(12) Goldberg, I. B.; Parry, E. P. *J. Electroanal. Chem.* **1974**, *54*, 427.
(13) Casalbore, G.; Di Marco, P. G.; Giro, G.; Mastragostino, M. *J. Electroanal. Chem.* **1980**, *111*, 369.
(14) Kaneko, N.; Nezu, H.; Ooka, K. *Denki Kagaku* **1981**, *49*, 302.
(15) Beevers, J. R.; Breyer, B. *J. Electroanal. Chem.* **1959/60**, *1*, 43.
(16) Kolthoff, I. M.; Lingane, J. J. "Polarography"; Interscience: New York, 1952; Vol. 2, p 442.
(17) Tanaka, K.; Morinaga, K.; Nakano, K. *Nippon Kagaku Zasshi* **1962**, *90*, 478.
(18) Toropova, V. F.; Vekslina, V. A.; Chovnyk, N. G. *Zh. Anal. Khim.* **1971**, *26*, 2132.
(19) Kaneko, H.; Ozawa, T. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 140.
(20) Blazek, A.; Koryta, J. *Collect. Czech. Chem. Commun.* **1953**, *18*, 326.
(21) Toropova, V. F.; Zabbarova, R. S. *Zh. Anal. Khim.* **1970**, *25*, 1059.
(22) Kaneno, H.; Kaneko, K. *Bunseki Kagaku* **1979**, *28*, 727.
(23) Pecsok, R. L.; Maverick, E. F. *J. Am. Chem. Soc.* **1954**, *76*, 358.
(24) Osteryoung, J.; Hasebe, K. *Rev. Polarogr.* **1976**, *22*, 1.
(25) Hasebe, K. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1056.
(26) Skogerboe, R. K.; Grant, C. L. *Spectrosc. Lett.* **1970**, *3*, 215.
(27) Yoe, J. H.; Armstrong, A. R. *Anal. Chem.* **1947**, *19*, 100.
(28) Nichols, D. N. R. *Analyst (London)* **1960**, *85*, 452.
(29) Easton, A. J.; Greenland, L. *Anal. Chim. Acta* **1963**, *29*, 52.

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Determination of Nitrated Polynuclear Aromatic Hydrocarbons in Particulate Extracts by Capillary Column Gas Chromatography with Nitrogen Selective Detection

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The highly complex matrix of a diesel particulate extract was analyzed for nitrated polynuclear aromatic hydrocarbons (nitro-PAH) by use of fused-silica capillary column GC/thermionic nitrogen–phosphorus (GC/NPD) analysis of HPLC fractions. These samples were found to contain at least 100 nitro-PAH. Positive isomer identification for 17 nitro-PAH has been made utilizing the GC retention times of authentic standards and low- and high-resolution mass spectra as criteria. An additional 45 nitro-PAH were tentatively identified by using one or more of these techniques. Quantitative GC/MS analysis of 1-nitropyrene, 1,3-dinitropyrene, 1,6-dinitropyrene, and 1,8-dinitropyrene was facilitated by the use of perdeuterated analogues of these compounds as internal standards. Detection limits by the GC/NPD method range between 0.2 and 0.5 ppm for the HPLC fractionated samples.

Interest in nitrated PAH (nitro-PAH) has grown since 1978 when Pitts et al. demonstrated that PAH react with oxides of nitrogen (NO_2) to form nitro-PAH (*1*). Some of these derivatives such as 1-nitropyrene and the dinitropyrenes have been shown to be potent direct acting mutagens in Ames assays using *Salmonella typhimurium* strains (*1–3*).

The soluble organic fractions (SOF) of environmental particulate samples are extremely complex chemical mixtures. Screening studies with high-resolution mass spectrometry (HRMS) (*4*, *5*), mass spectrometry/mass spectrometry (MS/MS) (*4*), and gas chromatography/mass spectrometry (GC/MS) (*4*, *6*) have shown that a large number of nitro-PAH may be present in diesel particulate extracts. However, only a few specific isomers of nitro-PAH including 9-nitroanthracene, 1-nitropyrene, 2-nitrofluorene, 3-nitrofluoranthene, 6-nitrobenzo[*a*]pyrene, and 7-nitrobenz[*a*]anthracene have been identified in particulates collected from ambient air, internal combustion and diesel engines, and aluminum smelter particulates (*7–20*). Oehme (*13*), Ramdahl (*14*), and Nielsen (*15*) have used the GC/NPD technique for the determination of nitro-PAH in environmental particulates but only the most abundant nitro-PAH species were identified. Schuetzle (*4*) and Newton (*6*) have used GC/MS to show the presence of several nitro-PAH whose extract isomeric structure was not determined.

Because there are potentially a large number of nitro-PAH compounds in these samples, an analytical technique which has a high degree of specificity and resolution is required for the definitive analysis of specific isomers. The two techniques which fulfill those requirements are high-resolution fused-silica capillary column chromatography combined with thermionic nitrogen–phosphorus (GC/NPD) and GC/MS detection.

The purpose of this work was to develop and validate GC/NPD techniques for the idenitfication of a large number of specific nitro-PAH isomers in particulate extracts. We have chosen for this study the rigorous analysis of a diesel particulate extract. Results obtained by the GC/NPD technique are confirmed by HPLC retention times and by GC/MS with acquisition of low- and high-resolution mass spectral data.

EXPERIMENTAL SECTION

Sample Collection. A light-duty diesel particulate sample was collected from a dilution tube and extracted with dichloromethane (DCM) as previously described (*21*).

HPLC Fractionation. The high-performance liquid chromatography (HPLC) fractionation scheme used in this study was slightly modified from that previously reported (*10*) in order to elute the moderately polar fraction with a greater degree of resolution. A normal-phase semipreparative 7.8 mm × 30 cm column packed with 10-μm μPorasil (Waters Associates) was used for the fractionation. Nominal sample masses loaded onto the column were 15 mg. The solvent flow was 4.5 mL/min. The

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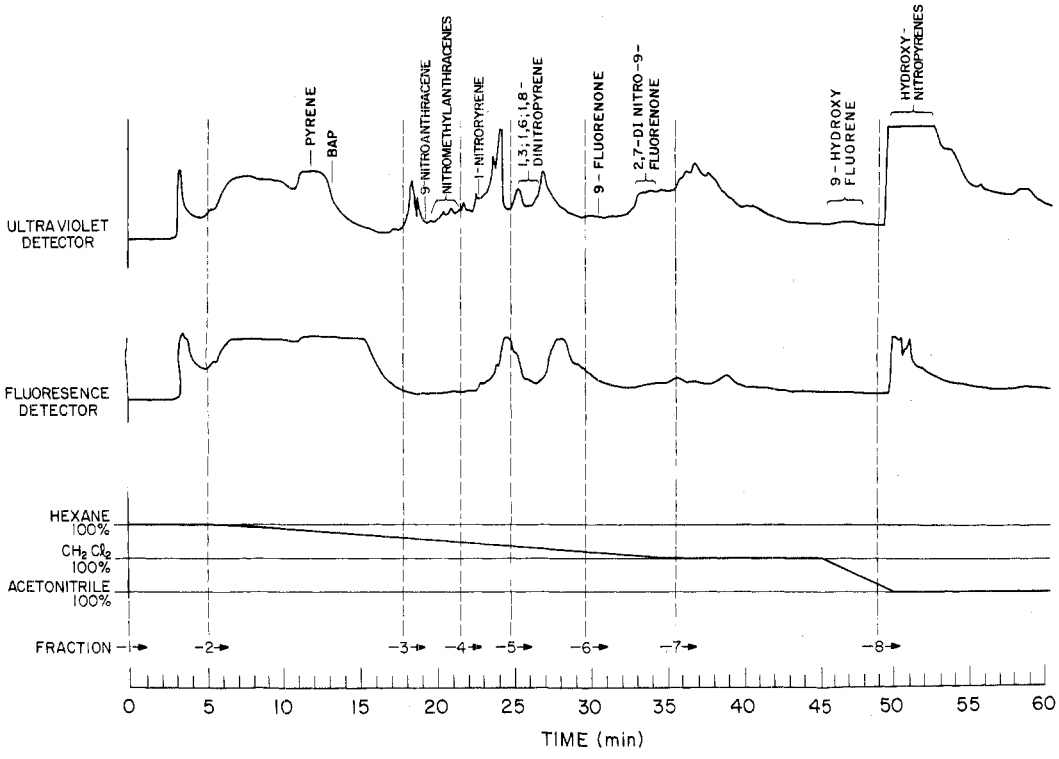

Figure 1. HPLC chromatogram for a light-duty diesel particulate extract. The elution times for some standards are shown.

programming scheme started with 100% hexane for 5 min, a linear gradient of 1% DCM/min for 5 min, a linear gradient of 4% DCM/min to 100% DCM for 25 min, held at 100% DCM for 10 min, a linear gradient of 20%/min acetonitrile to 100% acetonitrile for 15 min, and then step changed to 100% DCM and 100% hexane which were held for 10 min each. The fractions were collected during the HPLC run with an automatic collector (Valco AH12). A representative chromatogram, monitored by UV (λ = 254 nm) and fluorescence detectors (λ_{ex} = 254 nm, λ_{em} = 320 nm), is shown in Figure 1. The volume of solvent in these fractions was evaporated at reduced pressure under yellow-filtered light to minimize sample photooxidation. The concentrated samples were transferred to tared vials, blown down to dryness with dry nitrogen, and weighed after an equilibration period. Afterward, the weighed fractions were stored as DCM solutions in a freezer at −80 °C.

