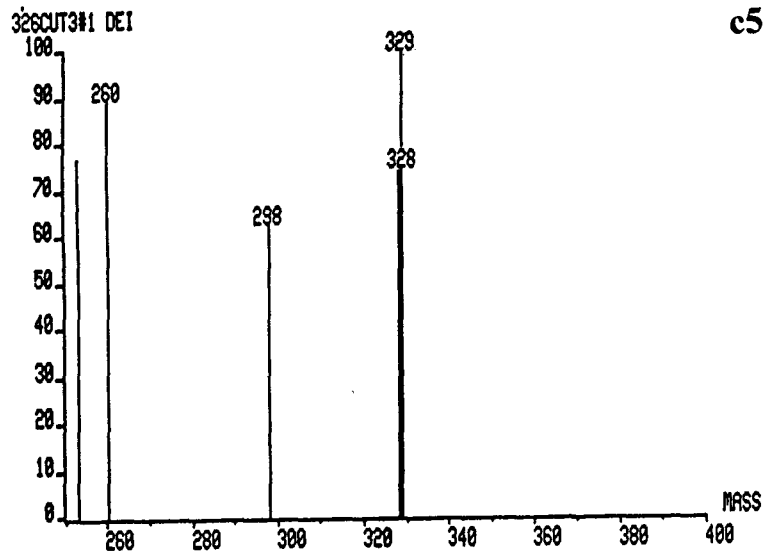
DEI Probe Mass Spectra of Peaks c1 to c35 Collected from RPLC Analyses of 326 amu PAH Fractions


