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April 1984

Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
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CHEMICAL AND TOXICOLOGICAL CHARACTERIZATION OF ORGANIC CONSTITUENTS IN FLUIDIZED-BED AND PULVERIZED COAL COMBUSTION: A TOPICAL REPORT

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the U.S. Department of Energy
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FOREWORD

The recently published "Status of Research on Physical, Chemical, and Biological Characterization of Particulate and Organic Emissions from Conventional and Fluidized-Bed Combustion of Coal: 1976 to the Present" (DOE/ER-0162), edited by C. H. Hobbs of the Inhalation Toxicology Research Institute (ITRI) for the U.S. Department of Energy, is a detailed summary of the major conclusions from research investigating the particulate and organic emissions from two modern coal combustion technologies. This report clearly indicates that, although particulate emissions from coal combustion have been analyzed in detail for physical properties, such as morphology, surface area, adsorption characteristics, and inorganic chemical composition (including trace metal analyses), very few data are available regarding the identities and quantities of organic compounds which are adsorbed on surface of the fly ash or those which remain in the vapor phase. Such data on organic emissions are needed in order to better assess the potential adverse health effects associated with commercial coal combustion, particularly in view of the worldwide increase in coal combustion.

Biological activity of such emissions can be assessed initially through the use of various *in vitro* bacterial and mammalian cellular assays, and further characterized through inhalation toxicological studies. It is the purpose of this report to detail the findings of research directed toward elucidating the identities and quantities of organic compounds found in fly ash extracts which have been shown to be biologically active through *in vitro* assays. This work has been carried out as a collaborative effort by the Laboratory for Energy-Related Health Research (LEHR) and the Pacific Northwest Laboratory (PNL).

EXECUTIVE SUMMARY

Coal combustion fly ash from both conventional pulverized coal combustion (PCC) and fluidized-bed combustion (FBC) have been characterized as to their organic constituents and microbial mutagenic activity. The PCC fly ash was collected from a commercial utility generating plant using a low sulfur coal. The FBC fly ash was from a bench-scale developmental unit at the Grand Forks Energy Technology Center. Bulk samples of each fly ash were extracted using benzene/methanol and further separated using high performance liquid chromatography (HPLC). Subfractions from the HPLC separation were analyzed by gas chromatography using both element-specific nitrogen-phosphorus detectors and flame ionization detectors.

Microbial mutagenicity assay results indicated that the crude organic extracts were mutagenic, and that both the specific activity and the overall activity of the PCC material was greater than that of the FBC material. Comparison of results from assays using S. typhimurium, TA1538NR indicated that nitrated polycyclic aromatic compounds (PAC) were responsible for much of the mutagenic activity of the PCC material. Similar results were obtained for assays of the FBC organic extract with standard and nitroreductase-deficient strains of S. typhimurium, TA100 and TA1538.

Mutagenically active HPLC fractions were analyzed using high resolution gas chromatography (HRGC) and GC mass spectrometry (GC/MS), as well as probe inlet low and high resolution MS. The PCC fly ash was found to contain several homologous series of nitrated polycyclic aromatic hydrocarbons (PAH), including alkylated nitrophenanthrenes and nitropyrenes and a series tentatively identified as alkylated nitrofluorenones. Later-eluting, mutagenically active HPLC fractions contained multi-nitrated PAH as well as nitrated nitrogen heterocycles. Several homologous series of oxygenated PAH were also found in certain mutagenically active HPLC fractions. The discovery and identification of nitrated, oxygenated PAC are important because the presence of both nitro and/or keto functionalities on certain PAC has been shown to confer or enhance mutagenic activity.

The FBC fly ash appeared to contain less organic material than did the PCC material. Mutagenic HPLC fractions from the FBC material contained mainly oxygenated PAH, including the known mutagen phenalen-1-one. Chemical analyses of the mutagenically active HPLC fractions failed to reveal the large quantities of nitro-PAC anticipated from the results of the mutagenicity assays using both parental and nitroreductase-deficient strains of S. typhimurium, although a small number of nitro-PAH were detected.

The studies reported here are preliminary, and caution should be used in drawing any conclusions regarding the relative potential for adverse health effects from exposure to PCC compared with exposure to FBC fly ash. The FBC fly ash was from a developmental combustor and does not necessarily represent that which might be obtained from a commercial-scale unit. Moreover, the possible effects of feed coal type and overall operating practices on the organic constituency of both the PCC and FBC materials are not known.

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Microbial Mutagenicity

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LIST OF ABBREVIATIONS

AAS =	atomic absorption spectroscopy
AFBC =	atmospheric fluidized-bed combustion
amu =	atomic mass unit
°C =	degrees Centigrade
cm =	centimeter
DOE =	U.S. Department of Energy
DPMS =	direct probe mass spectrometry
ESA =	electrostatic analyzer
eV =	electron volt
FBC =	fluidized-bed combustion
FID =	flame ionization detector
g =	gram
GC =	gas chromatography
GC/MS =	gas chromatography/mass spectrometry
HPLC =	high performance liquid chromatography
HRMS =	high resolution mass spectrometry
ICAP =	inductively coupled argon plasma emission spectroscopy
INAA =	instrumental neutron activation analysis
IR =	infrared
ITRI =	Inhalation Toxicology Research Institute
LEHR =	Laboratory for Energy-Related Health Research
LRMS =	low resolution mass spectrometry
LV/LRMS =	low voltage/low resolution mass spectrometry
m =	meter
μg =	microgram
ml =	milliliter
μl =	microliter
mm =	millimeter
μm =	micrometer
$M^{\ddagger}$ =	molecular radical cation (molecular ion)
MIKES =	mass-analyzed ion kinetic energy spectrometry
mmu =	millimass unit

MS = mass spectrometry

MS/MS = tandem mass spectrometry (multi-stage MS)

m/z = mass versus charge ratio.

ng = nanogram

N-PAC = nitrogen-containing polycyclic aromatic compounds

NMR = nuclear magnetic resonance spectrometry

NPD = nitrogen-phosphorus detector

OHER = Office of Health and Environmental Research

PAC = polycyclic aromatic compound(s)

PAH = polycyclic aromatic hydrocarbon(s)

PCB = polychlorinated biphenyl

PCC = pulverized coal combustion

PNL = Pacific Northwest Laboratory

ppm = parts per million

rev/mg = revertant colonies detected per milligram of test material in microbial mutagenicity assay

S-9 = metabolic enzyme system used in microbial mutagenicity assay

sec = second(s)

TA98 = designation for strain of Salmonella typhimurium used in microbial mutagenicity assay

TA100 = designation for strain of Salmonella typhimurium used in microbial mutagenicity assay