Gas Chromatographic/Nitrogen–Phosphorus Selective Detection. A Hewlett-Packard Model 5880A gas chromatograph equipped with a nitrogen–phosphorus detector (GC/NPD) and a Grob-type split/splitless injection system was used for the analysis. The HPLC fractionated DCM solutions were blown down with dry nitrogen and redissolved in toluene. The samples were analyzed on a 30 m × 0.25 mm fused-silica capillary DB-5 bonded-phase column (J and W Scientific) with a film thickness of 0.25 μm. The column was operated with a temperature profile initially held at 120 °C for 1 min followed by a temperature program of 8 °C/min to 310 °C and held for 8 min at 310 °C. The linear velocity of helium carrier gas was 26 cm/s. Samples were introduced in the splitless mode on the injector operating at 275 °C with the solvent vent closed for a period of 1.5 min after injection. The nitrogen–phosphorus detector was operated at 310 °C under 4.4 cm^3/min hydrogen, 110 cm^3/min air, and 22 cm^3/min helium auxiliary flow. The detector was maintained at 19–22 V. Standards were prepared to give concentrations that were approximately 2–3 μg/mL in toluene. The concentration of the HPLC fractions ranged from 0.5 to 1.0 μg/μL. The sample injection volume was ≈2 μL. A simple BASIC program on the level 4 integrator terminal was used to calculate retention times relative to a perdeuterated 1-nitropyrene internal standard following each chromatographic analysis.

Gas Chromatography/Mass Spectrometry. GC/MS analysis was performed in the EI mode on a Vacuum Generators ZAB-2F mass spectrometer interfaced directly to a Hewlett-Packard 5720A gas chromatograph. The interface temperature was 270 °C. Sample introduction into the DB-5 column was accomplished with a J and W fixed position on-column injector. A 1-μL aliquot was injected at room temperature; the GC column was then raised to 150 °C and programmed at 4 °C/min to 285 °C. The digital sampling rate of the Finnigan MAT Series 2000 computer was 66.7 kHz. During GC/MS data acquisition, perfluorokerosene was bled into the mass spectrometer to obtain high accuracy mass assignments at approximately 10 000 mass resolution. Mass deviations of between than ±1 mmu were obtained. For instance a 0.1 mmu deviation was obtained for nitroanthracene, accurate mass 223.063. For high sensitivity, low resolution (approximately 1000 mass resolution) analysis, the digital sampling rate was set to 40 kHz.

Source of Standards. Several compounds were synthesized and purified by HPLC, and the isomer structure was verified by using NMR and GC/MS. The synthesis procedures for these compounds were as follows:

1-Nitrofluoranthene. 1-Nitrofluoranthene was synthesized by the method of Campbell and Wilshire (22) and was purified by chromatography on neutral alumina by using hexane:benzene (1:1) as the eluant, followed by recrystallization from benzene:ethanol. The physical and spectroscopic data for this compound were as follows: mp 152–153 °C (lit. value (22) mp 151–153 °C); UV_{max} (95% C_2H_5OH), 251 nm (ϵ 12 000); NMR ($CDCl_3$), δ 8.49 (dd, 1, J(9,10) = 6.5 Hz, J(8,10) = 1.5 Hz, $C_{10}H$), δ 8.20 (d, 1, J(2,3) = 9 Hz, C_2H); δ 7.98–7.78 (m, 4, C_3H, C_4H, C_6H, and C_7H); δ 7.76–7.66 (dd, 1, J(4,5) = 8 Hz, J (5,6) = 7 Hz, C_5H); δ 7.52–7.38 (m, 2, C_8H and C_9H).

GC analysis of the 1-nitrofluoranthene and other isomers of nitrofluoranthene that follow was undertaken by using a Varian 3700 GC equipped with FID detector. A 50 m × 0.1 mm i.d. SP-2100 WCOT fused silica column was utilized with a temperature program of 180–270 °C at 4 °C/min. The retention order was 1-, 7-, 2-, 3-, and 8-nitrofluoranthene. The GC analysis indicated a purity of >99.5% for each isomer.

2-Nitrofluoranthene. 2-Nitrofluoranthene was synthesized by the method of Kloetzel et al. (23) and was purified by chromatography on neutral alumina using hexane:benzene as the eluant and was recrystallized from benzene:ethanol giving yellow needles. The physical and spectroscopic data for this compound were as follows: mp 156–157.5 °C (lit. value (23) mp 153–153.5 °C); UV_{max} (95% C_2H_5OH, 258 nm) (ϵ 38 000); NMR ($CDCl_3$) δ 8.82 (d, 1, J(1,3) = 2 Hz, C_1H), δ 8.65 (d, 1, C_3H), δ 8.07 (d, 1, J = 6.7 Hz, C_6H), δ 8.00–7.85 (m, 3, C_4H, C_7H, and $C_{10}H$), δ 7.78–7.70 (m, 1,

C_5H), δ 7.50–7.39 (m, 2, C_8H and C_9H).

3-Nitrofluoranthene. 3-Nitrofluroanthene was prepared by nitration of fluoranthene by the method of Kloetzel et al. (*23*) and purified by repeated recrystallization from nitroethane. The physical and spectroscopic data for this compound were as follows: mp 161–162 °C (lit. value (*23*) m.p. 159–160 °C); UV_{max} (95% C_2H_5OH) 273 nm (ε 18 250); NMR ($CDCl_3$), δ 8.65 (d, 1, J(4,5) = 9 Hz, C_4H), δ 8.58 (d, 1, J(1,2) = 8 Hz, δ 7.96–7.82 (m, 4, C_1H, C_6H, C_7H, and $C_{10}H$), δ 7.82–7.72 (m, 1, C_5H), δ 7.49–7.34 (m, 2, C_8H and C_9H).

7-Nitrofluoranthene. Nitration of fluoranthene was undertaken as described by Streitwieser and Fahey (*24*) and chromatography on neutral alumina using hexane:benzene gradient separated the crude mixture of isomers into a fraction containing the 1- and 7-isomers and another fraction containing the 3- and 8-isomers. 7-Nitrofluoranthene was separated from the 1-isomer by preparative HPLC on a 7.8 mm i.d. × 25 cm silica column using hexane/1% triethylamine as the mobile phase; the 7-nitrofluoranthene eluted after the 1-isomer. Recrystallization from benzene:ethanol gave yellow needles. The physical and spectroscopic data for the compound were as follows: mp 144–145 °C (lit. value (*24*) mp 144–144.5 °C); UV_{max} (95% C_2H_5OH) 234 nm (ε 41 000); NMR ($CDCl_3$) δ 8.66 (d, 1, J(5,6) = 7 Hz, C_6H), δ 8.20 (d, 1, J(8,9) = 8 Hz, C_8H), δ 8.12–7.94 (m, 4, C_1H, C_3H, C_4H, $C_{10}H$), δ 7.80–7.65 (m, 2, C_2H and C_5H), δ 7.52 (t, 1, C_9H).

8-Nitrofluoranthene. 8-Nitrofluoranthene was separated from a fraction enriched in the 8-isomer, using the same HPLC system as for 7-nitrofluoranthene. 8-Nitrofluoranthene eluted after the 3-isomer. Recrystallization from benzene:ethanol gave yellow needles. The physical and spectroscopic data for the compound were as follows: mp 162–164 °C (lit. value (*24*) mp 158–160 °C), UV_{max} (95% C_2H_5OH) 230 nm (ε 20 000); NMR ($CDCl_3$) δ 8.77 (d, 1, J(7,9) = 2 Hz, C_7H), δ 8.31 (dd, 1, J (9,10) = 8 Hz, C_9H), δ 8.15–8.06 (m, 2, C_1H and C_6H), δ 8.06–7.94 (m, 3, C_3H, C_4H and $C_{10}H$), δ 7.80–7.70 (m, 2, C_2H and C_5H).

