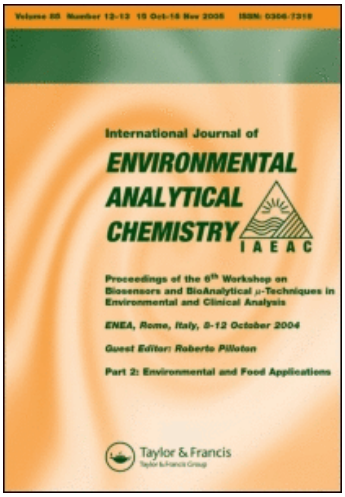

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Isolation and Characterization of Sulfur-Containing Polycyclic Aromatic Compounds (Thia-Arenes) from Complex Environmental Mixtures

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ISOLATION AND CHARACTERIZATION OF SULFUR-CONTAINING POLYCYCLIC AROMATIC COMPOUNDS (THIA-ARENES) FROM COMPLEX ENVIRONMENTAL MIXTURES

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A multi-dimensional chromatographic method was developed and applied to the separation of thia-arenes and polycyclic aromatic hydrocarbons (PAH) derived from coal tar. A thia-arene-rich fraction, prepared using a combination of alumina chromatography and palladium chloride/silica gel chromatography, was further separated using normal phase HPLC to isolate fractions containing thia-arenes with molecular masses ranging from 184 amu to 284 amu. These fractions were analysed using gas chromatography-mass spectrometry; approximately one-half of the thia-arenes in a coal tar extract were retained and separated by the palladium chloride-silica gel step. This methodology has been successfully applied to coal tar, air particulate material, sediments and biological samples.

Keywords: Thia-arenes; polycylic aromatic compounds; sulfur; source apportionment; thiophenes

INTRODUCTION

Sulfur-containing polycyclic aromatic compounds (PAC), or thia-arenes, are present in a variety of matrices including petroleum and coal products, combustion emissions, air particulate material and tobacco smoke[1]. Thia-arenes differ from polycyclic aromatic hydrocarbons (PAH) in that one of the six-membered rings is replaced by a five-membered thiophene ring. Levels of thia-arenes are generally low compared to levels of PAH; the ratio of three ring-PAH (178 amu)

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to three-ring thia-arenes (184 amu) measured in a coal tar standard reference material (SRM 1597) was approximately 25:1 [2] and the ratio of four-ring PAH (228 amu) to four-ring thia-arenes (234 amu) measured in an urban air particulate standard reference material (NIST SRM 1649) was approximately 7:1 [3]. The presence of a five-membered thiophene ring lowers the symmetry of these aromatic systems compared to the corresponding PAH; thus, there is a much larger number of possible thia-arene isomers than PAH isomers. For example, in cata-condensed systems, there are 12 five-ring PAH isomers and 51 five-ring thia-arene isomers [4]. Similarly, there is a greater number of alkylated thia-arene isomers compared to alkylated PAH isomers.

Thia-arenes are produced by the combustion of sulfur-containing fuels such as diesel fuel and coal, two fuels which comprise only a fraction of the sources that produce homocyclic PAH. We have previously demonstrated the utility of thia-arenes as source apportionment tracers in urban air particulate and aquatic sediments [5]. We used the profiles of alkylated dibenzothiophenes to assess the relative contributions of vehicular emissions and industrial emissions in respirable air particulate collected in Hamilton, Ontario. We have also used this methodology to assess suspended and bottom sediments from Hamilton Harbour (western Lake Ontario) for the relative contributions of contamination arising from the manufacturing of steel and from mobile emissions

Our interest in thia-arenes also stems from their potential genotoxicological properties about which relatively little is known [1]. The majority of the genotoxicity of heavily PAH-contaminated environmental samples such as coal tar can be attributed to higher molecular mass homocyclic PAH. Previously, we applied a bioassay-directed fractionation methodology to the analysis of coal tar-contaminated sediment samples from Hamilton Harbour and Sydney Harbour, Nova Scotia [6]. Compounds including benzo[a]pyrene, indeno[1,2,3-cd]pyrene and dibenzo[a,h]anthracene were determined to be primary mutagens in these samples as measured using the *Salmonella*/microsome assay. Despite the effectiveness of our multi-dimensional chromatographic fractionation method, we found it difficult to assess the contribution of thia-arenes to the genotoxicity of these complex mixtures due to their co-elution with PAH and their substantially lower abundances.