TA1538 = designation for strain of Salmonella typhimurium used in microbial mutagenicity assay

TA98nr = designation for strain of nitroreductase-deficient Salmonella typhimurium, TA98 used in microbial mutagenicity assay

TA100nr = designation for strain of nitroreductase-deficient Salmonella typhimurium, TA100 used in microbial mutagenicity assay

TA1538nr = designation for strain of nitroreductase-deficient Salmonella typhimurium, TA1538 used in microbial mutagenicity assay

TIC = total ion current

TCDD = tetrachlorodibenzo-p-dioxin

UV = ultraviolet

V = volt

v/v = volume to volume ratio

w/w = weight to weight ratio

XRF = X-ray fluorescence spectroscopy

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INTRODUCTION

The proposed increased use of coal as an energy source in the United States to supplement the petroleum fuels now in use has prompted questions regarding the potential health effects associated with current coal combustion technologies. The potential for adverse health effects from inadequately controlled coal combustion has been clearly demonstrated by the British experience, beginning during the industrial revolution in the mid 1800's and ending with the Clean Air Act of the 1950's. From epidemiological studies of that period, correlations were made relating increased incidence of respiratory diseases, such as emphysema and lung cancer, to coal smoke levels in the air of urban centers. Furthermore, these studies showed an approximate 20-year lag period between the beginning of substantial exposure and the onset of disease (Kennaway and Kennaway 1936). The Clean Air Act, which banned open coal fires in many urban centers, was adopted in 1954. By the beginning of the 1980's, lung cancer incidence in these centers was essentially indistinguishable from that of equivalent populations in rural areas (Waller 1981).

The loading of modern industrial environments with more than 5×10^4 tons of fly ash annually is of concern to those charged with maintaining the quality of the environment. Clearly, better decisions on appropriate control technologies and regulations can be made only if data are available concerning the chemical basis for potential adverse health effects. Through programs funded within the National Laboratories and in various other research facilities, the Department of Energy has compiled an extensive data base regarding the ecological and biological impact resulting from emissions produced by the coal combustion technologies of pulverized coal combustion (PCC) and fluidized-bed combustion (FBC).

Fluidized-bed combustion (FBC) technology has emerged as a possible alternative to the conventional pulverized coal combustion (PCC) for the direct utilization of coal. In fluidized-bed combustors, solid fuel is injected into a bed of inert material, such as sand or bentonite, which has been heated to a temperature higher than the ignition point of the fuel. Preheated air is forced through this bed. The constant mixing of bed material, fuel, and air

promotes more complete combustion and allows the use of fine-grained and low-grade fuels such as lignites, flycoke, and sawdust, as well as coal. FBC is also of interest because it allows the use of fuels with marginal heating values, such as certain mine tailings and biomass refuse, and because the quantities of stack emissions tend to be lower than those of comparable PCC facilities.

The results from several areas of coal combustion investigation have been summarized in recent reviews: the health and environmental research relative to solid wastes from coal conversion (Department of Energy 1982a), a risk analysis of FBC health and environmental effects in comparison with those from PCC (Seiter et al. 1982), the health and environmental research program with regard to coal conversion technology (Department of Energy 1982b), and the physical, chemical, and biological characterization of particulate and organic emissions (Hobbs 1983). This last review, prepared by C. H. Hobbs of the Inhalation Toxicology Research Institute, indicates that although a great deal is known relative to the physical and inorganic chemical characteristics of particulate emissions from coal combustion, very little detailed information regarding the qualitative and quantitative identification of organic species adsorbed on the fly ash was available for review. The purpose of this report is to make available additional chemical and toxicological information about organic compounds found in both PCC and FBC fly ash which have been examined in a joint effort by the Laboratory for Energy-Related Health Research (LEHR) and by Pacific Northwest Laboratory (PNL).

Most of the research regarding the biological health effects of coal combustion emissions has been focused on quantifying the mutagenicity and cytotoxicity of fly ash in in vitro microbial and mammalian cell assays. Work has also been carried out on the deposition, retention, and toxicity of the fly ash as determined by inhalation studies. The mutagenic activities of fly ash extracts in microbial studies have indicated the presence of mutagens, such as nitro-substituted polynuclear aromatic hydrocarbons (PAH), which do not require the addition of an external metabolic activation system (such as rat liver extract [S-9]) for expression of mutagenic activity. Nitro-PAH have also been

identified as major mutagenic species in diesel exhaust particle (Rappaport et al. 1983; Scheutzle et al. 1982; Newton et al. 1982; Yu and Hites 1981), and urban air (Bronstrom et al. 1982; Jager 1978; Ramdahl et al. 1982; Tokiwa et al. 1983; Talcott and Harger 1981; Gibson 1982). Thus, many recent efforts in analyzing the organic constituents adsorbed on fly ash have concentrated on identifying nitro-functional PAH species.

There are a number of difficulties in identifying compounds such as nitro-PAH that are responsible for the microbial mutagenicity. The total amount of organic material which can be extracted from fly ash is typically on the order of 10-1000 μg of material per gram of ash. Since this total mass may be composed of hundreds to thousands of organic compounds, the concentration of any individual component can easily be less than a part per million in an extract (or a part per billion in the fly ash). In addition to being found at trace levels, many of the compounds of interest are light-sensitive and/or thermally labile, causing problems in storage and analysis. The particulate collection process itself is involved in the production of chemical artifacts (Hanson et al. 1981a) which, when later extracted from the fly ash and analyzed, result in inaccurate assessments of the potential biological hazards associated with the particular emission. Finally, the overall lack of analytical-grade chemical standards for the higher molecular weight polynuclear aromatic compounds (PAC) and alkylated PAC, especially those containing functional groups such as carbonyl, hydroxyl and nitro-groups, often makes the task of positively identifying the components in combustion materials impossible and reduces the process of structure assignment to a "best guess" effort.

The approach taken by LEHR and PNL to the analysis of organic constituents found in coal fly ash has not been an attempt to catalogue a full list of organic compounds produced during coal combustion, but rather to identify and quantify those compounds which appear to pose the greatest threat of adverse health effects (especially carcinogenesis) from long-term, low-level exposure of fly ash to the general population and to utility power plant workers in particular. These data can be used to provide quantitative chemical information on the most active components. This is required for meaningful evaluation

of the relative health-effects risks associated with various types of fossil fuels and combustion technologies proposed and/or used for large-scale power production.