2-Nitropyrene. 2-Nitropyrene was synthesized by nitration of 4,5,9,10-tetrahydropyrene followed by aromatization using iodine in refluxing nitrobenzene (*25*). Purification by chromatography on neutral alumina with hexane:benzene as the eluant and recrystallization from benzene:ethanol gave 2-nitropyrene with a purity of 99.2% (GC). The physical and spectroscopic data for the compound were as follows: mp 197–199 °C (lit. value (*25*), mp 200–201 °C); UV_{max} (95% C_2H_5OH) 290 nm (ε 41 900); NMR ($CDCl_3$) δ 9.00 (s, 2, C_1H and C_3H), δ 8.34–8.08 (m, 7, ArH).

4-Nitropyrene. The synthesis employed was a modification of the procedure of Bavin (*26*). Nitration of 1,2,3,6,7,8-hexahydropyrene and then aromatization using DDQ in refluxing benzene was employed. Purification by chromatography on neutral alumina using a modified Soxhlet apparatus and hexane:benzene (9:1) as the eluant and then recrystallization from benzene:ethanol gave 4-nitropyrene with a purity of 99.4% (GC): The physical and spectroscopic data for the compound were as follows: mp 190–192 °C (lit. value (*26*) mp 196–197.5 °C); UV_{max} (95% C_2H_5OH) 318 nm (ε 15 640); NMR ($CDCl_3$) δ 8.92 (d, 1, J = 8 Hz, C_3H), δ 8.90 (s, 1, C_5H), δ 8.39–8.28 (m, 3, ArH), and δ 8.20–8.04 (m, 4, ArH).

1-Nitropyrene. The nitration procedure used was a modification of the method of Looker (*27*). To a solution of pyrene (2 g) in dioxane (100 mL) at 60 °C was added a solution of 15 mL of red fuming nitric acid:water (2:3). After 15 min the mixture was diluted with water and the product isolated by filtration. The crude product was purified by Soxhlet extraction through neutral alumina with isooctane:toluene (2:1). HPLC analysis of 1-nitropyrene and the dinitropyrenes was obtained by using a Waters 3.6 mm × 25 cm μBondapak CN column and a hexane/2-propanol gradient. The sample solvent was THF (uninhibited). Detection was at 280 nm. The material which crystallized from the extracts on cooling was found to be >99% pure. Further Soxhlet extraction followed by repeated recrystallizations yielded 1-nitropyrene of >99.5% purity which contained no detectable (detection limits: 0.01% of any isomer) dinitropyrenes. The physical and spectroscopic data for the compound were as follows: mp 151–152 °C (lit. value (*31*) mp 150 °C); UV_{max} (95% C_2H_5OH) 285 nm (ε 14 200), 364 nm (ε 11 700); NMR ($CDCl_3$) δ 8.91 (d, 1, J(9,10) = 9.6 Hz), δ 8.67 (d, 1, J(2,3) = 8.1 Hz, C_2H), δ 8.34–8.09 (m, 7, ArH).

1,3-Dinitropyrene. Pyrene was nitrated by the method of Vollman et al. (*28*) to give a mixture containing the three dinitropyrenes and also a substantial quantity of 1-nitropyrene. Chromatography with a modified Soxhlet column with neutral alumina and isooctane:toluene (2:1) as the eluant gave fractions enriched in 1,3-dinitropyrene. Latter fractions contained 1,6- and 1,8-dinitropyrene (see below). The material was chromatographed on silica gel and eluted with benzene:hexane (2:1). A column containing 2 kg of Merck silica gel 60 was used for separation of the dinitropyrenes. The mixture (~300 mg) was preadsorbed on silica (100 g) for loading on the column. Recrystallization from benzene gave orange needles. The physical and spectroscopic data for the compound were as follows: mp 295–297 °C; UV_{max} (THF) 415 nm (ε 19 800); NMR ($CDCl_3$) δ 9.36 (s, 1, C_2H), δ 8.97 (d, 2, J(4,5) = J(9,10) = 9.6 Hz, C_4H and C_9H), δ 8.54 (d, 2, C_5H and $C_{10}H$), δ 8.51 (d, 2, J(6,7) and J(7,8) = 7.7 Hz, C_6H and C_8H), δ 8.28 (t, 1, C_7H); HPLC analysis of the dinitropyrenes indicated a purity of >99% (*31*).

1,6-Dinitropyrene and 1,8-Dinitropyrene. A fraction enriched in the two isomers was separated on silica gel by using the silica gel column described above for 1,3-dinitropyrene. 1,6-Dinitropyrene eluted after a small amount of the 1,3-isomer and was purified by recrystallization from benzene, giving yellow needles. The physical and spectroscopic data for the compound were as follows: mp 309–310 °C (d); UV_{max} (THF) 410 nm (ε 17 600); NMR ($CDCl_3$) δ 9.02 (d, 2, J(4,5) = J(9,10) = 9.6 Hz, C_5H and $C_{10}H$), δ 8.76 (d, 2, J(2,3) = J(7,8) = 8.8 Hz, C_3H and C_8H), δ 8.40 (d, 2, C_3H and C_8H) and δ 8.40 (d, 2, C_4H and C_9H).

1,8-Dinitropyrene eluted following the 1,6-isomer. Recrystallization from benzene gave yellow platelets: mp 299–300 °C; UV_{max} (THF) 392 nm (ε 20 100); NMR ($CDCl_3$) δ 9.09 (d, 2, C_9H and $C_{10}H$), δ 8.76 (d, 2 J(2,3) = J(6,7) = 8.8 Hz, C_2H and C_7H), δ 8.38 (d, 2, C_3H and C_6H), δ 8.30 (s, 2, C_4H and C_5H).

1-Nitropyrene-d_9. This material was prepared from pyrene-d_{10} (KOR Inc., Cambridge, MA) by the nitration procedure described above. Following purification by column chromatography (silica gel/benzene:hexane (2:1)), the isotopic purity was determined to be 95.7%.

1,3-, 1,6-, and 1,8-Dinitropyrene-d_8 Mixture. This material was prepared by nitration of pyrene-d_{10} (KOR Inc., Cambridge MA) and purified by Soxhlet extraction as described above for the unlabeled compounds. The mixture was found to contain 15.4% 1,3-DNP-d_8, 33.1% 1,6-DNP-d_8, and 51.4% 1,8-DNP-d_8. HPLC analysis was undertaken with a Waters 3.6 mm × 25 cm μBondapak CN column and a hexane/2-propanol gradient. The sample solvent was THF (uninhibited). Detection was at 280 nm. The mixture had an isotopic purity of 95.3%.

1-Hydroxy-3-nitropyrene, 1-Hydroxy-6-nitropyrene, and 1-Hydroxy-8-nitropyrene. A mixture of the three isomers was obtained by nitration of 1-acetoxypyrene (using conditions described for pyrene) and then treated with methanol saturated with anhydrous HCl to remove the protecting group. The NMR of the 1-acetoxy-x-nitropyrene mixture (x = 3, 6, or 8) was consistent with a 1.0:1.5:2.0 isomer mixture (with 1-acetoxy-3-nitropyrene the minor compound). This was clearly indicated by the signals for protons ortho and peri to the nitro groups. These signals appeared as doublets (except for the C_2H proton in 1-acetoxy-3-nitropyrene, which was a singlet) and were strongly shifted downfield.

9-Methyl-10-nitroanthracene. To a solution of 500 mg of 9-methylanthracene in 3 mL of glacial acetic acid was added 0.2 mL of nitric acid. After 15 min, 3 mL of 12 M hydrochloric acid was added and the reaction mixture was stirred for an additional 30 min. The mixture was then filtered, and the residue triturated with hot concentrated potassium hydroxide for 5 min. Water (20 mL) was added and the product isolated by extraction with dichloromethane (50 mL). The organic layer was dried with anhydrous sodium sulfate and then absorbed onto 2 g of Florosil and the solvent removed in vacuo. This material was placed on a bed of Florisil in a modified Soxhlet extraction apparatus and eluted with hexane:benzene (99:1). The early eluting fraction containing a fluorescent impurity was discarded, and the orange band collected. The fraction was allowed to cool giving 53 mg (9% yield) of orange needles, mp 202–204 °C. The compound was further purified by chromatography on silica gel using a hexane:benzene gradient and then recrystallized from ethanol.

The physical and spectroscopic data for the compound were as follows: mp 203–205 °C; UV_{max} (95% C_2H_5OH) 252 nm (ϵ 112800); NMR ($CDCl_3$) δ 8.35 (d, 2, $J(3,4) = J(5,6) = 8$ Hz, C_4H, C_5H), δ 7.91 (d, 2, $J(1,2) = J(7,8) = 8$ Hz, C_1H, C_8H), δ 7.60 (m, 4, C_2H, C_3H, C_6H, C_7H), (s, 3, CH_3).