326CUT4#1 DEI
c1
326
273
270
MASS

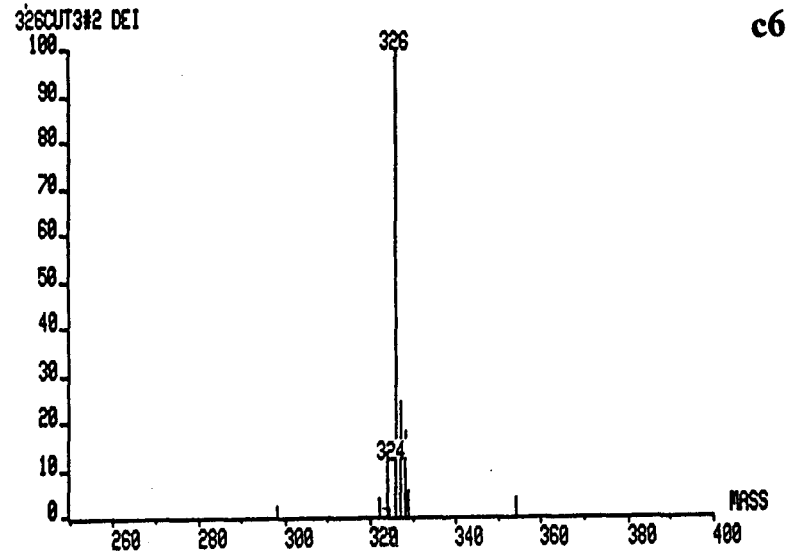

326 CUT 2 #1
c2
342
285
265
313
367
256
381
326
355
MASS


326CUT4#2 DEI
c3
326
270
347
MASS

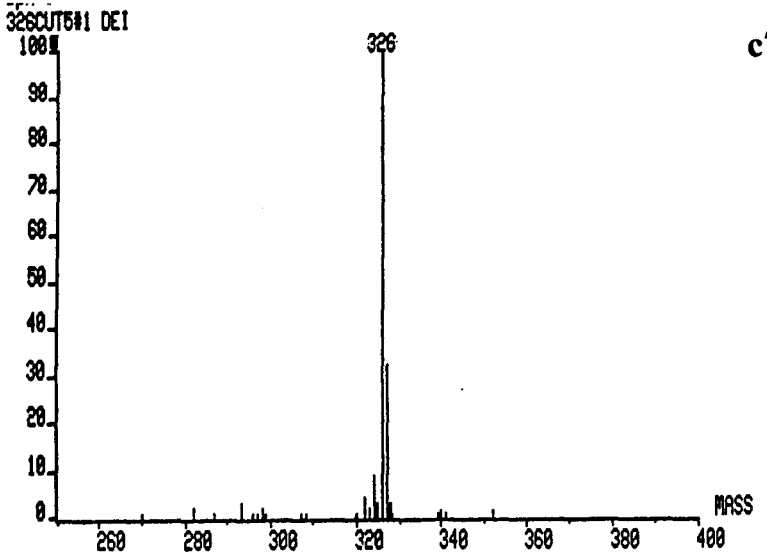

326CUT4#3 DEI
c4
328
MASS


326CUT3#1 DEI
329
260
328
298
MASS
260
280
300
320
340
360
380
400

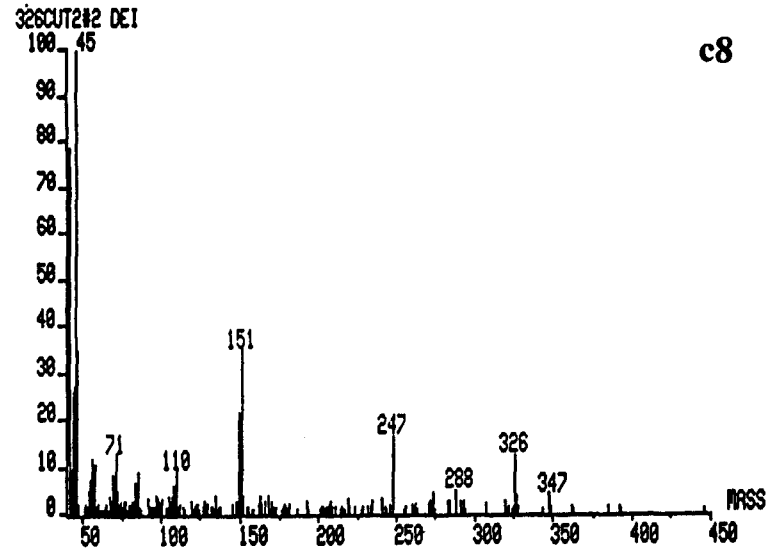
c5


326CUT3#2 DEI
326
324
MASS
260
280
300
320
340
360
380
400

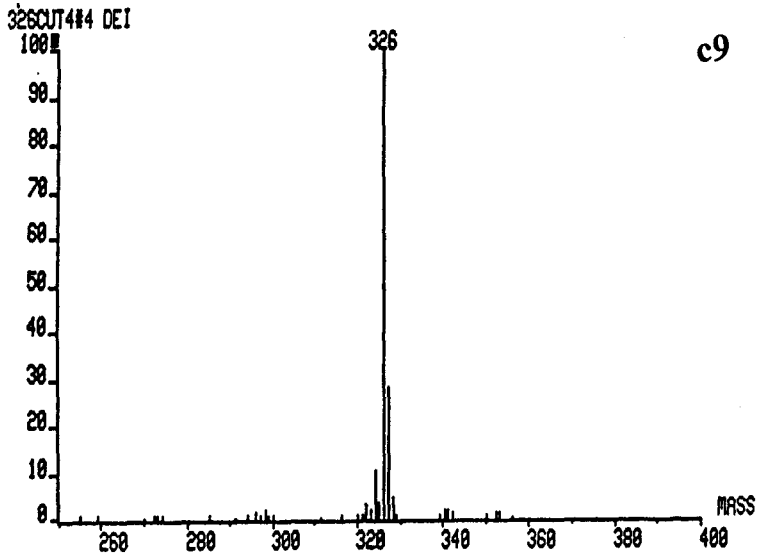
c6


326CUT5#1 DEI
326
MASS
260
280
300
320
340
360
380
400