The selection of compounds for isolation and identification has been based largely on mutagenic activity found in microbial mutagenicity assays using special strains of Salmonella typhimurium (Ames et al. 1975). Organic extracts from fly ash which exhibited mutagenic activity were separated by high performance liquid chromatography (HPLC), and individual fractions from this procedure were tested for mutagenic activity. By repeated HPLC fractionation, individual components or groups of chemically related compounds responsible for mutagenic response in the microbial assays were isolated, then submitted to further characterization by gas chromatography (GC), mass spectrometry (MS) and other instrumental analyses. Ultimately, individual compounds identified in the fly ash extract can be tested for mutagenic activity individually or in combinations to determine the identities of the compounds primarily responsible for the overall mutagenic activity of the fly ash itself. Fly ash collection, size-classification, extraction, mutagenicity testing, HPLC separation, and some GC have been the responsibility of LEHR. PNL has been responsible for MS and further gas chromatographic characterization. Although this report will concentrate primarily on the successful application of MS to the identification of organic constituents in mutagenic fractions, data comparing the microbial mutagenicity of an FBC fly ash to that of a PCC fly ash are also included.

INORGANIC CHEMISTRY

BACKGROUND

The compositions of PCC and FBC fly ashes with regard to inorganic constituents have been studied in detail, and the results recently were reviewed (Hobbs 1983). The concentrations of minor and trace elements were shown to be roughly comparable in fly ash resulting from PCC and FBC technologies. Based on elemental composition alone, the two technologies probably have similar environmental impacts (Weissman et al. 1982). However, greater trace element enrichment is found associated with the smaller diameter particles of PCC fly ash than with the FBC fly ash particles of comparable size, probably as a result of the lower temperatures associated with FBC (Weissman et al. 1982; Hobbs 1983). Thus, lower or comparable levels of potentially toxic trace elements have been associated with FBC emissions in comparison with those from PCC.

With regard to the particular PCC and FBC fly ashes examined in this study, detailed inorganic chemical and physical characterizations have been performed on both types of ash, and the results of these studies have been published elsewhere (PCC: Coles et al. 1979; FBC: Hall et al. 1982; Sears 1982). Only a brief summary of data for these two fly ashes will be presented here.

INORGANIC CHEMICAL COMPOSITION OF PCC FLY ASH

The concentrations of 42 minor and trace elements found in four size-classified fractions of stack fly ash from a Western PCC power plant were reported by Coles et al. (1979). The cut #2 ash, having a mass median diameter of 6.0 μm, was the size-fractionated ash used in the present study. The reported elemental compositions for the four size-classified fractions are summarized in Table 1; the data for fractions 1, 3 and 4 are reported here for comparison with fraction 2. The elemental composition of the input coal that produced this ash is summarized in Table 2.

TABLE 1. Comparison of Elemental Concentrations in Size-Classified PCC Fly Ash Fractions[a]

Element	Concentration, μg/g (or %, if indicated) Fraction 1 (18.5 μm)[b]	Fraction 2 (6.0 μm)[b]	Fraction 3 (3.7 μm)[b]	Fraction 4 (2.4 μm)[b]
INAA and AAS[c]				
Al (%)	13.8 ± 0.1	14.4 ± 0.1	13.3 ± 0.6	13.9 ± 0.3
Ba(%)	0.168 ± 0.001	0.245 ± 0.002	0.31 ± 0.01	0.41 ± 0.02
Ca(%)	2.1 ± 0.1	2.23 ± 0.08	2.30 ± 0.14	2.36 ± 0.09
Co	8.9 ± 0.2	17.7 ± 0.4	20.3 ± 0.7	21.8 ± 0.4
Cr	28 ± 3	53 ± 3	64 ± 3	68 ± 3
Fe (%)	2.51 ± 0.09	3.09 ± 0.02	3.04 ± 0.08	3.2 ± 0.1
K (%)	0.74 ± 0.01	0.80 ± 0.07	0.82 ± 0.08	0.81 ± 0.03
Mn	208 ± 5	231 ± 5	269 ± 6	309 ± 3
Na (%)	1.22 ± 0.03	1.75 ± 0.05	1.81 ± 0.06	1.85 ± 0.03
Ni	25 ± 3	37 ± 1	43 ± 4	40 ± 2
Ti (%)	0.62 ± 0.05	0.74 ± 0.05	0.73 ± 0.1	0.77 ± 0.05
Zn	68 ± 1	189 ± 4	301 ± 9	590 ± 98
AAS Only[d]				
Be	6.3 (0.2)	8.5 (0.2)	9.5 (0.3)	10.3 (0.5)
Cu	56 (1)	89 (1)	107 (4)	187 (1)
Cd	0.4 (0.2)	1.6 (0.3)	2.8 (0.4)	4.6 (0.2)
Mg (%)	0.47 (0.01)	0.56 (0.01)	0.60 (0.02)	0.63 (0.01)
Pb	73 (3)	169 (2)	226 (4)	278 (3)
Si (%)	29.6 (0.7)	28.0 (0.1)	27.5 (0.3)	26.8 (0.1)
INAA Only[e]				
As	13.7 ± 1.3	56 ± 14	87 ± 9	132 ± 22
Ce	113 ± 4	122 ± 5	123 ± 6	120 ± 5
Cs	3.2 ± 0.1	3.7 ± 0.2	3.7 ± 0.2	3.7 ± 0.2
Dy	6.9 ± 0.3	8.5 ± 0.9	8.1 ± 0.3	8.5 ± 0.8
Eu	1.0 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.4
Ga	43 ± 12	116 ± 52	140 ± 23	178 ± 90
Hf	9.7 ± 0.4	10.3 ± 0.3	10.5 ± 0.3	10.3 ± 0.5

TABLE 1. (contd)

Element	Concentration, μg/g (or %, if indicated)			
	Fraction 1 (18.5 μm)(b)	Fraction 2 (6.0 μm)(b)	Fraction 3 (3.7 μm)(b)	Fraction 4 (2.4 μm)(b)
La	62 ± 3	68 ± 4	67 ± 11	69 ± 3
Mo	9 ± 2	28 ± 1.4	40 ± 5	50 ± 9
Nd	45 ± 4	47 ± 4	49 ± 7	52 ± 6
Rb	51 ± 3	56 ± 4	57 ± 3	57 ± 8
Sb	2.6 ± 0.1	8.3 ± 0.4	13.0 ± 0.7	20.6 ± 0.7
Sc	12.6 ± 0.5	15.3 ± 0.6	15.8 ± 0.6	16.0 ± 0.2
Se	19 ± 2	59 ± 2	78 ± 2	198 ± 20
Sm	8.2 ± 0.3	9.1 ± 0.4	9.2 ± 0.4	9.7 ± 0.4
Sr	410 ± 60	540 ± 140	590 ± 140	700 ± 210
Ta	2.06 ± 0.09	2.3 ± 0.2	2.5 ± 0.3	2.7 ± 0.1
Tb	0.90 ± 0.05	1.06 ± 0.06	1.10 ± 0.07	1.13 ± 0.06
Th	25.8 ± 0.6	28.3 ± 0.6	29 ± 1	30 ± 2
U	8.8 ± 1.9	16 ± 3	22 ± 4	29 ± 4
V	86 ± 44	178 ± 17	244 ± 18	327 ± 40
W	3.4 ± 0.2	9 ± 2	16 ± 2	24 ± 2
Yb	3.4 ± 0.4	4.1 ± 0.4	4 ± 0.2	4.2 ± 0.3
S (%)	0.101	0.304	0.425	0.711