1-Methyl-9-nitroanthracene and 1-Methyl-10-nitroanthracene. 1-Methylanthracene (1 g) was nitrated by using the same conditions described above for 9-methylanthracene. The crude product isolated by extraction (0.5 g) was shown by TLC to consist of two main components ($R_f = 0.23$ (minor) and $R_f = 0.17$ (major)) with a ratio of ~1:2. Preparative HPLC on a Whatman Magmum 40 silica column and the previously described solvent system was used to separate the two components; recycle and peak shaving was employed to achieve the desired separation. The fractions containing the faster eluting component were concentrated and recrystallized from ethanol to give 1-methyl-9-nitroanthracene. The physical and spectroscopic data for the compound were as follows: mp 161–162 °C; UV_{max} (95% C_2H_5OH) 252 nm (ϵ 92500); NMR ($CDCl_3$) δ 8.58 (s, 1, $C_{10}H$), δ 8.04 (d, 1, ArH), δ 7.94 (m, a, ArH), δ 7.77 (d, 1, ArH), 7.58 (m, 2, ArH), δ 7.22 (d, 2, ArH), δ 2.72 (s, 3, CH_3). The mass spectrum of the compound showed a strong M – 17 peak which is characteristic for a nitro group para or ortho to a methyl group.

The slower eluting compound was concentrated and recrystallized from benzene:ethanol to give 1-methyl-10-nitroanthracene. The physical and spectroscopic data for this compound were as follows: mp 162–164 °C; UV_{max} (95% C_2H_5OH) 250 nm (ϵ 118000); NMR ($CDCl_3$) δ 8.74 (s, 1, C_9H), δ 8.10 (d, 1, ArH), δ 7.90 (d, 1, ArH), δ 7.78 (d, 1, ArH), δ 7.70–7.50 (m, 3, ArH), δ 7.38 (D, A, ArH), δ 2.86 (s, 3, CH_3). The mass spectrum showed the expected M – 30 and M – 46 fragments.

A number of nitro-PAH standards were synthesized for which the major isomers were not separated and purified by HPLC. This was accomplished by coating the inside surface of a 25-mL Erlenmeyer flask with approximately 2 mg of the parent PAH and injecting nitrogen dioxide and nitric acid fumes into the flask for a few seconds. Standards synthesized in this manner included *x*-nitrobenzocarbazoles (three major isomers), 6-nitrochrysene (one major isomer), 6-nitrocyclopenteno[*c,d*]pyrene (one major isomer), *x*-nitrodibenzothiophenes (two major isomers), *x*-nitro-9-hydroxyfluorene (two major isomers), *x*-nitroacridines (two major isomers), *x,y*-dinitroacridines (three major isomers), and *x*-nitrobenzo-5,6-quinoline (one major isomer). Another technique for quick synthesis of nitro-PAH compounds for MS analysis was accomplished by placing approximately 50 ng of the parent PAH on the mass spectrometer direct insertion probe (glass) and exposing the probe to concentrated nitrogen dioxide and nitric acid fumes for a few seconds.

2-Nitrobiphenyl, 2-methyl-1-nitronaphthalene, 2-nitronaphthalene, 9*H*-carbazole, 3-nitrobiphenyl, 4-nitrobiphenyl, 2-nitrofluorene, 1,5-dinitronaphthalene, 1,8-dinitronaphthalene, 2,5-dinitrofluorene, 2,7-dinitrofluorene, 3-nitro-9-fluorenone, and 2,7-dinitro-9-fluorenone were obtained from Aldrich Chemical Co. 1-Nitronaphthalene was obtained from Chem. Service, and 2,5-dinitrofluorene was obtained from C. White, Pittsburgh Energy Technology Center, Pittsburgh, PA.

2-Nitrophenanthrene and 2-nitroanthracene were obtained from J. Schribner at The University of Washington, Seattle, WA. 7-Nitrobenz[*a*]anthracene, 1 + 3-nitrobenzo[*a*]pyrene, and 6-nitrobenzo[*a*]pyrene (mixture 1:3) were obtained from P. Fu of The Toxicology Research Center of The U.S. Food and Drug Administration, Jefferson, AR.

RESULTS AND DISCUSSION

Advantages of HPLC Prefractionation. During initial investigations, raw diesel particulate extracts were directly injected onto the GC/NPD system. Major nitro-PAH components could be determined directly by this method (e.g., 1-nitropyrene above 100 ppm based on the weight of the soluble organic fraction). However, the raw extract contained large amounts of nonpolar (mostly aliphatic) and polar compounds which interfered with column performance and thereby decreased the sensitivity of the technique.

Prefractionation of the raw extract by semipreparative HPLC offered several advantages. Capillary column performance and lifetime were enhanced by the elimination of the noneluting compounds present in the extract. An injection of various nitro-PAH standard compounds revealed that the compounds elute from the column relative to increasing size and polarity (Figure 1). Therefore, prefractionation reduced the number of nitro compounds within a given fraction. This reduced background, lowered detection limits through sample concentration, and increased confidence in identifying nitro-PAH by retention times. Furthermore, the same fractions could also be analyzed by GC/MS without high background interferences, thus allowing direct confirmation of peak identities in many cases.

Compound Identification by GC/NPD Analysis. In Figures 2 and 3 NPD chromatograms are shown for HPLC fractions 3 through 8. Readily notable is the large number of nitrogen detector sensitive compounds in each fraction. Additional information was obtained by GC/MS which confirmed that most of these peaks are nitro derivatives of PAH and other related compounds. Identification of specific nitro-PAH isomers was made in those instances where the GC/NPD retention times (relative to 1-nitropyrene) of the standards and unknowns agreed to better than ±0.4% and were found to elute in the correct HPLC fraction. Thirty-four nitro-PAH were identified by this method and are listed in Table I. Retention times of standards not detected in the real sample are also included for reference.

A few N-heterocyclic compounds were also identified such as 9-methylcarbazole in fraction 3, and nitrated heterocyclics in later fractions such as two nitrodibenzothiophene isomers in fraction 6, a nitrobenzoquinoline isomer, and two nitroquinoline isomers in fraction 8. Because the sample had been extensively prefractionated, these compounds were not found to be a serious source of interference with the less polar nitro-PAH.

The important question at this point about the NPD technique is its susceptibility to interferences and misidentifications. Therefore, confirming analyses were undertaken with GC/MS.

Compound Identification by GC/MS. By use of chromatographic conditions similar to the GC/NPD study, GC/MS data were obtained for fractions 3 to 8. Figure 4 shows a detailed portion of the GC/NPD chromatogram for fraction 3 vs. a GC/MS summed ion current mass chromatogram for the molecular ions for nitroanthracene (m/z 223), the C_1–C_3 homologues of nitroanthracene (m/z 237, 251, 265), and nitropyrene (m/z 256). Positive identification was made for those compounds where the GC/MS retention times of the standards agreed to better than ±0.6%. Confirmation, however, was not possible for the low abundance peaks since the GC/MS sensitivity (~5 ppm for a 2 μg injection) was an order of magnitude lower than that of the GC/NPD.

Representative mass spectral data are compiled in Table II. Positive identification of compounds in Table I was confirmed when the mass spectral pattern was similar for standards and unknowns. Further identification was judged on the accuracy (better than ±10 ppm) of the molecular ion mass for the unknown. In some instances, tentative identification was made when standards were unavailable.

Mass spectra of the nitro-PAH characteristically give abundant M^+, $(M - NO)^+$, and $(M - NO_2)^+$ ions. Dinitro PAH give abundant $(M - 2NO_2)^+$ ions. Ion abundance contributions from direct loss of additional functionalities such as methyl, dimethyl, hydroxy, etc. are minimal. There are some spectral differences between isomers such as the dinitropyrenes. However, we have previously reported that fragmentation patterns are very dependent upon mass spectrometer source conditions (*24*) and the quantity of compound eluted through the GC/MS. Therefore, absolute identification of isomers by