To explore the potential of thia-arenes as source apportionment tracers, and to determine the potential of thia-arenes as mutagens and carcinogens, it was necessary to develop an efficient method to separate thia-arenes from their PAH counterparts in source samples and environmental samples. If the isolation of a sulfur-rich fraction or fractions were successful, chemical and biological analyses could be performed without potential complications due the presence of PAH. Unfortunately, the chromatographic properties of thia-arenes are very sim-

ilar to their PAH counterparts. A number of methods for separating sulfur-containing compounds from PAH have been described including those based on an oxidation/reduction sequence [7] and various ligand exchange methods using salts of mercury [8], copper [9], silver [10] and palladium [11,12]. However, these methods are generally less effective for separation of thiophenic compounds than for aliphatic sulfides.

In this paper, we present a multi-dimensional chromatographic method for the separation and analysis of thia-arenes derived from coal tar which utilizes a modification of the methods developed by Nishioka *et al.* [11] and Andersson [12] using palladium chloride-treated silica gel. Initial fractionation of coal tar was carried out using open-column alumina chromatography followed by a ligand-exchange chromatography procedure followed by normal phase (NP)-HPLC.

EXPERIMENTAL

Materials

High purity nitrogen and helium were purchased from Canada Liquid Air Ltd. (Toronto, Ont.). All solvents were HPLC grade or distilled in the laboratory. Distilled water was purified using a Milli-Q Water Purification System (Waters Associates, Milford, MA). Palladium chloride (99%) was purchased from Aldrich.

Instrumentation

Gas chromatography-mass spectrometry (GC-MS) experiments were performed on a Hewlett-Packard Model 5890 Series II gas chromatograph with a Model 5971A mass selective detector (Hewlett-Packard Co., Mississauga, Ont.). Reversed and normal phase HPLC analyses were carried out on a Hewlett-Packard model 1090 liquid chromatograph with a diode-array detector.

Compound class fractionation of coal tar

Neutral alumina (Fisher, Brockman activity I, 80–200 mesh) was activated by heating at 170°C for 48 hrs. Ten grams of coal tar was dissolved in 100 mL dichloromethane and treated with anhydrous magnesium sulfate. The mixture was filtered and added to 50 g neutral alumina and the solvent was evaporated

under reduced pressure which resulted in a free-flowing black material. The coated alumina was slurried in hexane and poured on top of 200 g of fresh alumina packed in an open column (5 cm × 40 cm). Elution of the column with 800 mL hexane afforded fraction Al (0.226 g, 2.26% of original coal tar); 2500 mL of benzene afforded fraction A2 (2.45 g, 24.5% recovery); 2300 mL dichloromethane afforded fraction A3 (0.554 g, 5.54% recovery); and 1540 mL of methanol afforded fraction A4 (2.11 g, 21.1% recovery). The remaining 47% of the mass of the original coal tar was not recovered. The A2 fraction was further fractionated using ligand-exchange chromatography.

Separation of thiaarenes and PAH by ligand-exchange chromatography

The ligand-exchange chromatography was a modification of the procedure of Nishioka *et al.* [11]. Silica gel (100 g, Merck, 230–400 mesh) 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°C overnight and finally activated at 170°C for 24 hrs before use. A sample of silica gel (5 g) impregnated with $PdCl_2$ slurried in hexane/dichloromethane (9:1 v/v) and packed in a glass column (10 mm i.d.) which contained the same solvent. A sample of coal tar fraction A2 (100 mg) was dissolved in dichloromethane (5 mL) to which was added 0.5 g of $PdCl_2$-silica gel. The solvent was removed by evaporation at reduced pressure and the $PdCl_2$-silica gel was suspended in the column packing solvent and the resulting slurry was added to the top of the column. Elution with hexane/dichloromethane (9:1 v/v, 60 mL) afforded fraction A2-P1; elution with dichloromethane (60 mL) afforded fraction A2-P2. Both fractions were reduced in volume to approximately 20 mL by rotary evaporation, transferred into separatory funnels and treated with 1 mL of 0.05 M NaCN solution. On shaking fraction A2-P2 with aqueous NaCN, the dark brown colour disappeared; no colour change was observed when fraction A2-P1 was similarly treated. The organic layers were collected and washed with water (2 × 5 mL), dried over anhydrous magnesium sulfate, evaporated at reduced pressure and then filtered into 10 mL volumetric flasks.