(a) From Coles et al. (1979).
(b) Mass median diameters (mmd) determined by centrifugal sedimentation.
(c) Weighted average for all determinations, both techniques; INAA = instrumental neutron activation analysis; AAS = atomic absorption spectroscopy.
(d) Errors in parentheses are the range for duplicated determination.
(e) INAA values are the weighted averages of three determinations. Uncertainties are the largest of: twice the weighted standard deviation, the range, or the best estimate of the accuracy.

INORGANIC CHEMICAL COMPOSITION OF FBC FLY ASH

The Beulah fly ash used in this work was the subject of a detailed material balance study of 24 major, minor and trace elements (Hall et al. 1982; Sears 1982). This combustion test (Number FB2-BA5-2581) was performed at the Grand Forks, ND atmospheric-pressure fluidized-bed combustion (AFBC) unit with

TABLE 2. Concentrations of Elements in the PCC Input Coal[a]

Elements	Concentration, μg/g ± σ[b]	Elements	Concentration, μg/g ± σ[b]
INAA Measurement			
Al	29500 ± 2390	Sb	0.61 ± 0.09
As	2.2 ± 0.8	Sc	3.0 ± 0.2
Ba	420 ± 170	Se	1.7 ± 0.2
Ca	5620 ± 860	Sm	1.8 ± 0.2
Ce	27 ± 2	Sr	98 ± 8
Cl	48 ± 17	Ta	0.51 ± 0.06
Co	2.1 ± 0.2	Tb	0.22 ± 0.02
Cr	7 ± 1	Th	6.2 ± 0.7
Cs	0.72 ± 0.16	Ti	1230 ± 180
Dy	1.6 ± 0.1	U	2.1 ± 0.2
Eu	0.26 ± 0.02	V	25 ± 3
Fe	6470 ± 570	W	0.9 ± 0.4
Ga	8 ± 1	Yb	0.84 ± 0.06
Hf	2.4 ± 0.1	Zn	16 ± 3
In	0.039 ± 0.0006	Zr	67 ± 10
K	1730 ± 260		
La	13.4 ± 0.8	AAS Measurements	
Lu	0.23 ± 0.03	Be	1.2 ± 0.6
Mg	2240 ± 750	Cd	0.22 ± 0.02
Mo	2.7 ± 0.3		
Mn	60 ± 20	XRF Measurements	
Na	2930 ± 250	Cu	12.7 ± 0.6
Nd	11 ± 1	Ni	4 ± 1
Rb	12 ± 2	Pb	12.1 ± 0.7

(a) From Coles et al. (1979).
(b) Errors are 1σ deviation from the mean of the replicates.

Beulah, North Dakota lignite, using a bed composed of granular Shakopee, Minnesota dolomite. No ash was recycled to the bed. Combustion conditions were 23.8% excess air, a superficial (bed fluidizing) air velocity of 1.8 m/s, and a mean bed temperature of 788°C. Flue gas concentrations were 28 ppm (vol) SO_2, 562 ppm (vol) NO_x, and 30 ppm (vol) CO. The total run composite (average of seven analyses) of the elements measured for the baghouse fly ash in the material balance study is given in Table 3. The elemental analysis for the feed coal used in a typical combustion test (in this case, run 4) is given in Table 4.

TABLE 3. Metal Analysis[a] of Total Run Composite of FBC Baghouse Fly Ash[b]

Metal	Concentration, µg/g
Aluminum	35,900
Arsenic	38
Barium	5,040
Beryllium	8.8
Cadmium	<0.5
Calcium	220,000
Chromium	41
Cobalt	7.7
Copper	17
Iron	32,800
Lead	<8
Magnesium	96,100
Manganese	1,230
Mercury	<0.3
Molybdenum	<1
Nickel	16
Potassium	7,000
Selenium	<11
Silicon	32,700
Silver	<0.5
Sodium	67,600
Titanium	2,100
Vanadium	32
Zinc	27.8

(a) All elements with the exception of mercury were analyzed by inductively coupled argon plasma emission spectroscopy (ICAP). Mercury was quantitated by means of flameless atomic absorption spectrometry (AA).
(b) From Hall et al. (1982).

TABLE 4. Concentrations of Elements[a] in the FBC Input Coal[b]

Metal	Concentration, μg/g
Aluminum	6,360
Arsenic	4.55
Barium	198
Beryllium	1.7
Cadmium	0.14
Calcium	14,100
Chromium	2.0
Cobalt	1.7
Copper	2.4
Iron	6,910
Lead	<1
Magnesium	3,540
Manganese	26.0
Mercury	<0.05
Molybdenum	<0.2
Nickel	19.6
Potassium	440
Selenium	<2.2
Silicon	7,970
Silver	<0.1
Sodium	7,380
Titanium	231
Vanadium	2.8
Zinc	1.4

(a) All elements with the exception of mercury were analyzed by ICAP. Mercury was quantitated by means of flameless AA.
(b) From Hall et al. (1982).

BACKGROUND FOR ORGANIC CHEMICAL CHARACTERIZATION OF FLY ASH

The majority of analyses of PCC and FBC fly ash for organic compounds have focused on the detection and quantitation of PAH. However, recent reviews (Hobbs 1983; GCA Corporation 1980; Hanson et al. 1981a) have concluded that very little information regarding the organic constituents adsorbed on fly ash is actually available. Studies dealing with the extraction, separation and recoveries of PAH from PCC fly ash have shown that serious losses can occur due to adsorptive effects of the analytes and extraction rates, and overall recoveries are largely dependent on ring system size, the larger ring systems being more difficult to analyze successfully (Griest 1980). One recent study provided a detailed analytical study of a typical fly ash and the associated ESP (electrostatic precipitator) hopper ash from a PCC facility to determine whether the more easily obtained hopper ash could be used as surrogate for fly ash. This investigation established that there were significant differences between fly ash and hopper ash as to quantities of PAH, other high molecular weight hydrocarbons, elemental composition, and ultimate biological activity (Griest et al. 1979).