Table I. GC/NPD Retention Time Data and GC/MS Spectral Data Used for Identification of Nitro-PAH in a Diesel Particulate Extract[a]

peak	compound[b]	mass	RRT (1NP = 1.00)[c] STD	RRT (1NP = 1.00)[c] sample	GC/MS confirm	HPLC fraction
2	1-nitronaphthalene	173.048	0.433	0.424	nd	3
3	2-methyl-1-nitronaphthalene	187.063	0.453	0.454	yes	3
nd	2-nitronaphthalene	173.048	0.459	nd	nd	3
nd	2-nitrobiphenyl	199.063	0.494	nd	nd	3
8	3-nitrobiphenyl	199.063	0.566	0.566	nd	3
10	4-nitrobiphenyl	199.063	0.586	0.584	yes	3
11	9-methylcarbazole	181.089	0.593	0.589	yes	3
23	2-nitrofluorene	211.063	0.722	0.722	yes	3
24	9-nitroanthracene	223.063	0.741	0.741	yes	3
25	*x*-nitrotrimethylnaphthalene	215.097	sna	0.759	yes	3
26	*x*-nitrotrimethylnaphthalene	215.097	sna	0.767	yes	3
27	*x*-nitrotrimethylnaphthalene	215.097	sna	0.771	yes	3
28	*x*-nitrophenanthrene	223.063	sna	0.781	yes	3
29	*x*-nitrophenanthrene	223.063	sna	0.788	yes	3
nd	1-methyl-9-nitroanthracene	237.079	0.804	nr	nr	3
31	1-methyl-10-nitroanthracene	237.079	0.809	0.811	yes	3
32	*y*-methyl-*x*-nitroanthracene	237.079	sna	0.817	yes	3
nr	2-nitrophenanthrene	223.063	0.824	nr	yes	3
33	*y*-methyl-*x*-nitroanthracene	237.079	sna	0.826	yes	3
nr	2-nitroanthracene	223.063	0.836	nr	yes	3
34	*y*-methyl-*x*-nitroanthracene	237.079	sna	0.838	yes	3
35	9-methyl-10-nitroanthracene	237.079	0.848	0.846	yes	3
36	*y,z*-dimethyl-*x*-nitroanthracene	251.095	sna	0.857	yes	3 + 4
37, 99	*y,z*-dimethyl-*x*-nitroanthracene	251.095	sna	0.861	yes	3 + 4
39, 100	*y,z*-dimethyl-*x*-nitroanthracene	251.095	sna	0.873	yes	3 + 4
40	*y,z*-dimethyl-*x*-nitroanthracene	251.095	sna	0.879	yes	3 + 4
41, 104	*y,z*-dimethyl-*x*-nitroanthracene	251.095	sna	0.885	yes	3 + 4
43	*y,z,z'*-trimethyl-*x*-nitroanthracene	265.111	sna	0.907	yes	3 + 4
44	*y,z,z'*-trimethyl-*x*-nitroanthracene	265.111	sna	0.917	yes	3 + 4
45	*y,z,z'*-trimethyl-*x*-nitroanthracene	265.111	sna	0.921	yes	3 + 4
46	*y,z,z'*-trimethyl-*x*-nitroanthracene	265.111	sna	0.928	yes	3 + 4
47, 111	*y,z,z'*-trimethyl-*x*-nitroanthracene	265.111	sna	0.936	yes	3 + 4
48	*y,z,z'*-trimethyl-*x*-nitroanthracene	265.111	sna	0.944	yes	3 + 4
54, 139	*x*-nitroterphenyl	275.095	sna	1.243	yes	3 + 4
64	1,5-dinitronaphthalene	218.033	0.619	0.617	nd	4
86	1,8-dinitronaphthalene	218.033	0.768	0.767	nd	4
113	1-nitrofluoranthene	247.063	0.950	0.950	nd	4
114	7-nitrofluoranthene	247.063	0.964	0.964	nd	4
nd	2-nitrofluoranthene	247.063	0.968	nd	nd	
115	3-nitrofluoranthene	247.063	0.971	0.970	nd	4
117	8-nitrofluoranthene	247.063	0.980	0.980	nd	4
nd	4-nitropyrene	247.063	0.996	nd	nd	4
120	1-nitropyrene	247.063	1.000	1.000	75 ppm[d]	4
nd	2-nitropyrene	247.063	1.006	nd	nd	
124	2,7-dinitrofluorene	256.048	1.031	1.029	nd	4
126	2,5-dinitrofluorene	256.048	1.035	1.035	nd	4
131	1-methyl-3-nitropyrene	261.079	1.075	1.074	yes	4
132	1-methyl-6-nitropyrene	261.079	1.081	1.081	yes	4
nr	1-methyl-8-nitropyrene	261.079	1.084	nr	yes	4
nd	7-nitrobenzo[*a*]anthracene	273.079	1.099	nd	nd	4
134	1-nitrochrysene	273.079	1.141	1.136	nd	4
135	6-nitrocyclopenta[*c,d*]pyrene	271.063	1.147	1.144	nd	4
165	3-nitro-9-fluorenone	225.043	0.733	0.734	nd	5
200	2,7-dinitro-9-fluorenone	270.028	1.041	1.043	nd	5
202	1,3-dinitropyrene[e]	292.048	1.178	1.174	0.30 ppm	5
204	1,6-dinitropyrene[e]	292.048	1.204	1.202	0.40 ppm	5
206	1,8-dinitropyrene[e]	292.048	1.222	1.226	0.53 ppm	5
207	1 + 3-nitrobenzo[*a*]pyrene	297.079	1.233	1.232	nd	5
208	6-nitrobenzo[*a*]pyrene	297.079	1.243	1.245	yes	5
209	3-nitroperylene?	297.079	sna	1.253	yes	5
214	nitro-$C_{12}H_8OH$	215.058	sna	0.672	yes	6
222	*x*-nitrodibenzothiophenes	229.020	0.729	0.729	yes	6
223	*x*-nitrodibenzothiophenes	229.020	0.735	0.738	yes	6
ne	*y*-hydroxy-*x*-nitrofluorene	227.058	ne	ne	ne	7
nd	*x*-nitroacridine	224.137	0.813	nd	nd	7
nd	*x*-nitroacridine	224.137	0.856	nd	nd	7
241	*x*-nitrobenzocinnoline	225.054	sna	0.786	yes	7
242	*x*-nitrobenzoquinoline	224.059	0.812	0.806	yes	7
247	5-nitroquinoline	175.051	0.377	0.379	yes	8
250	8-nitroquinoline	175.051	0.530	0.532	yes	8
ne	*x*-nitropyrene-3,6-quinone	277.037	ne	ne	ne	8
ne	*x*-nitropyrene-3,6-quinone	277.037	ne	ne	ne	8
ne	1-hydroxy-3-nitropyrene	263.058	ne	ne	ne	8
ne	1-hydroxy-6-nitropyrene	263.058	ne	ne	ne	8
ne	1-hydroxy-8-nitropyrene	263.058	ne	ne	ne	8

Table I (*Continued*)

peak	compound[b]	mass	RRT (1NP = 1.00)[c] STD	RRT (1NP = 1.00)[c] sample	GC/MS confirm	HPLC fraction
nd	x,y-dinitroacridine	269.044	1.019	nd	nd	8
nd	x,y-dinitroacridine	269.044	1.041	nd	nd	8
nd	x,y-dinitroacridine	269.044	1.054	nd	nd	8

[a] Abbreviations are as follows: sna, standard not available—tentative identification by GC/MS; ne, not eluted from GC column; nd, not detected is less than approximately 0.5 ppm by GC/NPD and less than approximately 5 ppm GC/MS; nr, not resolved from neighboring peaks. [b] x,y,z notations refer to isomer position unknown. [c] Relative retention times with respect to 1-nitropyrene (1NP = 1.00). [d] 1-Nitropyrene quantified by GC/MS using 1-nitropyrene-d_9 as an internal standard. See ref 30 for procedural details. [e] 1,3-, 1,6-, and 1,8-dinitropyrenes quantified by GC/MS using 1,3-, 1,6-, and 1,8-dinitropyrenes-d_8.