GC-MS analysis

The GC-MS operation conditions were as follows: transfer line temperature, 300°C; helium carrier gas velocity, 30 cm/sec; injection volume, 1 μL in toluene. The following temperature program was used: initial temp., 90°C; 90–300°C at 3°C/min; hold at 300°C for 20 min. The column was a 30 m × 0.25 mm i.d.

DB-17ht column with a 0.15 μm stationary phase film coating (J&W Scientific). An internal standard method employing pyrene-d_{10} and perylene-d_{12} was used for quantitation.

Normal phase HPLC analysis

The HPLC operating conditions were as follows: diode array UV absorption detection over a wavelength range of 230–440 nm; column temperature 40°C; injection volume 100 μL. A Whatman Partisil M9 PAC 4.6 mm i.d. × 25 cm column containing a 5 micron packing (Whatman, Clifton, NJ) at a flow rate of 4.2 mL/min was used with the following gradient elution program: 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% dichloromethame; 70 min, 100% dichloromethame; 75 min, 95% hexane and 5% dichloromethane.

RESULTS AND DISCUSSION

Open column alumina chromatography

The coal tar sample used in the study was a dark black oil obtained from a local steel manufacturer. Recovery of organic material from the alumina column was roughly 50%, based on two replicate samples. These low recoveries were probably due to irreversible adsorption of high molecular weight asphaltenes, phenolics and insoluble tars onto the alumina stationary phase. Each of the four fractions collected from the alumina column was analysed by GC-MS and NP-HPLC using a Whatman PAC (polyaminocyano) column. Figure 1 shows the multi-dimensional chromatographic scheme used in the sample preparation and the weight percentages of organic material in each fraction. Figure 2 shows the NP-HPLC chromatograms from the analysis of the four fractions (A1-A4) prepared using the open-column alumina procedure.

GC-MS analysis of PAH and thia-arenes

The linear retention index system of Lee et al. [13] was adopted for GC-MS analysis; naphthalene (2-rings), phenanthrene (3-rings), chrysene (4-rings), and picene (5-rings) were assigned retention index values of 200, 300, 400 and 500, respectively. Retention indices of compounds eluting before naphthalene were

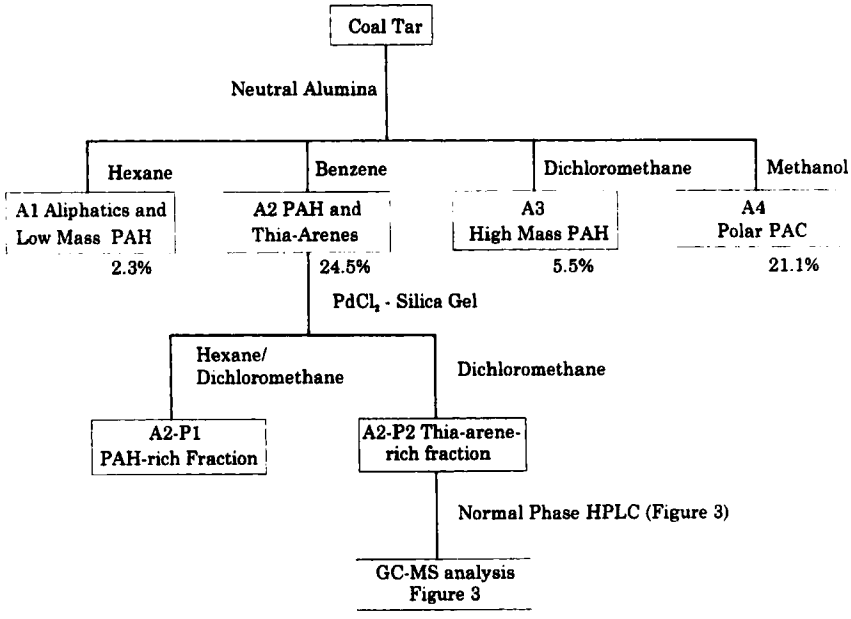

FIGURE 1 Multi-dimensional chromatographic sample preparation scheme showing weight percentages of organic material in each fraction