PCC fly ash has also been analyzed for tetrachlorodibenzo-p-dioxins (TCDD), with negative results (Kimble and Gross 1980), and for the presence of polychlorinated biphenyls (PCB), with positive results (Richard and Junk 1981). Organic extracts from PCC fly ash have also exhibited direct-acting mutagenicity in standard bacterial tests (Hansen et al. 1981). Other studies on similar ash, using nitroreductase-deficient strains of S. typhimurium, have shown that most of the mutagenic activity is due to nitro-substituted organic compounds which express mutagenic activity in the absence of external metabolic activation systems such as rat liver extract (S-9; Wei et al. 1982). Recently, nitropyrene has been positively identified and quantitated in both FBC (Mumford and Lewtas 1982) and PCC fly ash (Mumford and Lewtas 1982; Harris et al. in press).

Both vapor-phase and particulate-bound organic compounds found in FBC emissions have been sampled in recent studies (Newton et al. 1979; Hanson et al. 1979, 1980, 1981a, b, 1983). In general organic compounds have been

quantified in much higher concentrations in the vapor phase than in organic extracts of FBC particulate materials (Hanson et al. 1980). GC/MS was used to tentatively identify organic compounds in dichloromethane extracts of atmospheric FBC (AFBC) fly ash (Hanson et al. 1983; Merryman et al. 1977). Several classes of mutagenic organic compounds were identified in organic extracts from small experimental FBC, including 4- to 6-ring aromatic and highly polar compounds such as phenols, nitriles, ketones, and carboxylic acids (Kubitschek and Haugen 1980). Nitro aromatic compounds, corresponding to products from the reactions between NO_x and PAH previously determined in the sample by GC/MS, were tentatively identified using tandem mass spectrometry (MS/MS) to analyze extracts of FBC baghouse fly ash. Among these compounds were the dinitro-products of pyrene and fluoranthene, which were highly mutagenic in microbial frame-shift tests (Hanson et al. 1983).

EXPERIMENTAL

The following section deals specifically with the extraction, separation, and analyses performed on PCC and FBC fly ashes in a collaborative effort by PNL and LEHR. It should be noted at the outset that our goal has not been to generate a comprehensive list of organic compounds found adsorbed to coal fly ash; rather, we have directed the research toward the identification and quantitation of those compounds which are mutagenic in laboratory bioassays. All of the compounds described were found in extract fractions which expressed mutagenicity without metabolic activation; however, not all of the materials that have expressed mutagenicity have been examined. These initial studies were performed on fractions of fly ash organic extracts which were mutagenic and which could be isolated in reasonably large (ng to μg) quantities. Data on paraffins and PAH for example, are not included, even though some of the latter are known mutagens, because these compounds, although present in the fly ash extracts, were not found in the fractions examined in this study.

MATERIALS AND METHODS

Previous mutagenesis studies on the PCC fly ash samples clearly indicated that a significant fraction of the mutagenic activity was due to higher-molecular-weight nitro-PAC (Wei et al. 1982), which can be thermally unstable and difficult to analyze by GC. Rather than rely on GC for separating the fly ash components, we chose to use high performance liquid chromatography (HPLC) to separate the components in benzene/methanol extracts of the fly ash. By using repeated HPLC fractionation on a series of different preparative and analytical columns, it was possible (in some cases) to isolate individual components in essentially pure form. More often, the fractions generated contained mixtures of closely related compounds. A microbial mutagenicity assay was used throughout this study as a specific detector for mutagenic compounds in the initial HPLC fractions, identifying those fractions with high activity for further subfractionation and subsequent analysis by analytical HPLC, GC, GC/MS, and MS.

Collection and Preparation: PCC Fly Ash

The collection and size separation of the PCC fly ash has been described by McFarland et al. (1977). The ash used in this study (cut 2) was reported to have a volume median diameter of 6.3 μm (McFarland et al. 1977). The ash was extracted for 15 minutes with a mixture (60/40, w/w) of benzene/methanol, using an ultrasonic probe to disperse the particles. The use of the probe significantly increased the mutagenic activity of the extract over that obtained by simply shaking or stirring. In the first experiment, in which the mutagenicity of the HPLC fraction was initially determined by *in vitro* assays, 52 g of ash were extracted with 480 mL benzene/methanol, which was then evaporated at 30°C to 4 mL by a stream of nitrogen. In the second experiment, which generated samples for GC and MS analyses, 97 g of ash were extracted in 900 mL of benzene/methanol. The solution was then blown to dryness, and the residue was taken up in 100 μL of 50/50 (v/v) hexane/methylene chloride.

To measure the amount of nitropyrene (see page 50) in the PCC ash, a third sample of 95 g of fly ash was extracted and fractionated on the CN-10 preparatory column. The nitropyrene cut from the CN-10 column, corresponding to Fraction B (see page 45), was collected and analyzed on an MCH-10 analytical HPLC column. Calibration curves were prepared from commercially available 1-nitropyrene (Analabs, New Haven, CT; >99.7% pure). All laboratory manipulations used chromic-acid-cleaned glassware. The extraction procedure removed 1.9 mg of material per g of ash. During the concentration step, a clear, resin-like material precipitated from solution. No analytical studies were performed with this resin-like material, which represented 0.37 mg per g of ash or 19% of the total extracted mass. However, for completeness in assessing the biological activity of the fly ash, the material was redissolved in 1 mL of DMSO for mutagenicity studies.

Liquid Chromatography: PCC Fly Ash

Liquid chromatography was performed with a Varian (Palo Alto, CA) 5020 chromatograph equipped with a Varian Vari-chrom variable-wavelength ultraviolet (UV) detector and a Varian Vista 401 data system for disk storage and processing of data. The concentrated solution from the extraction of 52 g of PCC fly ash was fractionated at a flow rate of 3 mL/min on a 30-cm Varian MicroPak

CN-10 (alkylnitrile bonded to 10 μm diameter LiChrosorb) preparative HPLC column maintained at 30°C. The solvent program consisted of 100% hexane (Burdick and Jackson, Muskegon, MI; UV grade) for 2 min, programming to 5% methylene chloride (Burdick and Jackson, UV grade) at 1%/min, then programming to 100% methylene chloride at 4.75%/min. This column and program were used primarily to separate the mutagenic components from the remaining phthalate esters, which appeared at ~30-40 min.

Twelve fractions were collected for mutagenicity testing from the CN-10 preparatory column, as depicted in Figure 1. Fraction four was concentrated and then subfractionated on a 30-cm Varian MicroPak MCH-10 (monomeric octadecylsilane bonded to 10 μm diameter LiChrosorb Si60) analytical column (30 cm x 4 mm i.d.) to improve the resolution of the hydrocarbon components. The column was operated at a flow rate of 2 mL/min at 30°C, with a solvent program from 20% acetonitrile (Burdick and Jackson, UV grade): 80% water (Baker, Phillipsburgh, NJ; HPLC grade) to 100% acetonitrile in 20 min.