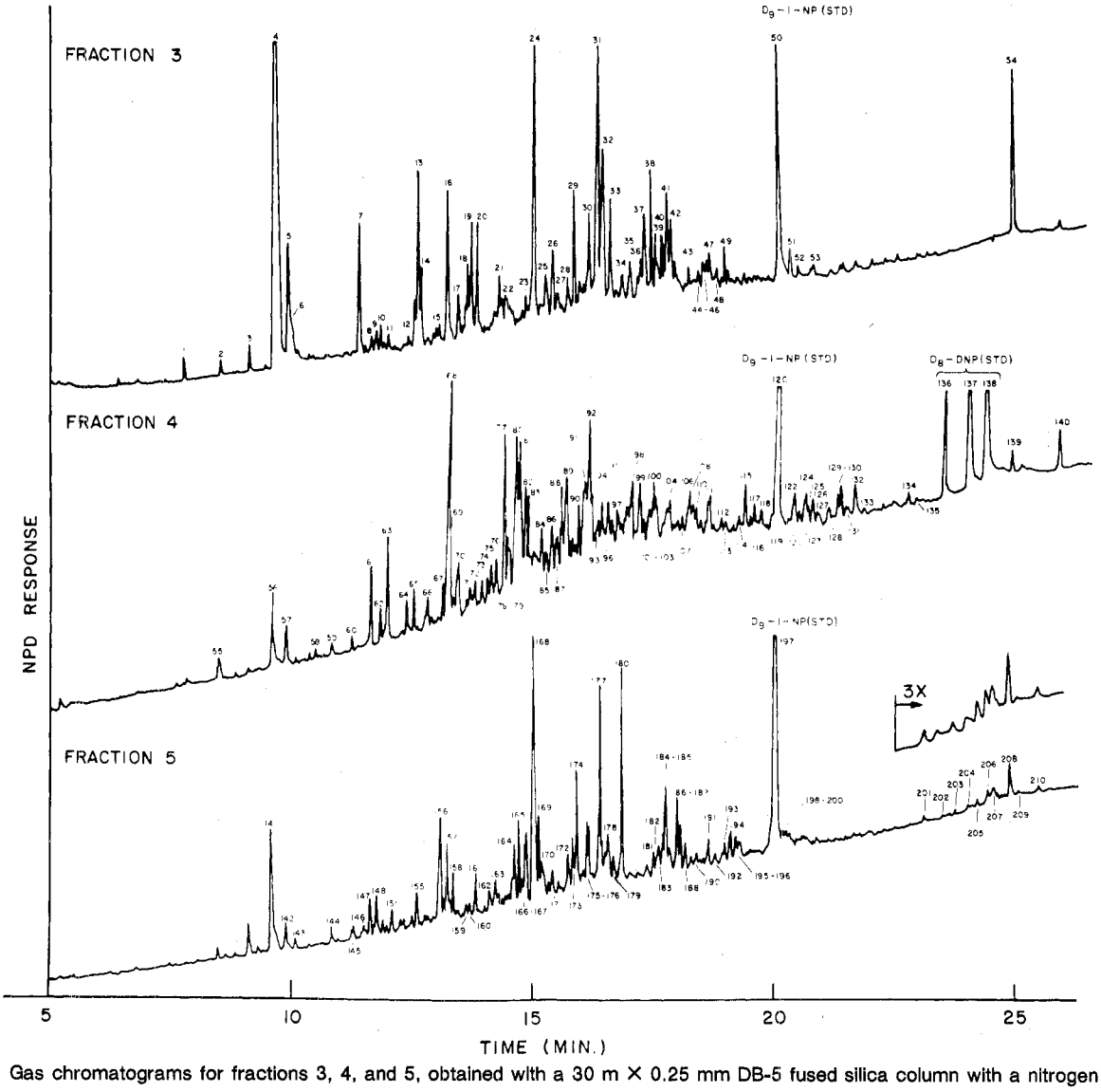

Figure 2. Gas chromatograms for fractions 3, 4, and 5, obtained with a 30 m × 0.25 mm DB-5 fused silica column with a nitrogen selective detector.

comparison of fragmentation patterns was not found to be reliable.

Positive isomer identification for 15 nitro-PAH, two nitrated nitrogen-containing heterocyclics, and a nitrogen-containing heterocyclic have been made by using the criteria of closely matching retention times, low-resolution mass spectra, and high-resolution mass spectra of authentic standards. The 15 nitro-PAH include 2-methyl-1-nitronaphthalene, 4-nitrobiphenyl, 2-nitrofluorene, 9-nitroanthracene, 9-methyl-10-nitroanthracene, 2-nitroanthracene, 2-nitrophenanthrene, 1-methyl-9-nitroanthracene, 1-methyl-3-nitropyrene, 1-methyl-6-nitropyrene, 1-methyl-8-nitropyrene, 1,3-dinitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, and 6-nitrobenzo[*a*]pyrene. The two nitrated nitrogen containing heterocyclics include 5-nitroquinoline and 8-nitroquinoline. The nitrogen containing heterocyclic was 9-methylcarbazole.

Two isomers of nitrodibenzothiophenes and one isomer of nitrobenzoquinoline were synthesized (exact isomer positions not known). These compounds were found to be present in the sample by GC/MS and GC/NPD. Further work will be necessary to determine the exact isomer structure of these three compounds. Three of the isomers, 2-nitrophenanthrene, 2-nitroanthracene, and 1-methyl-8-nitropyrene, were not chromatographically resolved from other components using

Table II. Gas Chromatography/Mass Spectrometry Data Used for Identification of Nitro-PAH in a Diesel Particulate Extract

compound	mass (rel abundance)[a] M	(M – NO)	(M – NO_2)	(M – HNO_2)	(M – CNO_2)
Nitro-PAH					
2-nitrofluorene	211 (0.29)	181 (0.14)	165 (1.00)	164 (0.27)	153 (0.04)
9-nitroanthracene	223 (0.98)	193 (1.00)	177 (0.95)	176 (0.78)	165 (0.83)
1-nitropyrene	247 (0.67)	217 (0.98)	201 (1.00)	200 (0.53)	189 (0.50)
7-nitrobenz[*a*]anthracene	273 (0.78)	243 (1.00)	227 (0.20)	226 (0.23)	215 (0.68)
6-nitrobenzo[*a*]pyrene	297 (0.49)	267 (1.00)	251 (0.48)	250 (0.39)	239 (0.41)
x-nitroterphenyl	275 (1.00)	245 (0.97)	229 (0.27)	228 (-)	217 (0.19)

compound	mass (rel abundance)[a] M	(M – NO)	(M – 2NO)	(M – N_2O_3)	(M – $2NO_2$)
Dinitro-PAH					
1,5-dinitronaphthalene	218 (0.49)	188 (-)	158 (0.13)	142 (0.08)	126 (1.00)
1,3-dinitropyrene	292 (0.37)	262 (0.09)	232 (0.06)	216 (0.12)	200 (1.00)
1,6-dinitropyrene	292 (0.76)	262 (0.14)	232 (0.66)	216 (0.51)	200 (1.00)
1,8-dinitropyrene	292 (0.69)	262 (-)	232 (0.79)	216 (0.38)	200 (1.00)

compound	mass (rel abundance)[a] M	(M – NO)	(M – NO_2)	(M – HNO_2)	(M – CNO_2)
Methyl-Nitro-PAH					
9-methyl-10-nitroanthracene	237 (1.00)	207 (0.58)	191 (0.24)	190 (0.39)	179 (0.17)
1-methyl-9-nitroanthracene	237 (1.00)	207 (0.12)	191 (0.43)	190 (0.41)	179 (0.32)
1-methyl-10-nitroanthracene	237 (1.00)	207 (0.25)	191 (0.34)	190 (0.30)	179 (0.24)
1-methyl-3-nitropyrene	261 (0.45)	231 (1.00)	215 (-)	214 (-)	203 (-)
1-methyl-6-nitropyrene	261 (0.49)	231 (1.00)	215 (0.20)	214 (-)	203 (-)

compound	mass (rel abundance)[a] M	(M – NO)	(M – $NOCH_3$)	(M – NO_2)	(M – $C_2H_3NO_2$)
Nitro-Dimethyl-PAH					
x-nitro-*y*,*z*-dimethylanthracene	251 (1.00)	221 (0.47)	206 (0.55)	205 (-)	178 (0.52)
x-nitro-*y*,*z*-dimethylanthracene	251 (1.00)	221 (0.67)	206 (0.18)	205 (0.25)	178 (0.22)

compound	mass (rel abundance)[a] M	(M – O)	(M – NO)	(M – NO_2)	(M – $C_2H_4NO_2$)
Nitro-Hydroxy-PAH, Nitro-PAH Ketones					
x-nitro-1-hydroxypyrene (*x* = 3, 6, and 8 isomers in the ratio 1.0/1.5/2.0)	263 (1.00)	247 (0.05)	233 (0.58)	217 (0.77)	189 (0.51)
3-nitro-9-fluorenone	225 (0.89)	209 (-)	195 (0.28)	179 (0.31)	151 (1.00)

compound	mass (rel abundance)[a] M	(M – NO)	(M – NO_2)	(M – HNO_2)	(M – CNO_2)
Nitro-Heterocyclics					
x-nitrodibenzothiophene	229 (1.00)	199 (0.95)	183 (-)	182 (-)	171 (0.16)
x-nitrobenzoquinoline	224 (1.00)	194 (0.22)	178 (0.99)	177 (0.31)	166 (0.96)

[a] (-) designates intensity less than 5% of base peak.

GC/NPD analysis alone. In these cases, GC/MS analysis was necessary for identification.

Fifteen additional nitro-PAH isomers were identified by GC/NPD determined by the matching of peak retention times in the sample with those from authentic standards but were present in concentrations below 5 ppm and were not detected by GC/MS. These compounds included: 1-nitronaphthalene, 3-nitrobiphenyl, 1,5-dinitronaphthalene, 1,8-dinitronaphthalene, 1-nitrofluoranthene, 7-nitrofluoranthene, 3-nitrofluroanthene, 8-nitrofluoranthene, 2,7-dinitrofluorene, 2,5-dinitrofluorene, 1-nitrochrysene, 6-nitrocyclopenteno[*c*,*d*]pyrene, 3-nitro-9-fluorenone, 2,7-dinitro-9-fluorenone, and 1 + 3-nitrobenzo[*a*]pyrene.

Forty-five nitro-PAH compounds were identified by their characteristic mass spectra from the GC/MS analysis. In addition, there were peaks present in the GC/NPD chromatograms whose retention times correlated very closely with those obtained by GC/MS. These compounds are given in Table I with the designation sna (standard not available).