calculated by linear extrapolation from the naphthalene-phenanthrene interval. In the same manner, indices for compounds with longer retention times than picene were calculated by linear extrapolation from the chrysene-picene interval. Table I lists the compounds identified in fraction A2 of coal tar sample and their concentrations and retention indices on the DB-17ht column. Standard deviations of retention indices for individual PAC based on four injections were less than 0.4. The estimated detection limits (2:1 S/N) for PAC identified in this study were in the range of 3–30 pg injected. We have used a DB-17ht column (50% phenyl methylpolysiloxane) instead of a DB-5 ms column (5% phenyl methylpolysiloxane) because we have found this stationary phase to provide improved separation of PAH and thia-arenes. Mossner and Wise [14] have also found the DB-17 phase to be superior for most thia-arene separations. An example of the advantage of the DB-17 phase is the separation of dibenzothiophene and naphtho[1,2-b]thiophene (retention index values of 295.68 and 297.09, respectively); these compounds were poorly resolved on the DB-5 column (retention index values of 295.72 and 295.55, respectively). Mossner et al. have also reported gc retention indices for thia-arenes on different stationary phases [15].

TABLE I Quantitation of polycyclic aromatic hydrocarbons and thia-arenes in coal tar fractions A2, A2-P1 and A2-P2

Mol Wt.	*Compound*	*Frac. A2 (μg/g)*	*Frac. A2-P1 (μg/g)*	*Frac. A2-P2 (μg/g)*	*Retention Index*
Polycyclic Aromatic Hydrocarbons					
142	2-methylnaphthalene	1,400	1,210	1.5	216.99
142	1-methylnaphthalene	574	496	0.5	221.14
154	Biphenyl	820	707	0.5	234.06
152	Acenaphthylene	4430	3230	36.8	248.56
154	Acenaphthene	267	238	ND	252.67
166	Fluorene	6240	5580	4.9	267.70
178	Phenanthrene	32,900	32,200	24.5	**300**
178	Anthracene	8,080	7,900	17.2	300.61 ± 0.15
202	Fluoranthene	28,200	26,400	27.0	343.28 ± 0.08
202	Pyrene	21,900	19,700	19.6	352.89 ± 0.07
216	Benzo[a]fluorene	1,790	1,570	1.8	363.95 ± 0.29
216	Benzo[b]fluorene	1,550	1,340	3.1	366.03 ± 0.27
226	Benzo[ghi]fluoranthene	1,240	1,100	6.1	389.80 ± 0.12
228	Benzo[c]phenanthrene	687	614	2.8	391.57 ± 0.19
228	Benz[a]anthracene	8,890	7,550	14.7	396.98 ± 0.18
226	Cyclopenta[cd]pyrene	385	15	24.5	398.54 ± 0.15
228	Chrysene	8,430	7,220	14.7	**400**
252	Benzo[b]fluoranthene	4,010	3,610	4.9	440.29 ± 0.19
252	Benzo[k]fluoranthene	2,710	2,510	4.9	441.31 ± 0.02