These same chromatography conditions were also used to fractionate an extract from 97 g of PCC fly ash to produce samples for mass spectrometric analysis. In this second fractionation, various cuts were taken to bracket the retention times of compounds suspected of being present and isolate well-defined chromatographic peaks. These collections are depicted as the cross-hatched areas in Figure 1. Additional subfractionation steps were carried out on the individual, collected fractions as described below.

Fraction A: The 15 to 16-min fraction from the CN-10 column was further purified by subfractionation on a 30-cm Varian TSK 1000H gel permeation column (7.5 mm i.d.), using benzene as the mobile phase at a flow rate of 1 mL/min at 30°C with the UV-visible detector set at 322 nm. The major peak from the gel column eluted from 7.6 to 8.6 min, and these components were collected and concentrated by slow evaporation.

Fraction B: The 17.6 to 19-min fraction from the CN-10 column was further purified using the same column and conditions described for Fraction A, except that the detector wavelength was set at 400 nm. The major components, which eluted as a single peak from the gel column between 8.5 and 9.5 min, were collected and concentrated by slow evaporation.

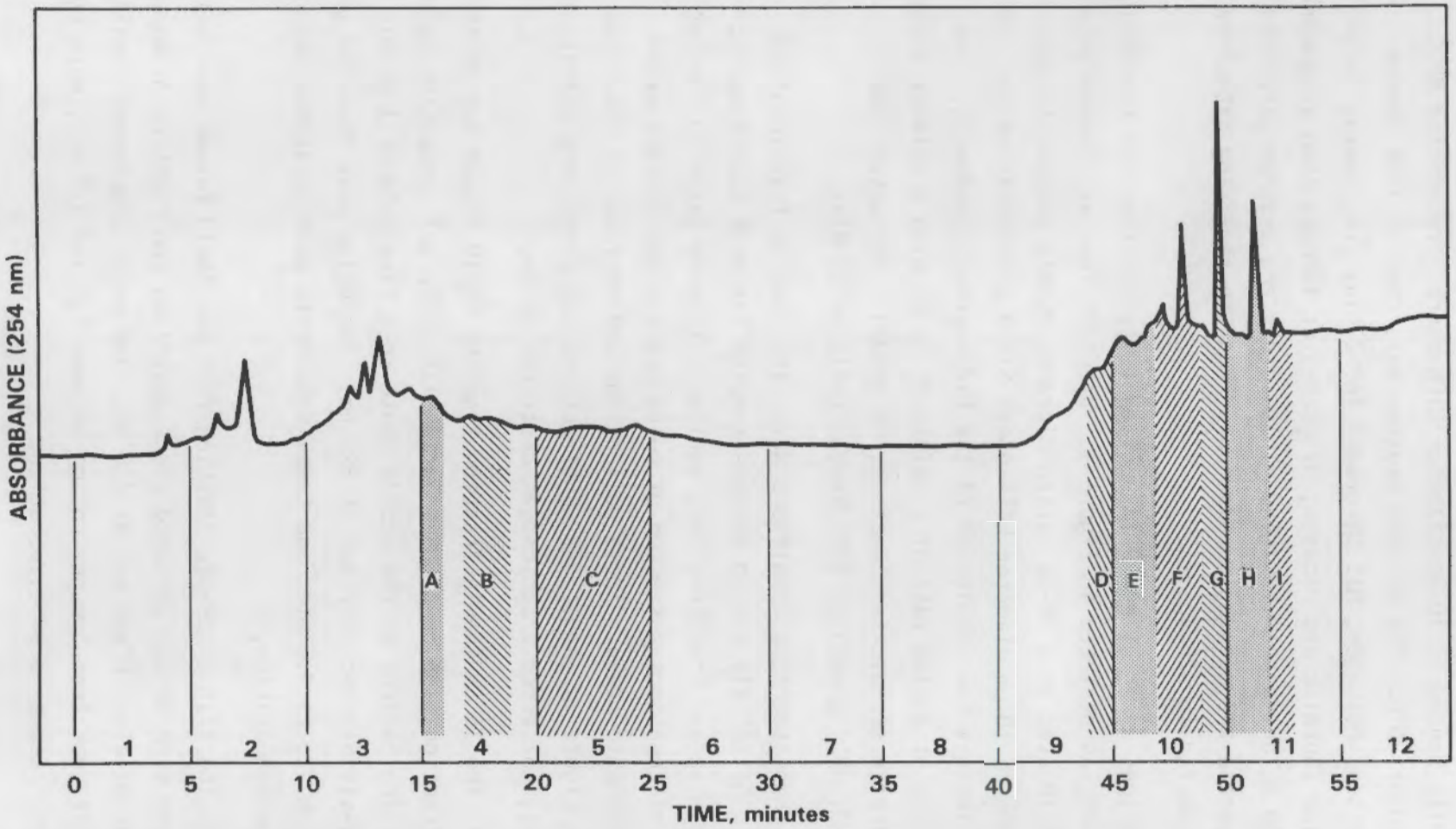

FIGURE 1. HPLC Chromatogram for the Fractionation of a Benzene/Methanol Extract of PCC Coal Fly Ash Using a Preparative CN-10 Column. The numbered segments show the range of elution times represented by each fraction used in the mutagenesis assays. The lettered cross-hatched segments show the range of elution times represented by each fraction collected for chemical characterization.

Fraction C: The 20 to 25-min fraction from the CN-10 column was further purified using the same column and conditions described for Fraction A, except that the detector wavelength was set to 295 nm. The major components, which eluted as a single peak between 6.6 and 7.7 min, were collected and concentrated by slow evaporation.

Fraction D: The 20 to 44-min fraction from the CN-10 column was blown dry under a steam of dry N_2 gas, redissolved in 50/50 hexane/methylene chloride, and rechromatographed under the same conditions as the first fractionation. The largest peak in the chromatogram, measured at 400 nm, eluted at 44-45 min; these components were collected, blown to dryness, and then dissolved in benzene. The extract was fractionated for a final time using the gel permeation column under the conditions described for Fraction A, except that the detector wavelength was set to 400 nm. The major components, which eluted between 7.1 and 8.7 min as a single peak, were collected and concentrated under a stream of dry N_2 gas. This fraction contained a subset of the materials found in Fraction E.

Fraction E: The 44 to 46-min fraction from the CN-10 column was further purified using the gel permeation column with a detector wavelength of 325 nm under the conditions given for Fraction A. The components eluting between 7.5 and 8.5 min were collected and blown dry under a stream of dry N_2 gas.