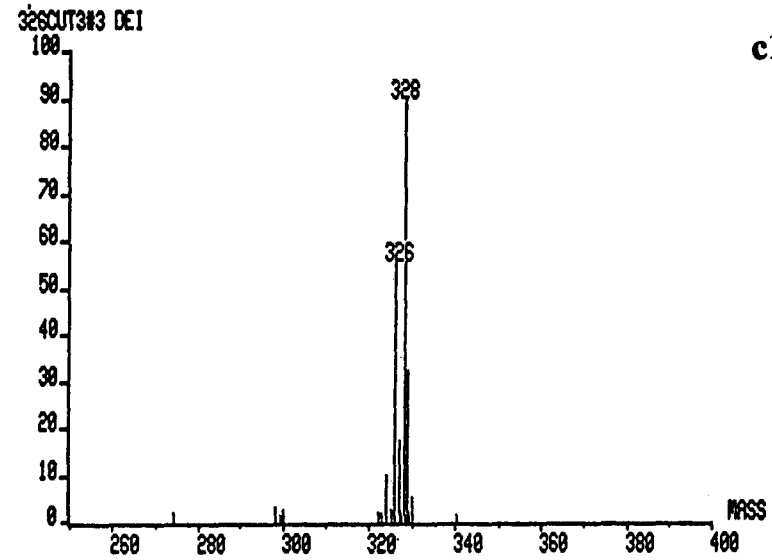
c7


326CUT2#2 DEI
45
151
71
110
247
326
288
347
MASS
50
100
150
200
250
300
350
400
450

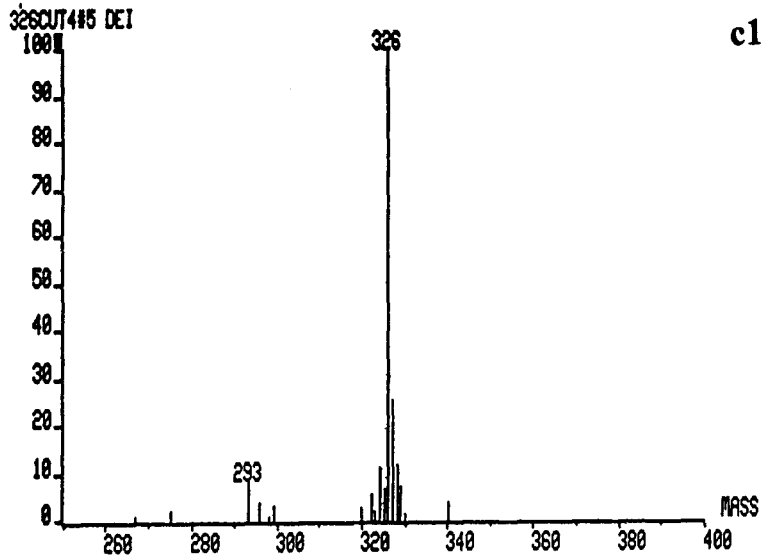
c8



c9

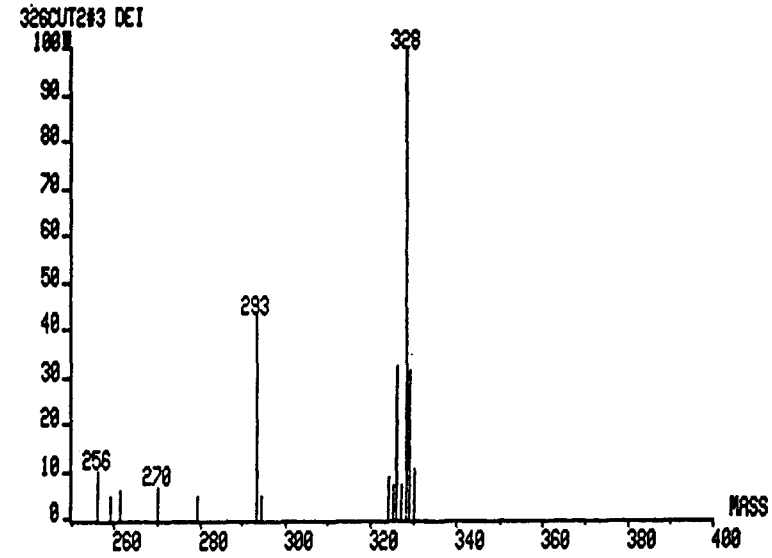


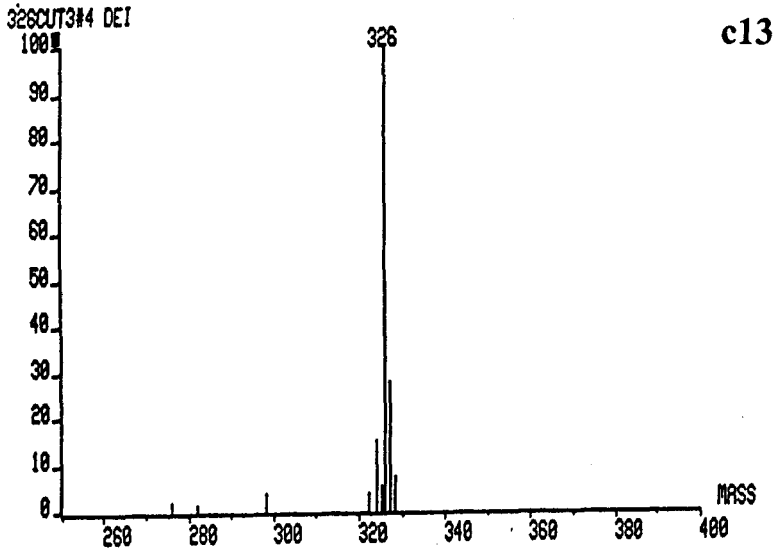
c10



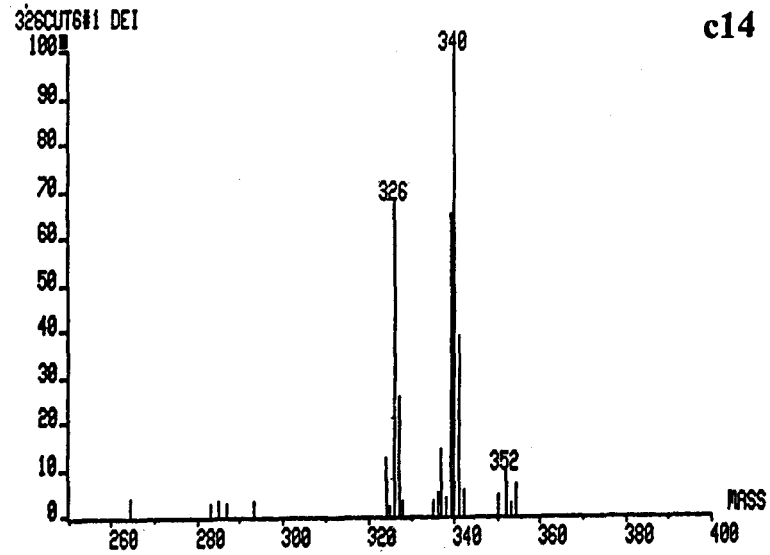
c11

c12

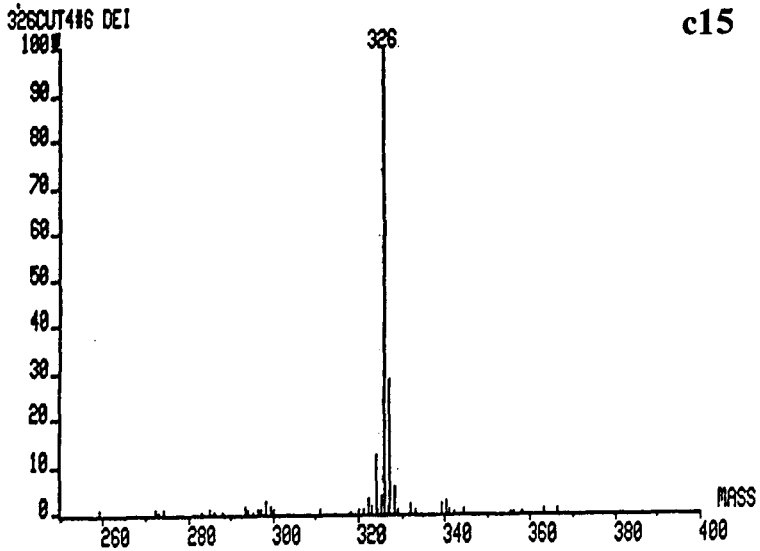




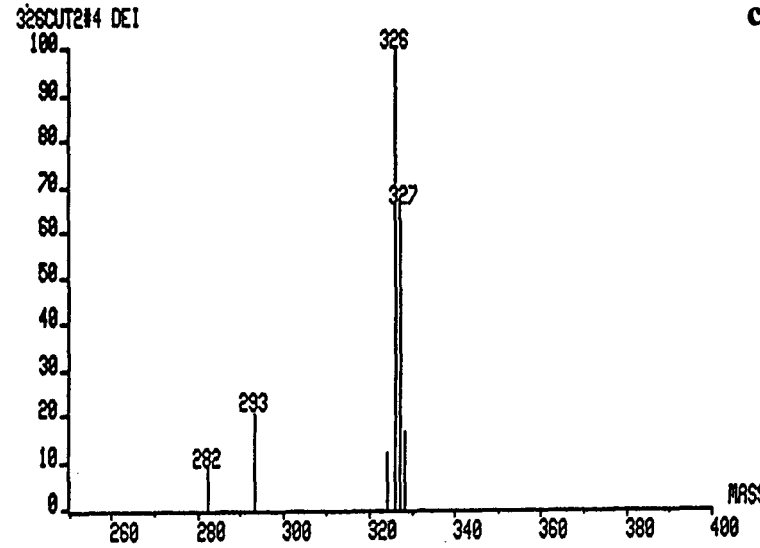
c13



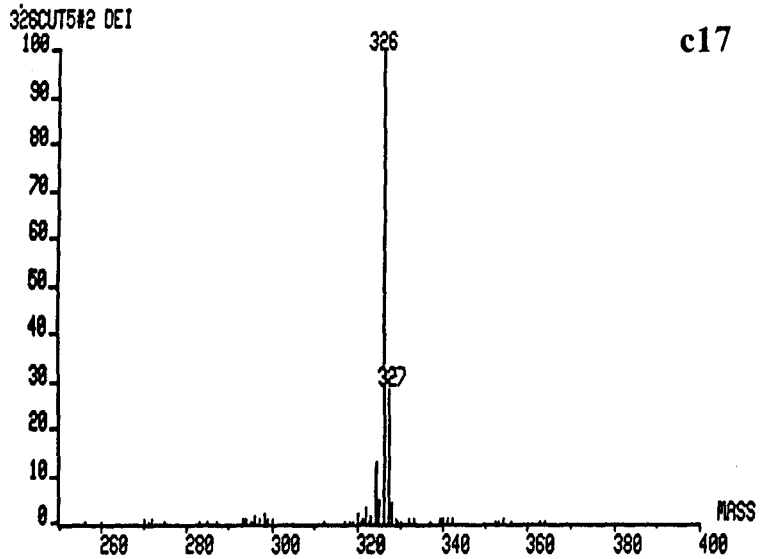
c14



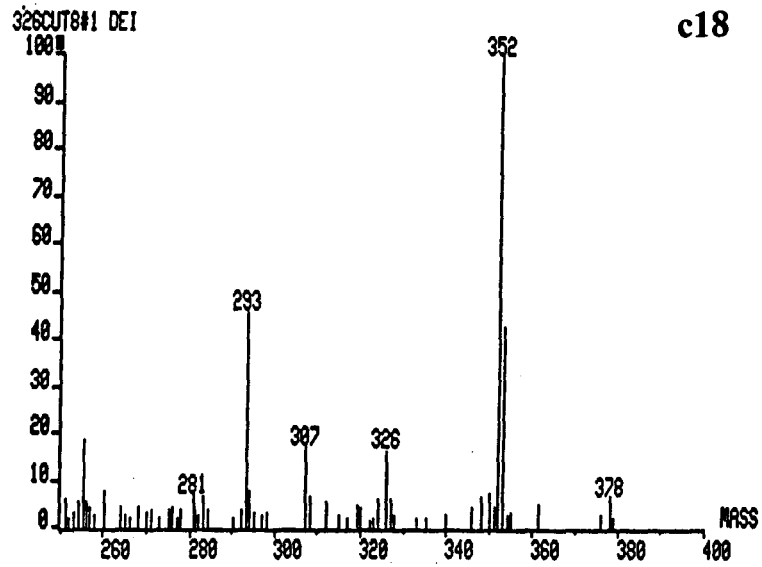
c15



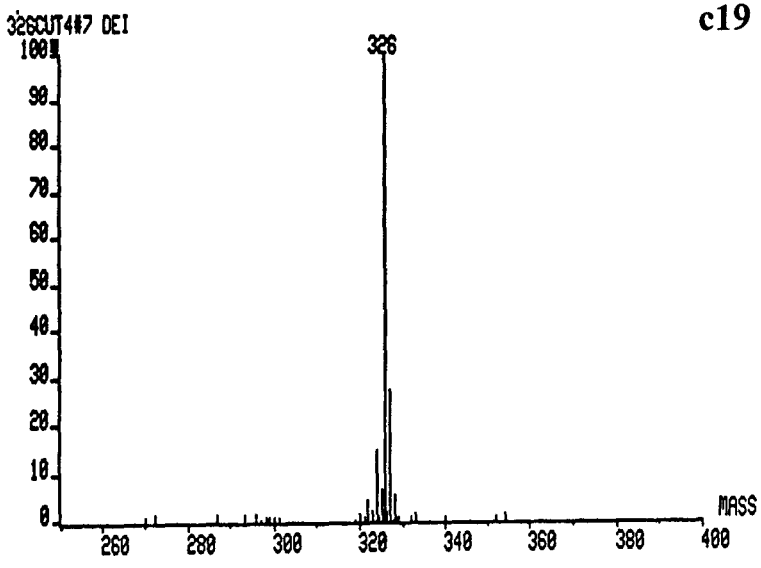