Mol Wt.	*Compound*	*Frac. A2 (μg/g)*	*Frac. A2-P1 (μg/g)*	*Frac. A2-P2 (μg/g)*	*Retention Index*
252	Benzo[j]fluoranthene	2,340	2,180	4.9	442.80 ± 0.09
252	Benzo[e]pyrene	3,160	3,000	1.3	453.62 ± 0.12
252	Benzo[a]pyrene	5,750	5,120	13.4	455.31 ± 0.14
252	Perylene	1,570	1,360	5.4	460.05 ± 0.12
276	Indeno[1,2,3-cd]pyrene	3,660	3,350	7.4	492.28 ± 0.18
278	Dibenz[a,c]anthracene	587	638	ND	492.86 ± 0.19
278	Picene	429	334	4.9	**500**
276	Benzo[ghi]perylene	3,580	3,270	7.4	502.26 ± 0.33
300	Coronene	601	223	ND	549.86
302	Dibenzo[a,e]pyrene	466	294	63.8	549.33
302	Dibenzo[a,i]pyrene	734	378	81.0	556.55
302	Dibenzo[a,h]pyrene	761	533	191	560.91
Total	**PAH**	**158,400**	**144,000**	**591**	
Thia-Arenes					
184	Dibenzothiophene	1,300	753	344	295.68 ± 0.10
184	Naphtho[1,2-b]thiophene	530	474	52.3	297.09 ± 0.15
184	Naphtho[2,1-b]thiophene	337	299	2.6	302.59 ± 0.14
184	Naphtho[2,3-b]thiophene	157	3	106	307.22 ± 0.35
208	Phenanthro[4,5-bcd]thiophene	560	160	368	351.19 ± 0.13
208	Phenaleno[6,7-bc]thiophene	169	ND	1.2	357.87 ± 0.27
234	Benzo[b]naphtho[2,1-d]thiophene	1,020	856	49.1	388.26 ± 0.19

Mol Wt.	*Compound*	*Frac. A2 (μg/g)*	*Frac. A2-P1 (μg/g)*	*Frac. A2-P2 (μg/g)*	*Retention Index*
234	Benzo[b]naphtho[1,2-d]thiophene	199	140	24.5	393.03 ± 0.22
234	Benzo[b]naphtho[2,3-d]thiophene	379	ND	295	394.87 ± 0.16
234	Phenanthro[1,2-b]thiophene	96	69	ND	397.51 ± 0.27
234	Anthra[2,1-b]thiophene	42	29	ND	400.13 ± 0.22
234	Phenanthro[2,1-b]thiophene	60	ND	22.1	403.38 ± 0.21
234	Phenanthro[2,3-b]thiophene	36	ND	34.4	403.97 ± 0.28
258	258 amu thia-arene	24	15	0.8	439.05 ± 0.10
258	258 amu thia-arene	24	17	0.7	439.95 ± 0.21
258	Benzo[2,3-phenanthro][4,5-bcd] thiophene	139	83	29.4	443.48 ± 0.13
258	Triphenyleno[4,5-bcd]thiophene	84	ND	47.1	450.05 ± 0.36
258	Chryseno[4,5-bcd]thiophene	157	12	100	452.66 ± 0.25
258	258 amu thia-arene	72	39	ND	454.78 ± 0.23
258	258 amu thia-arene	48	49	ND	459.08 ± 0.13
284	Dinaphtho[1,2-b:1′2′-d]thiophene	60	27	1.1	485.14 ± 0.34
284	Benzo[b]phenanthro[3,4-d]thiophene	42	20	ND	485.98 ± 0.32
284	Dinaphtho[1,2-b:2′,3′-d]thiophene	48	7	30.9	487.64 ± 0.28
284	Benzo[b]phenanthro[3,2-d]thiophene	108	20	127	488.16 ± 0.33
284	284 amu thia-arene	36	ND	35.8	492.96 ± 0.33
Total	**Thia-Arenes**	**5,727**	**3,072**	**1,672**	

ND denotes not detected

Both full scan and selected ion monitoring (SIM) were used in the characterization and identification of PAC; SIM was used for quantitative analyses. Fragmentation of thia-arenes yielded a prominent peak corresponding to the loss of sulfur (M-32) and often exhibited loss of a CHS unit (M-45). As in the mass spectra of PAH, unsubstituted thia-arenes exhibited intense molecular ions and weak fragment ions corresponding to elimination of hydrogen (M-1, M-2) and a C_2H_2 moiety (M-26). The (M-2) ion peak was generally more intense than the (M-1) ion peak. Doubly-charged ions were also frequently observed in these spectra. Spectra of monomethyl-substituted PAH and thia-arenes were dominated by the (M-1) peak resulting in a one mass unit shift of the fragment ions observed in spectra of the parent compounds. The (M-1) peaks were much more intense than the (M-2) peaks in spectra of methyl-substituted derivatives.