Fraction F: The 46 to 47-min fraction from the CN-10 column was fractionated for a final time using the gel permeation column as described for Fraction A, except that 400 nm was used as the detector wavelength. The largest peak eluted between 6.5 and 7.5 min, and these components were collected and blown dry under a stream of dry N_2 gas.

Fraction G: The 49 to 50-min fraction from the CN-10 column was not further purified.

Fraction H: The 50 to 52-min fraction from the CN-10 column was chromatographed for the final time using the gel permeation column with a detector wavelength of 400 nm under the conditions given for Fraction A. The components from the largest peak, which eluted between 6.5 and 8.0 min, were collected and blown dry under a stream of dry N_2 gas.

Fraction I: The 52.5 to 53.6-min fraction from the CN-10 column fractionated a final time using a reverse-phase MCH-10 preparatory column with a detector wavelength of 395 nm. The column was operated at a flow rate of 2.5 mL/min, and the solvent was programmed linearly from 20% acetonitrile/80% water to 100% acetonitrile over a 20 minute period. The components from the largest peak, eluting from 19.2-20.5 min, were collected and then blown dry using a stream of dry N_2 gas.

Collection and Preparation: FBC Fly Ash

The FBC fly ash examined in this study was generated at the Grand Forks experimental atmospheric-pressure fluidized-bed combustion (AFBC) unit, details of which are published elsewhere (Goblirsch and Sondreal 1979; Talty et al. 1981). The particulate control system consisted of two cyclones, followed by a pulse-jet baghouse. Coarse particles larger than ~5 μm mass median aerodynamic diameter were captured by the two cyclones. Therefore, the particulate matter presented to the baghouse was largely in the respirable range and had a high specific surface area. The particulate AFBC combustion test in which the Beulah fly ash used in this study was collected was the subject of a detailed material balance study of 24 major, minor, and trace elements (Hall et al. 1982; Sears 1982). Consequently, the experimental conditions and ash composition are well documented.

Fly ash was collected from the hopper of the pulse-jet baghouse, equipped with Teflon-coated fiberglass fabric bags. An ash composite from the entire 288-hr run was blended by slow tumbling, overnight, in a polyethylene container. This resulted in a bulk volume reduction of 67% as a result of ash particle aggregation. The ash (100 g) was extracted for 15 min with a mixture (60/40, w/w) of benzene/methanol, using an ultrasonic probe to provide agitation. A volume of extract representing 5 g of the ash was reserved for mutagenicity testing. The remaining extract, representing 95 g of fly ash, was blown dry under dry N_2 gas and taken up in a methylene chloride/hexane mixture (50/50, v/v) for chromatography.

Liquid Chromatography: FBC Fly Ash

Liquid chromatography on the FBC fly ash extract was performed using the same equipment described previously for the PCC extracts. The first fractionation of the total extract from 67 g of FBC fly ash was performed using the 30-cm Varian MicroPak CN-10 preparative HPLC column operated at 30°C with a flow rate of 3 mL/min. The binary solvent program consisted of 100% hexane for 2 min, programming to 5% methylene chloride at 1%/min, holding at 95% hexane for 28 min, then programming to 100% methylene chloride at 4.75%/min and holding at 100% methylene chloride for 12 min. The six fractions collected corresponded to the elution of discrete components as detected in the chromatogram and measured at 390 nm. (See Figure 2.) The decision to collect each of these fractions was made either because the component peak was large and well-defined (fractions 1 and 3), or because the retention time closely matched one of the major component peaks seen in the PCC fly ash separation (fractions 2, 4, 5 and 6). Because this separation was performed using a different solvent program than that used for the initial fractionation of the PCC fly ash extract, the chromatograms in Figures 1 and 2 are not directly comparable. To allow for a direct comparison, a small amount of PCC fly ash extract was chromatographed using the same solvent program as for the FBC fly ash extract; the two comparable chromatograms are shown in Figure 45.

Subfractionation of the six fractions collected from the CN-10 column was carried out using the Varian MicroPak MCH-10 analytical column operated at 30°C and with a 2 mL/min flow rate. The solvent program was the same for each subfractionation, consisting of programming from 80% water to 100% acetonitrile at 4%/min. The detector was set to monitor 390 nm. The largest peak in each chromatogram was collected, and the retention times for the various peaks on the MCH-10 analytical column are given in Table 5. Note that fraction 5 had two major components, denoted as 5a and 5b; both were collected.

To provide samples of FBC fly ash extract for mutagenicity studies, a total of 15.07 g of ash were extracted as described above, and the volume of benzene/methanol was reduced to 3 mL by evaporation under a stream of dry N_2 gas. One mL was evaporated to dryness and the residue taken up in 1.6 mL DMSO for testing the mutagenicity of the total extract. The remaining 2 mL were