c16



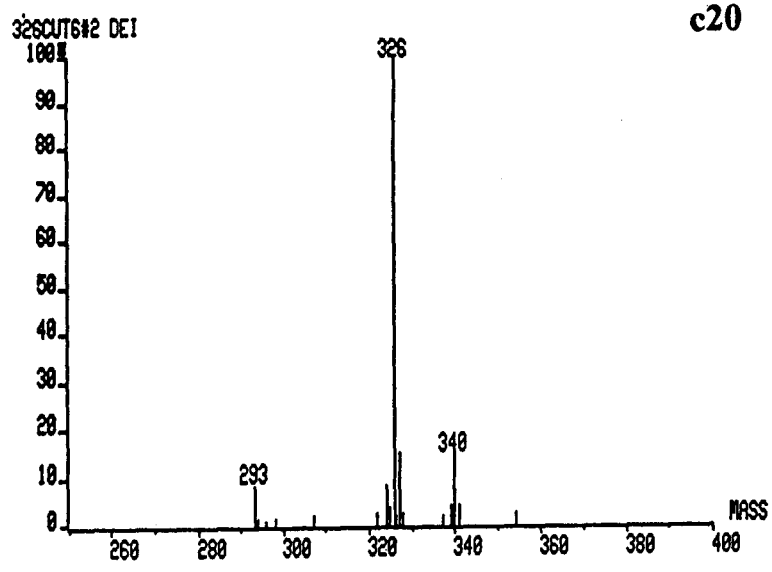
c17



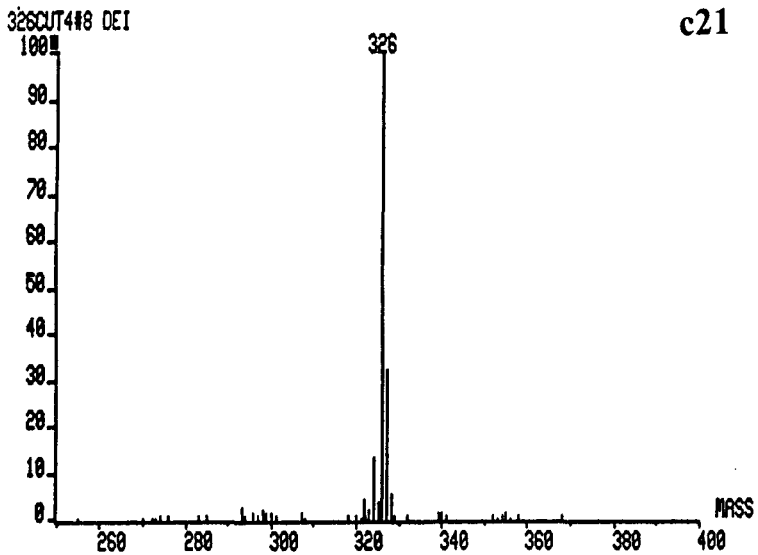
c18



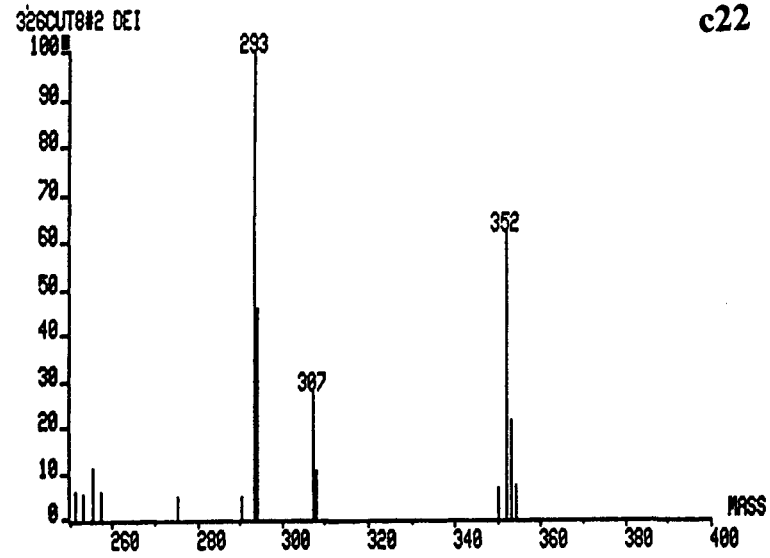
c19



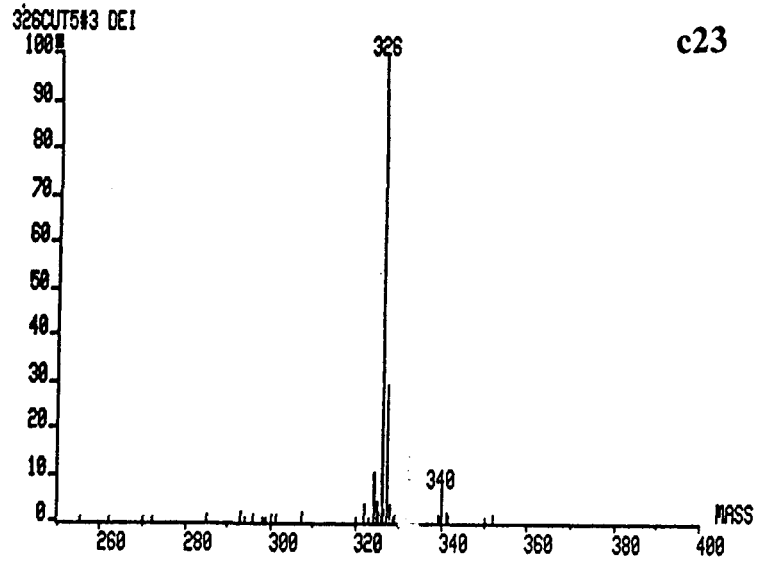
c20



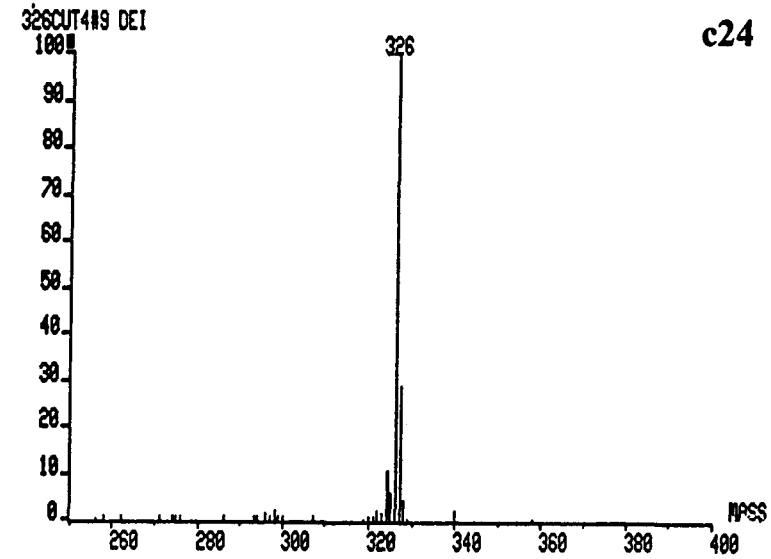
c21



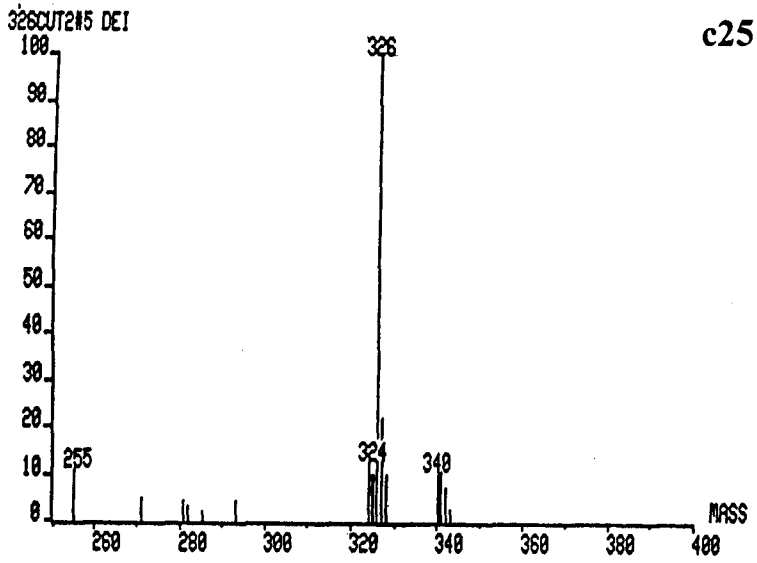
c22



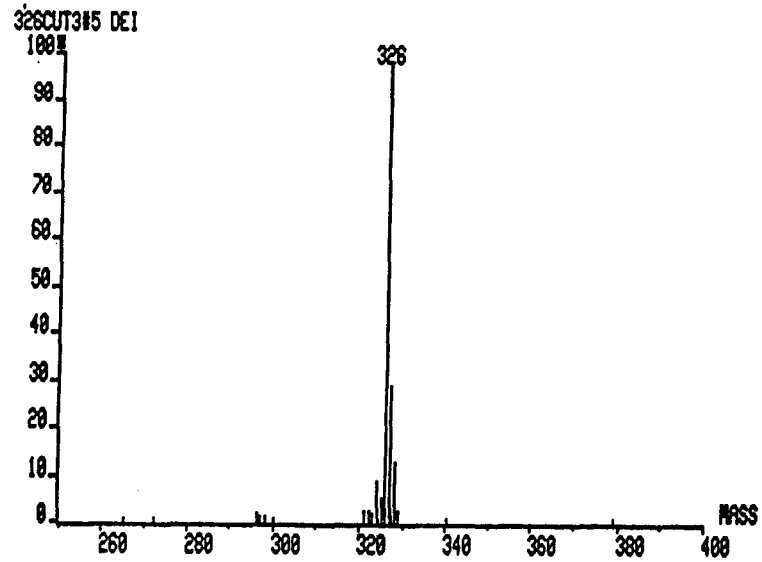
c23



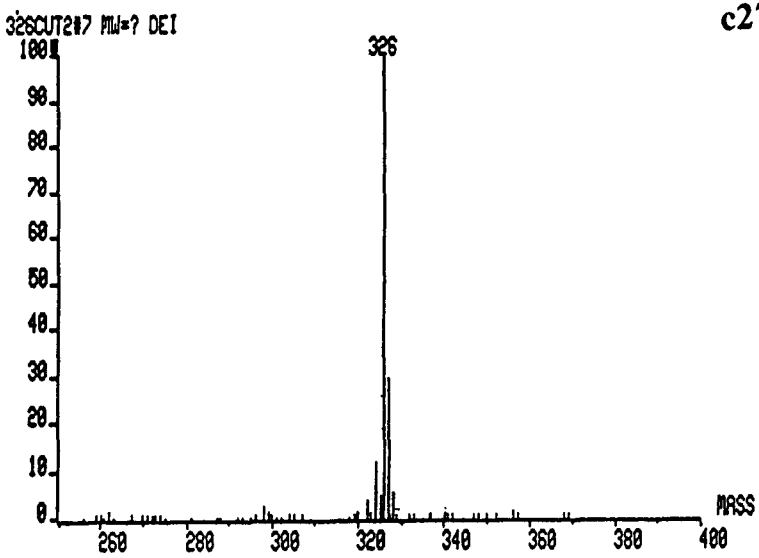
c24



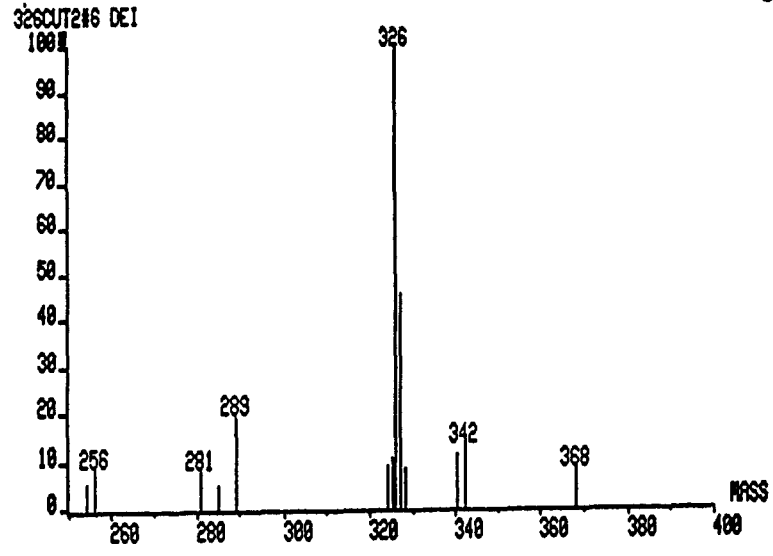
c25



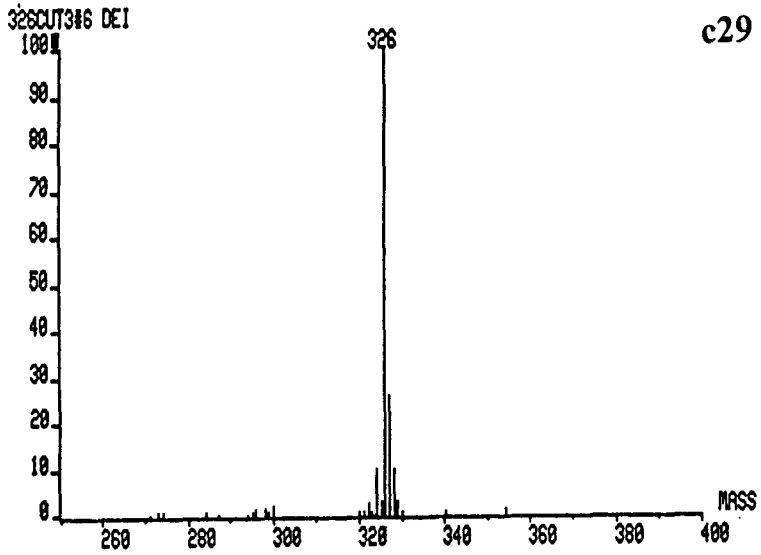
c26



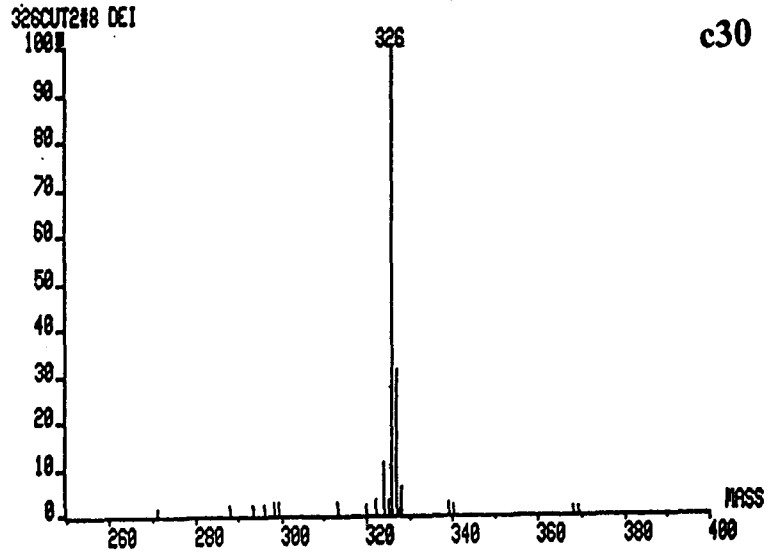