Separation of PAH and thia-arenes by ligand-exchange chromatography

The basis for separation of thia-arenes by ligand exchange chromatography lies in the thiophilic nature of heavy transition metal salts. When a thia-arene containing mixture is applied to a chromatographic support containing a thiophilic transition metal salt, it is expected that the thia-arenes will be retained more strongly by the adsorbent. Following elution of the non-thia-arene components, the solvent strength is increased to elute the more strongly adsorbed thia-arenes. We chose to examine the $PDCl_2$-based method of Nishioka *et al.* [11] because it appeared to offer the best separation of thia-arenes from PAH. In our hands, the solvent elution protocol of Nishioka *et al.* [11] did not result in satisfactory separation of PAH and thia-arenes. We investigated a variety of solvent systems and optimum separation of PAH and thia-arenes was achieved by eluting the $PdCl_2$-silica gel column with 1:9 dichloromethane/hexane to afford a PAH-rich fraction (fraction A2-P1) followed by dichloromethane to afford a thia-arene-rich fraction (fraction A2-P2). Fraction A2-P1 typically contained approximately 70% of the mass of the parent A2 fraction (corresponding to 18% of coal tar) while fraction A2-P2 typically contained approximately 15% of the mass of the A2 fraction (4% of coal tar), the remainder could be eluted with 10% methanol in dichloromethane. Analysis of fractions A2, A2-P1 and A2-P2 was accomplished using GC-MS. However, prior to analysis, any residual Pd complexes needed to be destroyed. The method of Nishioka *et al.* [11] required treatment of the column effluent with diethylamine to destroy any Pd complexes prior to analysis. We were concerned about treatment with diethylamine and possible carry-over of this basic substance into the GC analyses. Thus, we developed an alternative treatment with aqueous cyanide to destroy the Pd/thia-arene complexes; the resulting $Pd(CN)_4^{2-}$ complex would partition into the aqueous phase thereby