The nitration of acridine was found to produce two major mononitrated isomers and three dinitro isomers, all of whose exact isomer positions are not yet known. None of these isomers were detected in fractions 7 and 8 of the sample.

The major mononitrated isomers of 3,6-pyrenequinone, 1-hydroxypyrene, and 9-hydroxyfluorene were synthesized. None of these isomers eluted by using the GC conditions described in this paper. Six nitro-PAH including 2-nitrobiphenyl, 2-nitronaphthalene, 2-nitrofluoranthene, 4-nitropyrene, 2-nitropyrene, and 7-nitrobenz[*a*]anthracene were not detected with either GC/NPD or GC/MS.

Quantitation of Nitro-PAH by GC/NPD and GC/MS. In a related study, two raw diesel extract samples were quantitatively analyzed for 1-nitropyrene by GC/NPD and were found to contain 14.1 ± 3.3 ppm and 124.0 ± 8.0 ppm, respectively. Comparable results were obtained in another laboratory where identical samples were analyzed by an HPLC

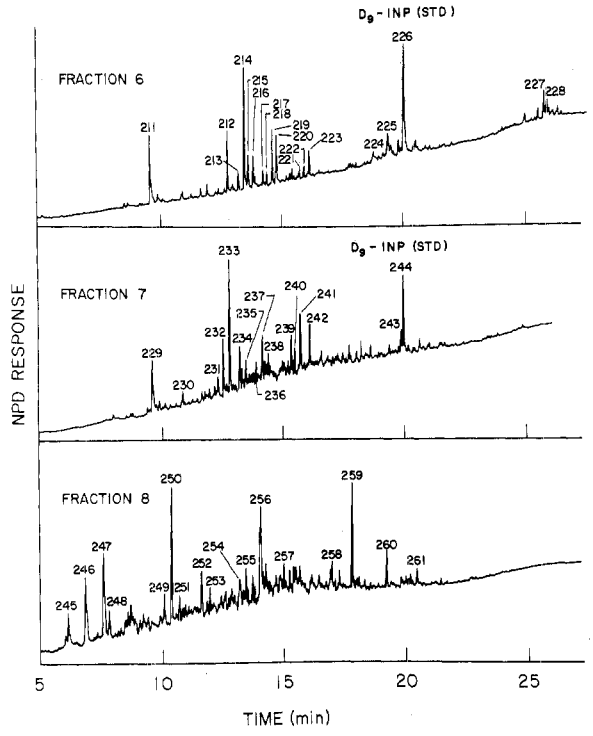

Figure 3. Gas chromatograms for fractions 6, 7, and 8 obtained with a 30 m × 0.25 mm DB-5 fused silica column with a nitrogen selective detector.

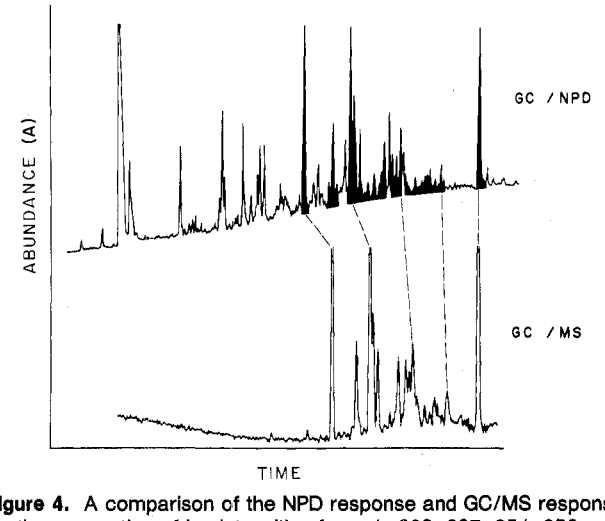

Figure 4. A comparison of the NPD response and GC/MS response for the summation of ion intensities for *m/z* 223, 237, 251, 256, and 265 for nitro(anthracenes and phenanthrenes), C_1–C_3-methylnitro(anthracenes and phenanthrenes), and 1-nitropyrene-d_9.

catalyst reduction method (*8*, *18*) and were found to contain 13 ppm and 94 ppm of 1-nitropyrene. Further results of these investigations, together with an improved prefractionation method will be discussed in a forthcoming paper.

By use of 1-nitronaphthalene and 1,3-dinitropyrene standards, detection limits were determined and estimated to range between 0.2 and 0.5 ppm (200–500 pg) at a signal/noise ratio of 3/1 for the NPD method. For most nitro-PAH compounds observed, relative NPD responses were approximately proportional to the number of nitrogen atoms present per molecule. This was generally true for compounds with similar relative retention times (e.g., 2,5-dinitrofluorene gave approximately twice the response of 7-nitrofluoranthene for equal molar concentrations (*20*)). Once identified, the concentrations of nitro-PAH compounds were estimated by comparing their peak height with that of an internal standard in the same retention time range. A study of the accuracy and precision of quantitation by using peak heights vs. peak areas was undertaken by using nine identical sets of samples. The use of peak heights gave the best quantitative results.

GC/MS was used to quantify 1-nitropyrene (1-NP), 1,3-dinitropyrene (1,3-DNP), 1,6-dinitropyrene (1,6-DNP), and 1,8-dinitropyrene (1,8-DNP) in fractions 4 and 5 using per-deuterated analogues of those compounds as described previously (*29*). The concentration of 1,3-DNP, 1,6-DNP, and 1,8-DNP was found to be 0.30 ± 0.20 ppm, 0.40 ± 0.20 ppm, and 0.53 ± 0.20 ppm. The concentration of 1-nitropyrene was found to be 75 ± 10 ppm. The dinitropyrenes were quantified by GC/NPD using an external calibration mixture of the deuterated analogues for these compounds. The quantitative results were slightly more accurate than those for the GC/MS yielding 0.27 ± 0.15 ppm, 0.42 ± 0.15 ppm, and 0.66 ± 0.15 ppm (see Table I) for the 1,3-DNP, 1,6-DNP, and 1,8-DNP, respectively. The concentration of the 1-NP was determined by using 3-nitrofluoranthene as an internal standard. The concentration of 1-NP was found to be 75 ± 10 ppm. These results are comparable to those obtained for the GC/MS analysis.

CONCLUSIONS

This study has shown that the chemical composition of diesel particulate extracts are much more complex than had been previously anticipated. In this study we have chosen to concentrate on the analysis of a specific class of compounds—the nitrated polynuclear aromatic hydrocarbons (nitro-PAH)—because of their potential for accounting for most of the direct-acting mutagenicity in diesel particulate extracts and possibly other environmental samples. We have shown that at least 60 specific nitro-PAH compounds are present in diesel particulate extracts. High-resolution capillary GC/NPD analysis has been shown to be a valuable screening technique for these compounds following a prefractionation technique such as HPLC. However, because these samples are extremely complex, there is still the potential for misidentification. We have confirmed, using GC/MS and HRMS procedures, that the GC/NPD technique gives the correct identification of specific isomers with a high degree of reliability. GC/NPD identification of nitro-PAH at concentrations above 5 ppm can be determined with a high level of confidence (90–95% accuracy) when used with HPLC elution information and with a level of confidence (near 100%) when used in conjunction with GC/MS and HRMS.

Registry No. 1-Nitropyrene, 5522-43-0; 1,3-dinitropyrene, 75321-20-9; 1,6-dinitropyrene, 42397-64-8; 1,8-dinitropyrene, 42397-65-9; 1-nitronaphthalene, 86-57-7; 2-methyl-1-nitronaphthalene, 881-03-8; 2-nitronaphthalene, 581-89-5; 2-nitrobiphenyl, 86-00-0; 3-nitrobiphenyl, 2113-58-8; 4-nitrobiphenyl, 92-93-3; 9-methylcarbazole, 1484-12-4; 2-nitrofluorene, 607-57-8; 9-nitroanthracene, 602-60-8; nitrotrimethylnaphthalene, 80182-40-7; nitrophenanthrene, 68455-92-5; 1-methyl-9-nitroanthracene, 86695-76-3; 1-methyl-10-nitroanthracene, 86689-95-4; methylnitroanthracene, 80191-43-1; 2-nitrophenanthrene, 17024-18-9; 2-nitroanthracene, 3586-69-4; 9-methyl-10-nitroanthracene, 84457-22-7; dimethylnitroanthracene, 80191-45-3; trimethylnitroanthracene, 86689-92-1; nitroterphenyl, 86695-75-2; 1,5-dinitronaphthalene, 605-71-0; 1,8-dinitronaphthalene, 602-38-0; 1-nitrofluoroanthene, 13177-28-1; 7-nitrofluoranthene, 13177-31-6; 2-nitrofluoranthene, 13177-29-2; 3-nitrofluoranthene, 892-21-7; 8-nitrofluoranthene, 13177-32-7; 4-nitropyrene, 57835-92-4; 2-nitropyrene, 789-07-1; 2,7-dinitrofluorene, 5405-53-8; 2,5-dinitrofluorene, 15110-74-4; 1-methyl-3-nitropyrene, 86689-96-5; 1-methyl-6-nitropyrene, 86689-97-6; 1-methyl-8-nitropyrene, 74869-47-9; 7-nitrobenz[*a*]anthracene, 20268-51-3; 1-nitrochrysene, 81316-77-0; 6-nitrocyclopenta[*c*,*d*]pyrene, 86689-98-7; 3-nitro-9-fluorenone, 42135-22-8; 2,7-dinitro-9-fluorenone, 31551-45-8; 1-nitrobenzo[*a*]pyrene, 70021-99-7; 3-nitrobenzo[*a*]pyrene, 70021-98-6; 6-nitrobenzo[*a*]pyrene, 63041-90-7; nitrodibenzothiophene, 86689-93-2; hydroxynitrofluorene, 82322-45-0; nitroacridine,