c27



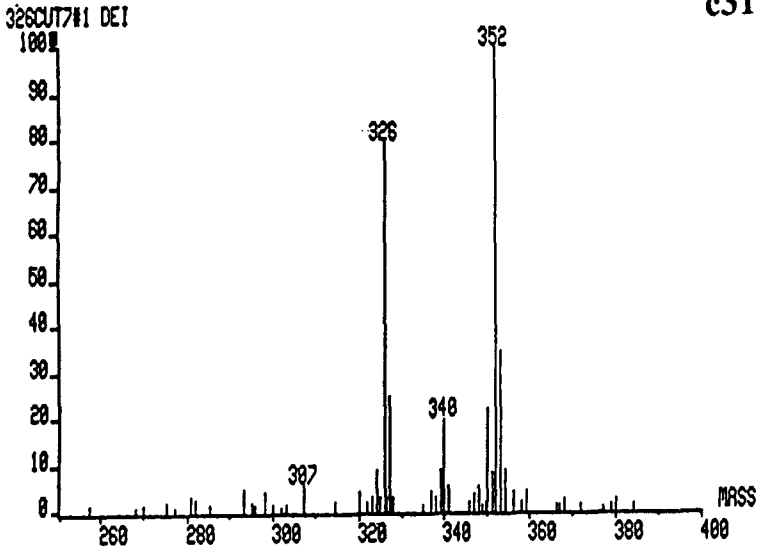
c28



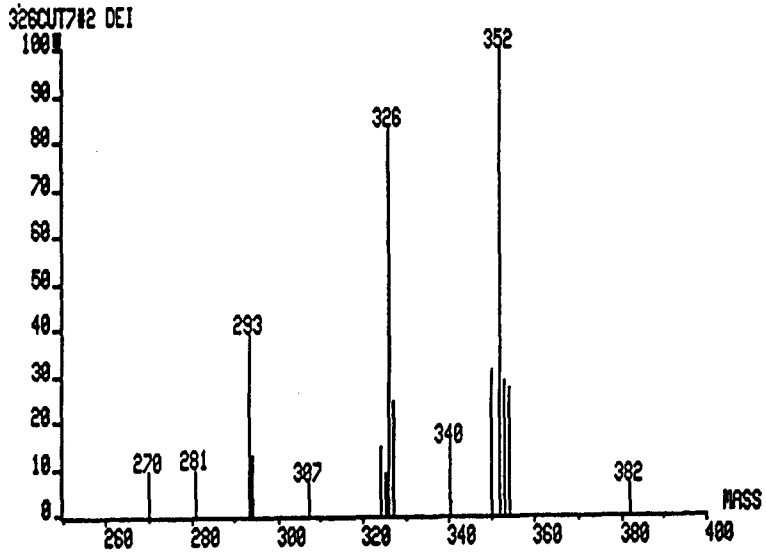
c29



c30

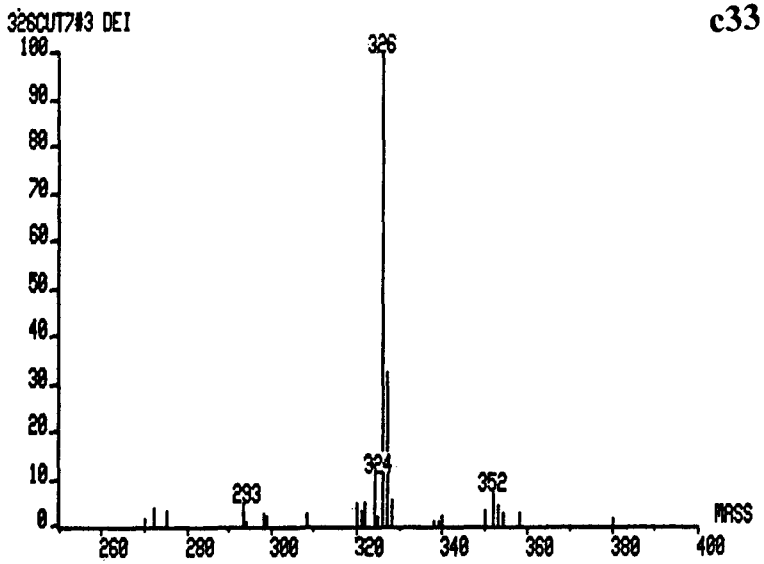


c31

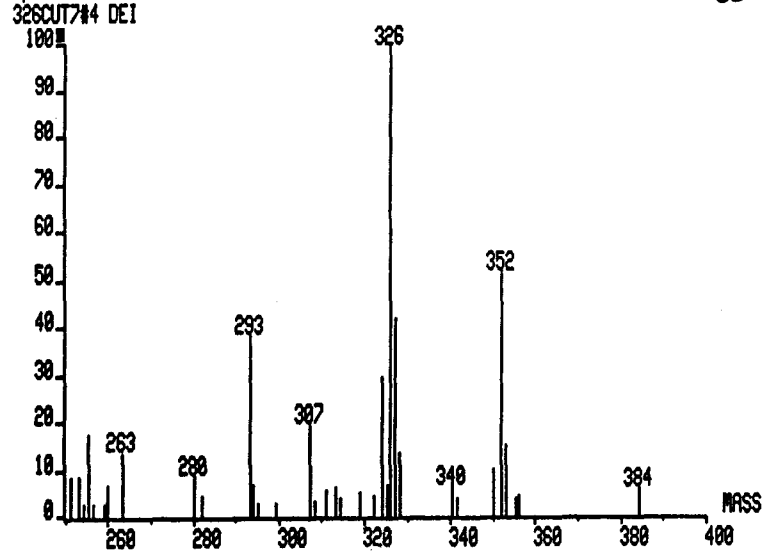


c32

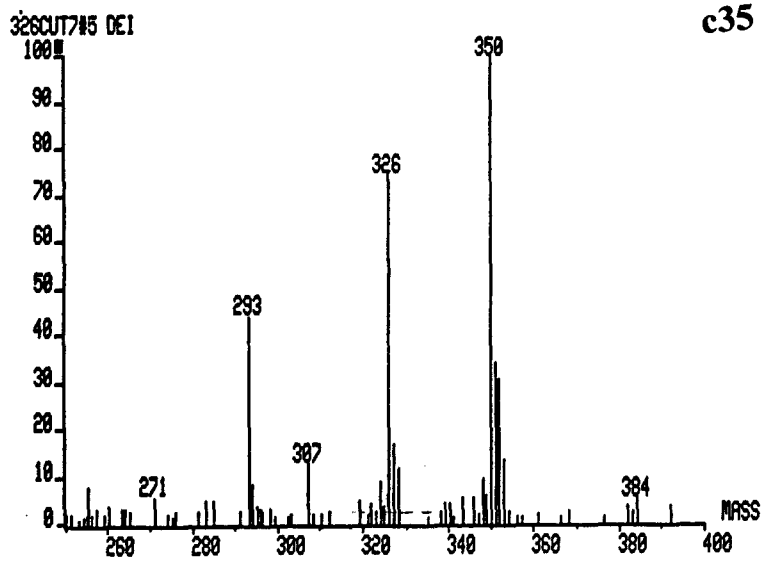
c33


326CUT7#3 DEI
100
90
80
70
60
50
40
30
20
10
0
326
324
293
352
MASS
260
280
300
320
340
360
380
400

c34

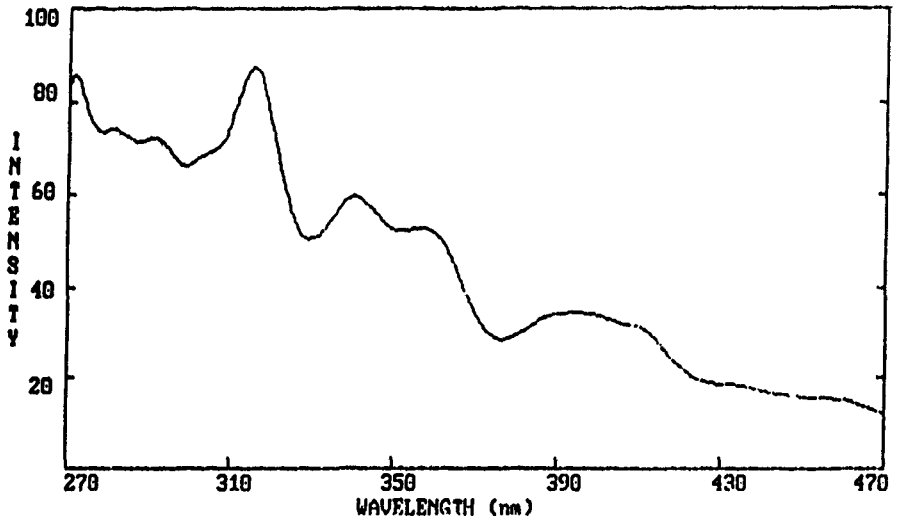

326CUT7#4 DEI
100
90
80
70
60
50
40
30
20
10
0
326
352
293
307
263
280
340
384
MASS
260
280
300
320
340
360
380
400