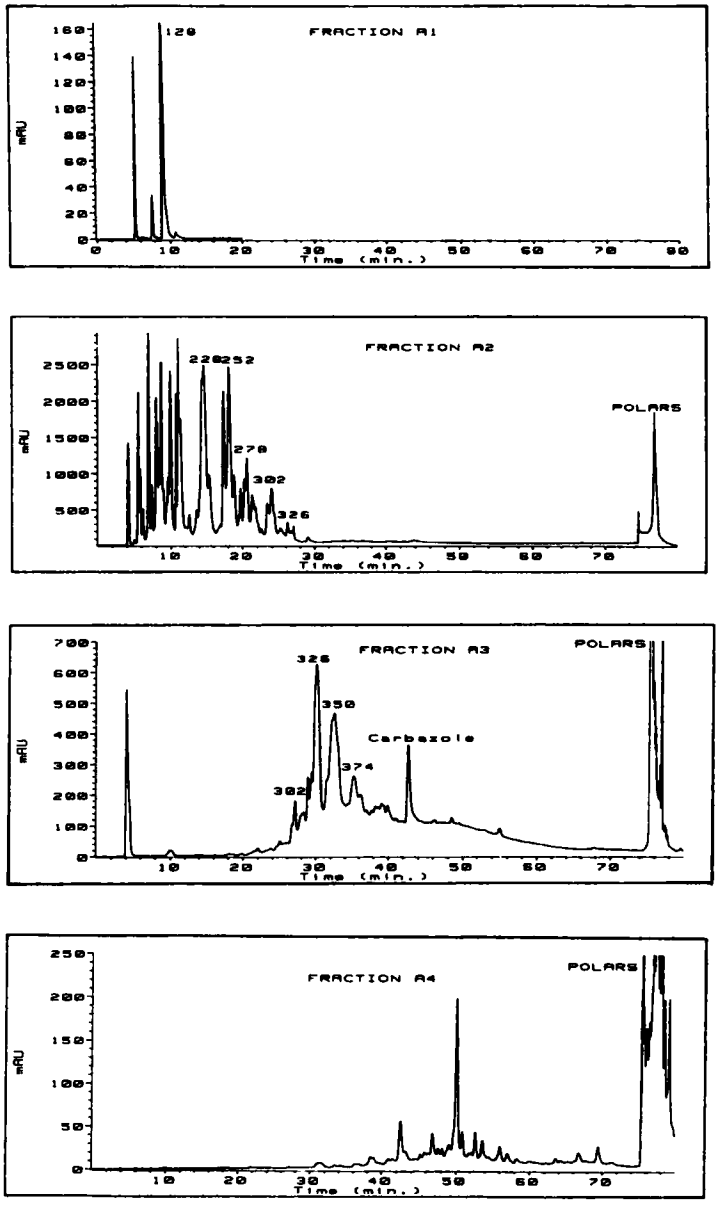

FIGURE 2 Normal phase HPLC chromatograms of coal tar fractions A1-A4 prepared using open-column alumina chromatography. Peak numbers correspond to the molecular masses of PAH in each fraction

avoiding subjecting the GC column to exposure to Pd complex or strong basic conditions.

Table I shows the quantitative data for PAH and thia-arenes in fractions A2, A2-P1 and A2-P2. In general, the percentage recovery of each PAH eluting in fraction A2-P1 and the overall recovery from the $PdCl_2$/silica gel column exceeded 85%; six of thirty-one PAH with poorer recoveries were either reactive PAH or had molecular masses exceeding 278 amu, in particular three PAH with molecular masses of 302 amu (dibenzo[a,e]pyrene, dibenzo[a,i]pyrene and dibenzo[a,h]pyrene). Thia-arenes constituted only 3.6% of fraction A2 while PAH constituted the rest.

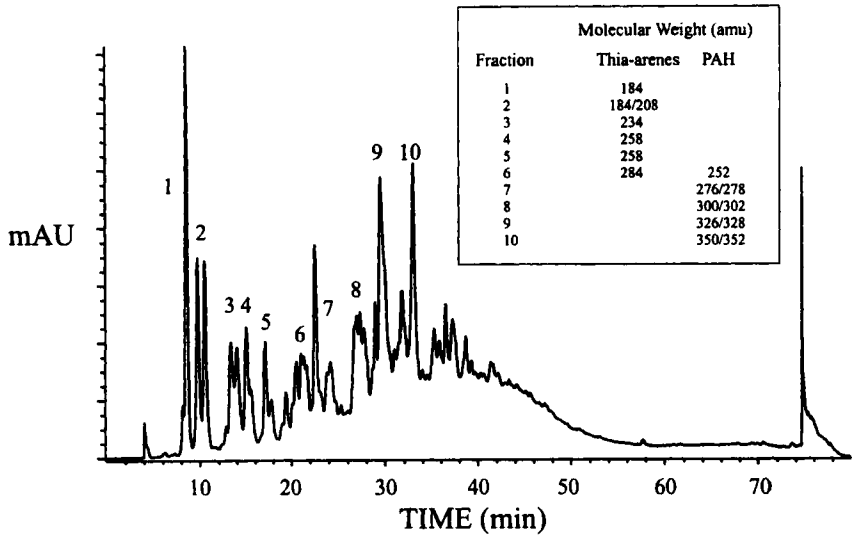

FIGURE 3 Normal phase HPLC chromatogram of coal tar fraction A2-P2 showing the molecular masses of the principal thia-arenes and PAH in the numbered fractions collected during analysis. Data in the inset were derived from GC-MS analyses of fractions 1–8 (Figure 4)

The retention characteristics of thia-arenes in fraction A2 on the $PdCl_2$/silica gel column differed significantly from those of PAH and recoveries from the column were satisfactory for most thia-arenes (>70%). The recovery of phenalo[6,7-bc]thiophene from the column was very low (0.7%) and the recoveries of five others were in the range of 37%-57% (Table I). We attribute these low recoveries to incomplete elution from the column. Of the 25 thia-arenes in fraction A2, 12 were not retained by the $PdCl_2$ column and eluted primarily in fraction A2-P1, 9 were more strongly retained by the $PdCl_2$ column and eluted primarily in fraction A2-P2, 3 eluted in both fractions and one compound was

not recovered in either fraction (Table I). It has been reported that thia-arenes with terminal thiophene rings are poorly retained by $PdCl_2$/silica adsorbents while compounds with internal thiophene rings are more strongly adsorbed [12]. In this study, the $PdCl_2$ column did not clearly discriminate between terminal-thiophene and internal-thiophene compounds.