76025-15-5; 5-nitroquinoline, 607-34-1; 8-nitroquinoline, 607-35-2; nitropyrene-3,6-quinone, 86689-94-3; 1-hydroxy-3-nitropyrene, 86674-49-9; 1-hydroxy-6-nitropyrene, 1767-28-8; 1-hydroxy-8-nitropyrene, 1732-29-2; dinitroacridine, 50764-83-5.

LITERATURE CITED

(1) Pitts, J. N., Jr.; Cauwenberghe, K. A.; Grosjean, D.; Schmid, J. T.; Fitz, D. R.; Belser, W. L., Jr.; Knudson, G. B.; Hynds, P. M. *Science* **1978**, *202*, 515–519.
(2) Pederson, T. C.; Siak, J.-S. *J. Appl. Toxicol.* **1981**, *1*, 54–66.
(3) Mermelstein, R.; Kiriazides, D. K.; Butler, M.; McCoy, E. C.; Rosenkranz, H. S. *Mutat. Res.* **1981**, *89*, 187.
(4) Schuetzle, D.; Riley, T.; Prater, T. J.; Harvey, T. M.; Hunt, D. *Anal. Chem.* **1982**, *54*, 265–271.
(5) Xu, X. B.; Nachtman, J. P.; Jin, Z. L.; Wei, E. T.; Rappaport, S. W. *Anal. Chim. Acta* **1982**, *136*, 163–174.
(6) Newton, D. L; Erickson, M. D.; Tomer, K. B.; Pellizzari, E. D.; Gentry, P. *Environ. Sci. Technol.* **1982**, *16*, 206–213.
(7) Jager, J. *J. Chromatogr.* **1978**, *152*, 575–578.
(8) Wang. C. Y.; Lee, M. S.; King, C. M.; Warner, P. O. *Chemosphere* **1980**, *9*, 83–87.
(9) Ramdahl, T.; Becher, G.; Bjorseth, A. *Environ. Sci. Technol.* **1982**, *16*, 861–865.
(10) Schuetzle, D.; Lee, F. S.-C.; Prater, T. L.; Tejada, S. B. *Int. J. Environ. Anal. Chem.* **1981**, *9*, 93–144.
(11) Tejada, S. B.; Zweidinger, R. B.; Sigsby, J. E.; Paper 820775 presented at SAE Passenger Car Meeting, Troy, MI, June 7–11, 1982, and references cited therein.
(12) Schuetzle, D. *EHP, Environ. Health Perspect.* **1983**, *47*, 65–80.
(13) Oehme, M.; Mano, S.; Stray, H. *HRC CC J. High Resolut. Chromatogr. Chromatogr. Commun.* **1982**, *5*, 417.
(14) Ramdahl, T.; Urdall, K. *Anal. Chem.* **1982**, *54*, 2256–2260.
(15) Nielsen, T. *Anal. Chem.* **1983**, *55*, 286–290.
(16) Gibson, T. *Atmos. Environ.* **1982**, *16*, 2037–2040.
(17) Pitts, J. N., Jr.; Lokensgard, D. M.; Harger, W.; Fisher, T. S.; Mejia, V.; Schuler, J. J.; Scorziell, G. M.; Katzenstein, Y. A. *Mutat. Res.* **1982**, *103*, 241–249.
(18) Rappaport, S. M.; Jin, Z. L.; Xu, X. B.; *J. Chromatogr.* **1980**, *240*, 140–154.
(19) Yergey, J. A.; Risby, T. H.; Lestz, S. S. *Anal. Chem.* **1982**, *54*, 354–357.
(20) Ramdahl, T.; Kveseth, K.; Becher, G. *HRC CC J. High Resolut. Chromatogr. Chromatogr. Commun.* **1982**, *5*, 19–26.
(21) Lee, F. S.-C.; Schuetzle, D. In "Handbook of Polycyclic Aromatic Hydrocarbons"; Bjorseth, A., Ed.; Marcel Dekker: New York, 1983: Chapter II.
(22) Campbell, N.; Wilshire, J. F. K. *J. Chem. Soc.* **1954**, 867–869.
(23) Kloetzel, M. C.; King, W.; Menkes, J. H. *J. Am. Chem. Soc.* **1956**, *78*, 1165–1168.
(24) Streitweser, A.; Fahey, R. C. *J. Org. Chem.* **1962**, *27*, 2352–2355.
(25) Bolton, R. *J. Chem. Soc.* **1964**, 4637–4638.
(26) Bavin, P. M. G. *Can. J. Chem.* **1959**, *37*, 1614–1615.
(27) Looker, J. J. *J. Org. Chem.* **1972**, *37*, 3379–3381.
(28) Vollman, H.; Becker, H.; Correll, M.; Streeck, H. *Justus Liebigs Ann. Chem.* **1937**, *53*, 1–159.
(29) Sweetman, J.; Karasek, F. W.; Schuetzle, D. *J. Chromatogr.* **1982**, *247*, 245–254.
(30) Schuetzle, D.; Riley, T. L.; Prater, T. J.; Salmeen, I.; Harvey, T. M. In "Analytical Techniques In Environmental Chemistry 2"; Albaiges, J., Ed.; Pergamon Press: New York, 1982; pp 259–280.

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Comparison of Fast Atom Bombardment and Field Desorption Mass Spectrometry of Coordination Complexes

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Fast atom bombardment (FAB) and field desorption (FD) mass spectrometry of neutral, 1+, and 2+ cationic transition metal complexes suggest that FAB is preferable for 1+ complexes, producing both parent and useful fragment ions. For neutral complexes FD is better for molecular weight determination while FAB can provide fragment ion information. Both techniques yield a minimum of information when applied to dicationic complexes although some knowledge of the major fragmentation pathways can be obtained. Ligand loss and ease of reduction in FAB parallel ground state solution substitutional and redox chemistry for complexes where the latter data are available. The hypothesis of ion formation in FAB in the condensed phase where the matrix could play a significant role is consistent with these observations. The experimental results indicate FAB fragmentations may be useful in predicting solution chemistry of complex ions.

The general interest in design of oxidation and reduction catalysts (*1*, *2*), the stoichiometric reactions of coordinated ligands (*3*, *4*), and the design of new types of molecular and polymeric excited states (*5*) require increased sophistication in the complete characterization of cationic or involatile neutral metal complexes containing a wide variety of ligand environments.

The mass spectrometric analysis of involatile or thermally labile inorganic complexes has generally proved so difficult that mass spectrometry is an uncommon structural tool of the transition-metal coordination chemist. This is not to say, however, that its use is unknown. Field desorption (FD) (*6*) has been developed as a technique for involatile materials which has led to its application to inorganic complexes (*7–9*). Fast atom bombardment (FAB) (*10*, *11*) has been developed for large polar molecules, but there have been fewer applications to coordination complexes of transition metals (*12–14*).

The combination of FD and FAB mass spectrometry applied to coordination complexes should provide information concerning the parent ion molecular weight, an indication of structural complexity though fragmentation patterns, and perhaps a prediction of chemical reactivity. Such information is particularly important for complexes containing, for example, paramagnetic sites where NMR spectral studies may be of limited value.

In this paper we compare the two techniques of FAB and FD for a series of neutral, 1+ cationic, and 2+ cationic complexes of transition metals. These include many different metals in various formal oxidation states and a diverse group of ligands, including polypyridyl, phosphine, and oxo ligands.

EXPERIMENTAL SECTION

Instrumentation. Field Desorption. A Du Pont 21-492B mass spectrometer modified for field desorption was operated at 3 kV accelerating potential and the cathode was at −7 kV. Samples were dissolved in methylene chloride and placed on the emitter by the dipping technique. Cobalt dendrites were used with heating currents of 15–30 mA. Their preparation has been described

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