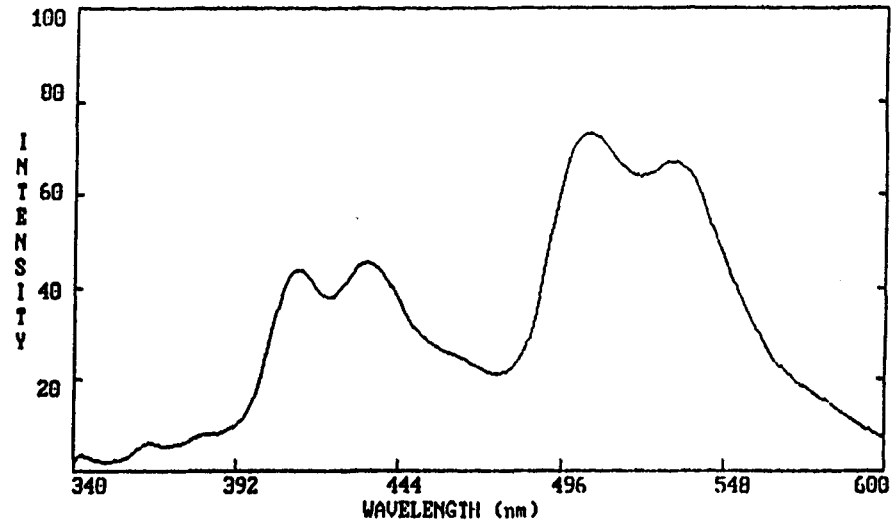
c35


326CUT7#5 DEI
100
90
80
70
60
50
40
30
20
10
0
350
326
293
307
271
384
MASS
260
280
300
320
340
360
380
400

Appendix V

Excitation and Emission Fluorescence spectra of 326 amu PAH compounds



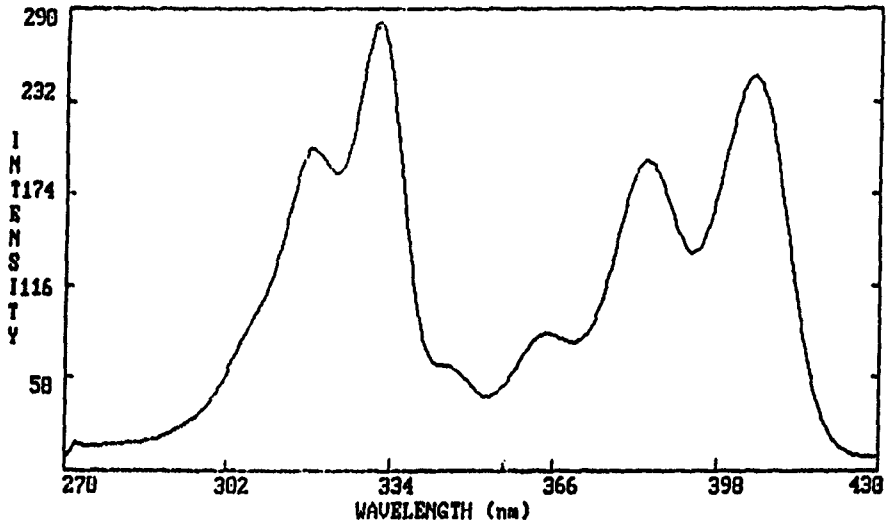


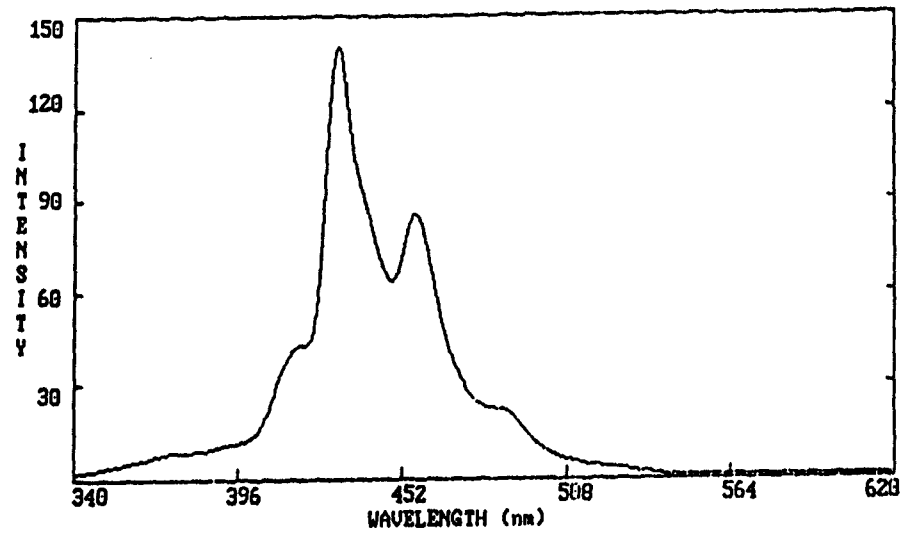
Excitation spectrum of c7

wavelength (nm)	relative intensity
280	84.5
291.5	82.5
316	100
340	68.1
357	60.5
395	39.4

Emission spectrum of c7

wavelength (nm)	relative intensity
365	8.0
380	10.5
412	60.1
434	62.2