Normal phase HPLC separation of the thia-arene fraction

The $PdCl_2$-silica gel chromatographic method was partially successful in separating the entire suite of thia-arenes from PAH. On average, greater than 85% of the PAH eluted in fraction A1-P1; however, the total PAH content of the parent A2 fraction was roughly thirty-fold higher than the total thia-arene content (Table I). Thus, PAH which eluted in the thia-arene fraction (A2-P2), which were primarily compounds of molecular mass 302, represented only 0.08% to 1.1% of the total PAH in fraction A2 but were significant compared to the individual thia-arene concentrations.

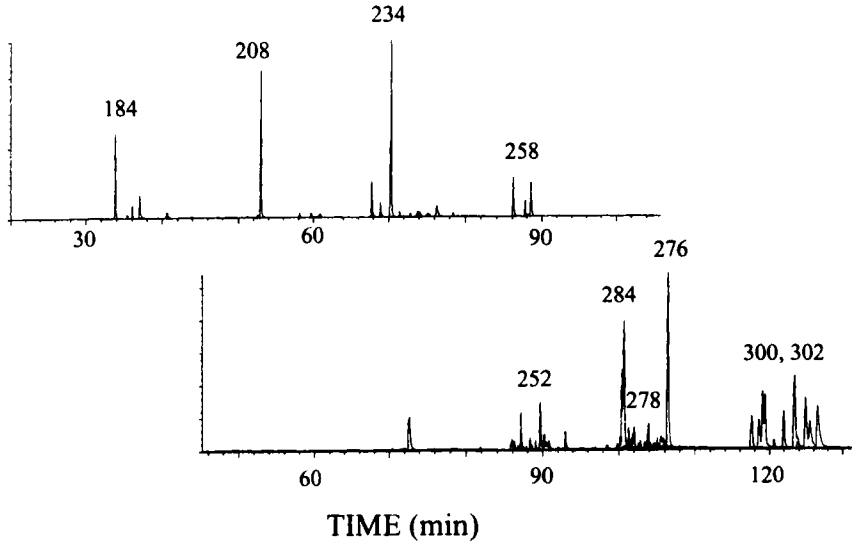

FIGURE 4 Superposition of total ion chromatograms from GC-MS analyses of the numbered fractions collected during normal phase HPLC analysis of fraction A2-P2. The top panel represents GC-MS analyses of fractions 1–5; the bottom panel represents GC-MS analyses of fractions 6–8. The numbers above the peaks represent molecular masses

Normal phase HPLC has been very useful in our hands for the separation of PAH mixtures into individual benzologue classes [6]. Since most of the

thia-arenes we identified in this study had molecular masses of 284 amu and less, we decided to use this approach. To separate thia-arenes from PAH, fraction A2-P2 was subjected to NP-HPLC and fractions corresponding to the numbered chromatographic peaks (Figure 3) were collected and analysed by GC-MS. These data showed that thia-arenes with molecular masses ranging from 184 amu to 258 amu were isolated in fractions eluting before 22 min. Fractions eluting after 22 min exhibited some co-elution of thia-arenes and PAH. Figure 4 shows the superposition of the GC-MS analyses of fractions 1–5 (top panel) and fractions 6–8 (bottom panel). Fractions corresponding to peaks 9 and 10 in NP-HPLC contained PAHs of molecular masses 326 amu, 328 amu, 350 amu and 352 amu. Subfractions 9 and 10 were not amenable to analysis by GC-MS due to the low volatilities of these compounds; the masses of these compounds were determined using probe EI-MS.

Acknowledgements

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