504	100
531	91.9



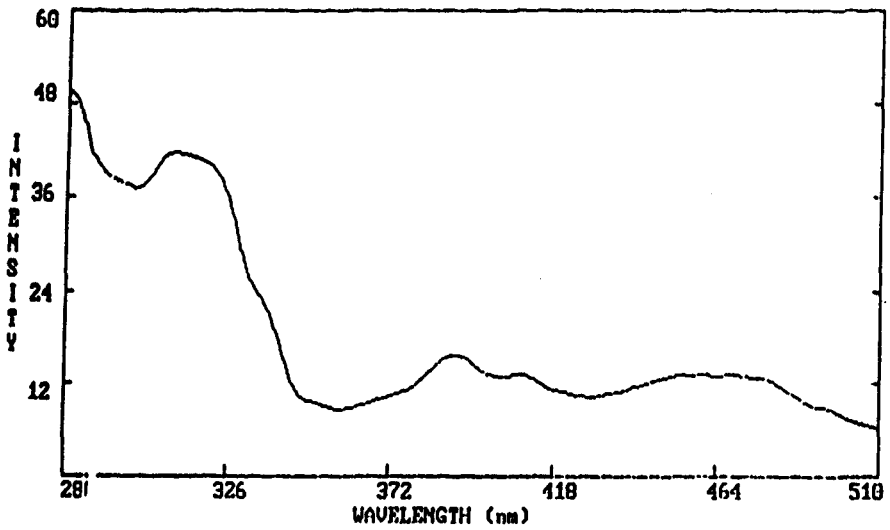


Excitation spectrum of c15

wavelength (nm)	relative intensity
319	72
331.5	100
345.5 (sh)	23
365	30.3
384	69.2
405.5	87.9

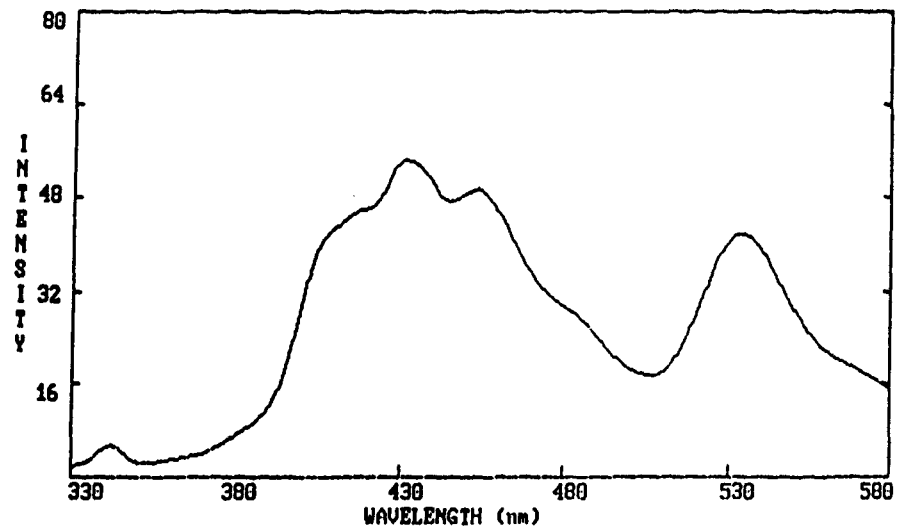
Emission spectrum of c15

wavelength (nm)	relative intensity
416 9 (sh)	30
430.5	100
457	61.2
486.5 (sh)	15.8



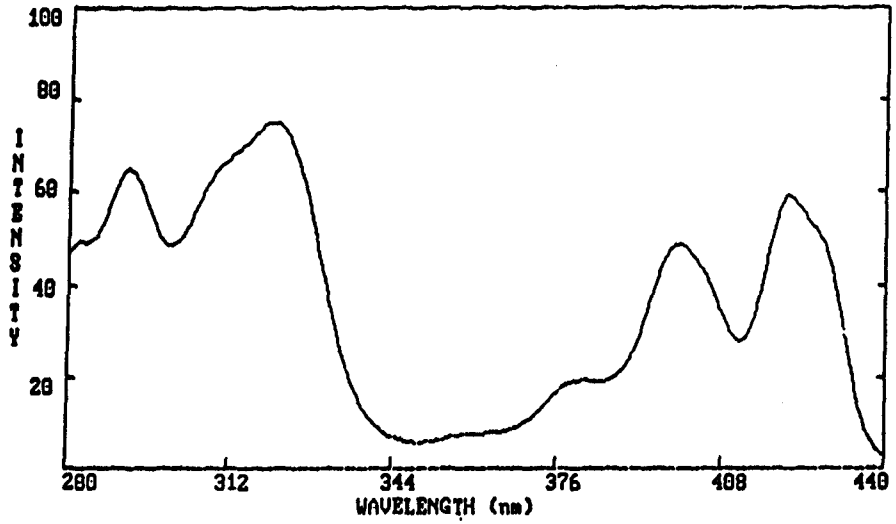
Excitation spectrum of c17

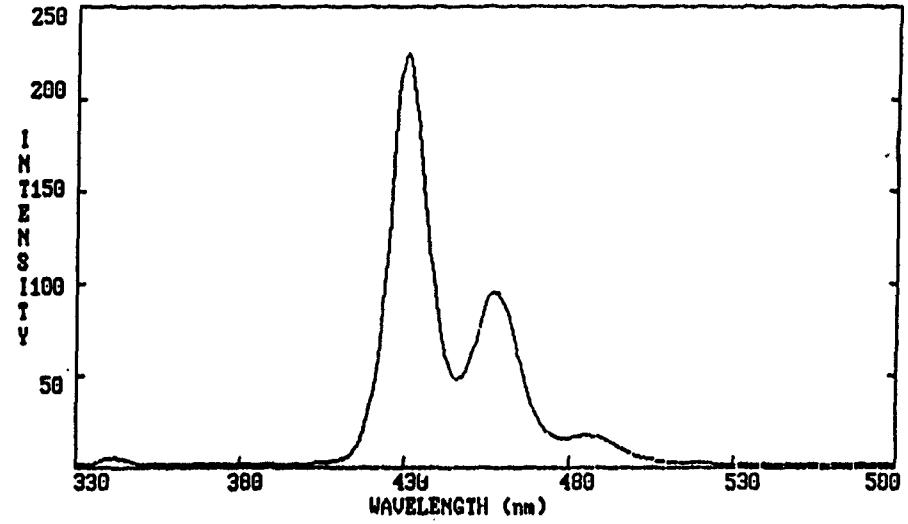
wavelength (nm)	relative intensity
312	100
391	38
408	31.7
459	31.7
469	31.1



Emission spectrum of c17

wavelength (nm)	relative intensity
414.5 (sh)	81
431	100
453	90.8
533	76.6



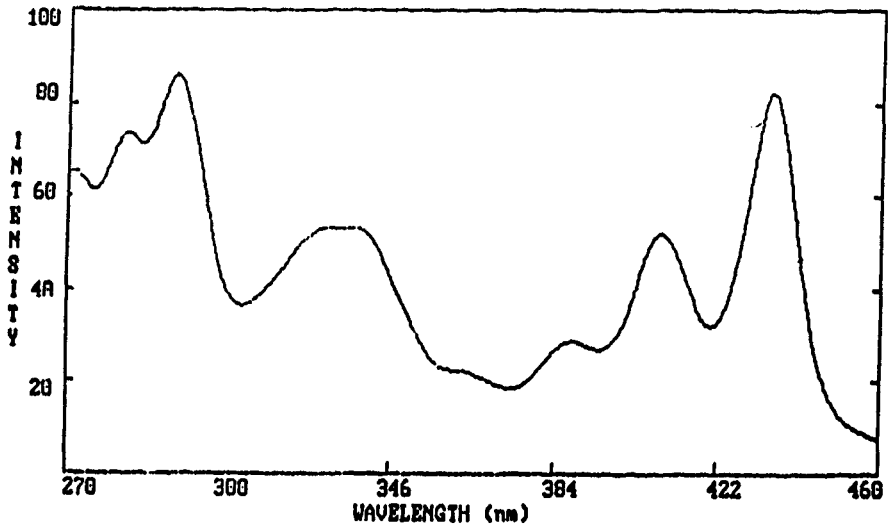


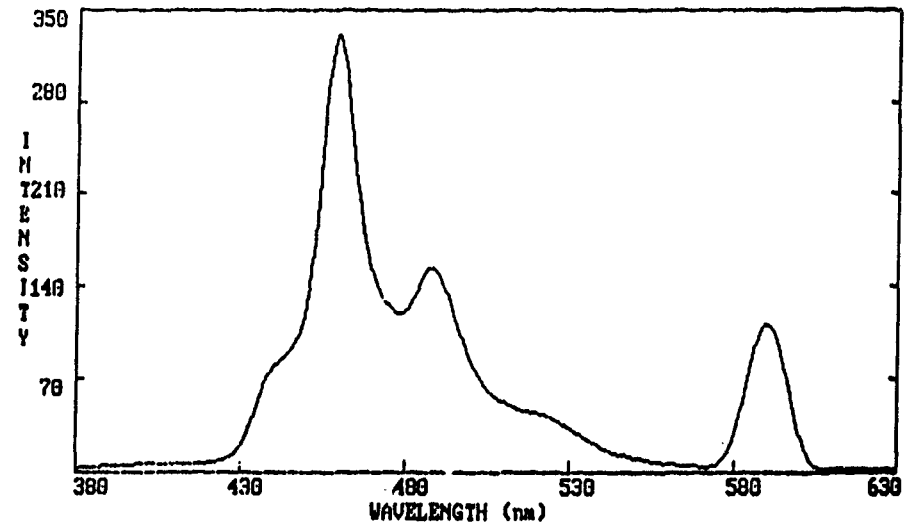
Excitation spectrum of c19

wavelength (nm)	relative intensity
292	86.5
321	100
382	26.1
400	64.8
421	78.9

Emission spectrum of c19

wavelength (nm)	relative intensity
431	100
457	42.8
486	7.8



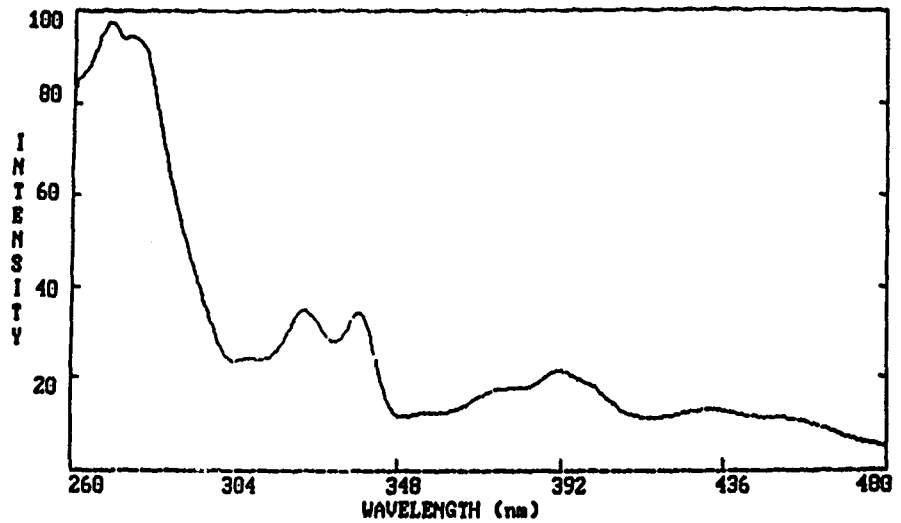


Excitation spectrum of c28

wavelength (nm)	relative intensity
284.5	85.5
295.5	100
332.5	61.9
364.5	25.6
388	33.3
408.5	60.3
434.5	95.5

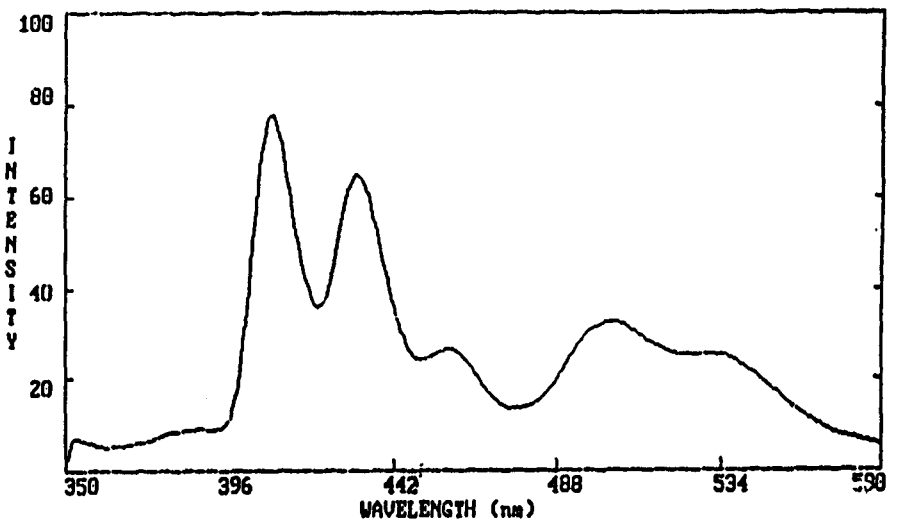
Emission spectrum of c28

wavelength (nm)	relative intensity
440 (sh)	24
459	100
488	46.3
521 (sh)	13
589.5	33.5



Excitation spectrum of c29

wavelength (nm)	relative intensity
270	100
277.5	96
323	35.8
338	35.1
391	22
434	13



Emission spectrum of c29

wavelength (nm)	relative intensity
407.5	100
431.5	83.5
458	34.2
504	41.9
533 (sh)	32.9