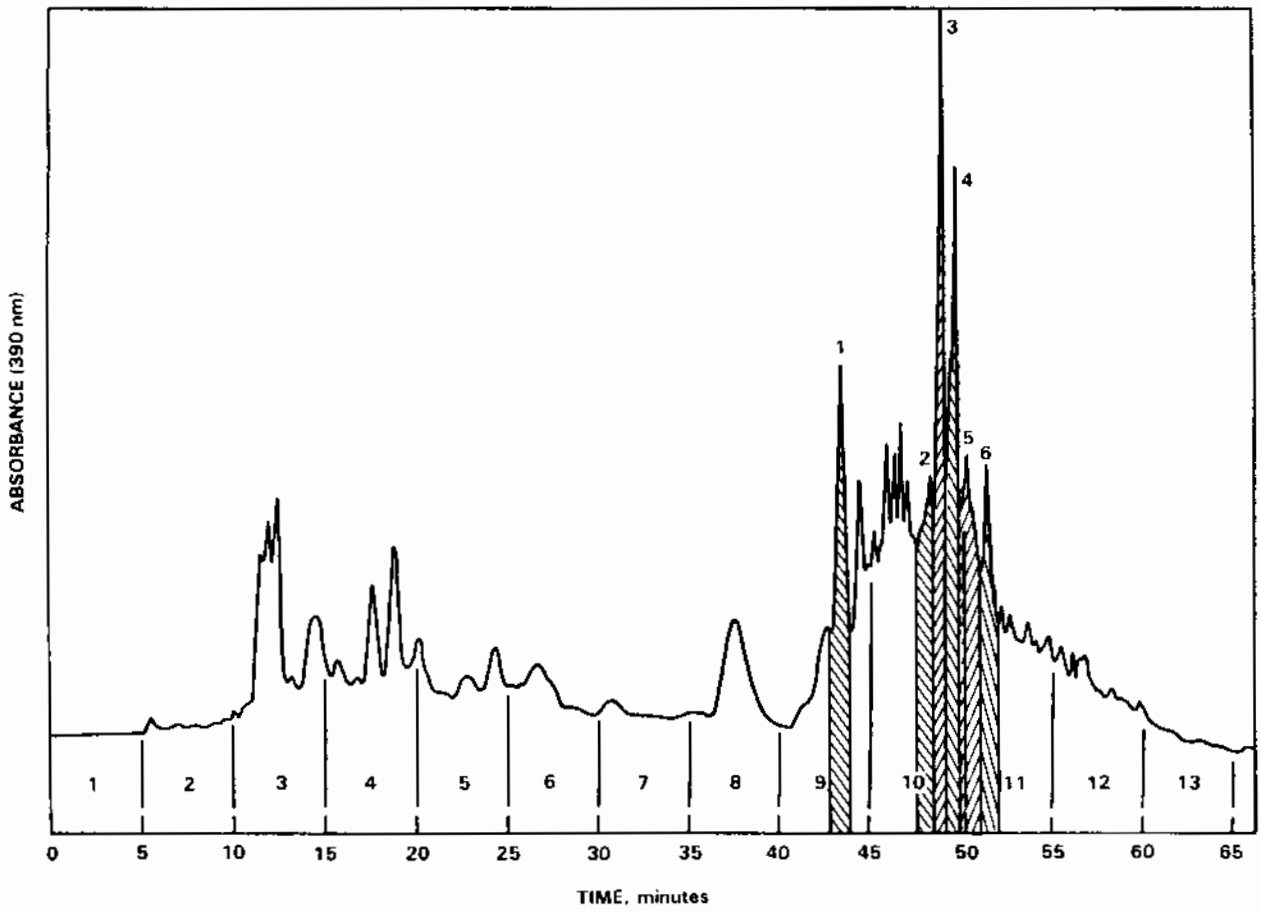

FIGURE 2. HPLC Chromatogram for the Fractionation of a Benzene/Methanol Extract of FBC Coal Fly Ash Using a Preparative CN-10 Column. The numbered segments show the range of elution times represented by the first 13 fractions used in the mutagenesis assays. The numbered, cross-hatched segments show the range of elution times represented by each fraction collected for chemical characterization.

TABLE 5. Retention Times for the Seven FBC Fly Ash HPLC Fractions Collected from the MCH-10 Analytical Column

Peak No.	T_R, min
1	15.014
2	10.474
3	8.993
4	12.731
5a	10.345
5b	11.010
6	14.856

evaporated to dryness, and the residue taken up in 100 μL of methylene chloride/hexane (25/75, v/v). Fifty-μL aliquots of this solution were fractionated using the CN-10 preparatory column operated under the conditions previously described for the FBC fly ash separation. Fractions were collected every 5 min, resulting in a total of 15 fractions; the first 13 are depicted in Figure 2. Each fraction was blown to dryness under a stream of nitrogen, and the residue dissolved in 1.2 mL DMSO.

Gas Chromatography

Gas chromatograms of the twelve PCC fly ash HPLC fractions were obtained with a Varian model 3740 chromatograph equipped with a 30-m SE-54 fused-silica capillary column (J&W Scientific, Rancho Cordova, CA), an effluent splitter, and dual flame ionization and thermionic detectors, the latter being selective for nitrogen and phosphorus compounds. The data were stored and manipulated with the Varian Vista 401 chromatography data system.

A Hewlett-Packard (Palo Alto, CA) 5880 gas chromatograph equipped with a 25-m x 0.25-mm Durabond-5 (DB-5) (polymethyl [5% phenyl] siloxane, bonded phase) fused-silica capillary column (J&W Scientific) was used to determine the retention indices of all the possible mononitro isomers of pyrene and fluoranthene. Retention index measurements for benzo[c]cinnoline, 9-fluorenone, phenalen-1-one, nitrophenanthrenes and the nitromethylphenanthrenes were measured using a 30-m x 0.25-mm DB-5 column. Splitless vaporization was the sample introduction mode. The retention index system employed for these measurements has been described by Lee et al. (1979) and Vassilaros et al. (1982). The 1-, 2-, 3-, 7-, and 8-nitrofluoranthene standards were obtained from M. Lee (Dept. of Chemistry, Brigham Young University) and Raymond Castle (Dept. of Chemistry, University of South Florida) and their coworkers. 1-Nitropyrene, benzo[c]cinnoline, and 9-fluorenone were acquired from Aldrich Chemical Company (Milwaukee, WI); 2- and 4-nitropyrene standards were provided by W. P. Duncan of the Midwest Research Institute. Phenalen-1-one was supplied by J. A. Leary (Dept. of Chemistry, M.I.T.). All other standards used in these studies were acquired through commercial sources.

Mass Spectrometry

Capillary column GC/MS of the PCC-derived fractions was conducted with a Hewlett-Packard 5982A integrated GC/MS system. The gas chromatograph was equipped with a 15-m x 0.25-mm DB-5 fused-silica column (J&W Scientific). The end of the column was inserted through modified transfer lines to within 5 to 10 mm of the source ionization beam. For the more volatile samples, and the nitropyrene samples in particular, the GC oven was held at 50°C during sample introduction onto the column via a split/splitless vaporization injector held at 250°C and operated in the splitless mode. The capillary column was then programmed from the initial oven temperature to 270°C at 8°C/min. The best results for the less volatile samples were obtained by using a shortened capillary column (approximately 7-10 m), on-column GC injection, and programming to temperatures exceeding 300°C. The mass spectra were obtained in the electron impact mode with an electron energy of 70 eV. Spectra were acquired and processed with a Hewlett-Packard 5934A data system.

Direct probe low resolution mass spectra (LRMS) of both the PCC-and FBC-derived samples were recorded on a VG Micromass ZAB-1F mass spectrometer, a double-focussing instrument of reverse Nier-Johnson geometry (i.e., magnetic analyzer precedes the electrostatic analyzer), operated with an accelerating potential of 6000 or 7000 V and a nominal dynamic mass resolution of 1:2000. The ion source temperature was 250°C. Most analyses performed in the electron impact ionization mode used an electron energy of 70 eV and a trap current of 500 μA. Chemical ionization (CI) experiments were conducted using methane (99.99%, Matheson, East Rutherford, NJ) as the ionizing reagent at a measured pressure of 5×10^{-5} torr in the source housing (approximately 0.1-1 torr source pressure). Low voltage LRMS (LV/LRMS) analyses, using ionizing electron potentials in the 10- to 14-eV range and a trap current of 50 μA, reduced fragmentation and produced simplified spectra consisting primarily of molecular ions. The LV/LRMS results were very useful for indicating the species to look for in subsequent GC/MS analyses, which provided fragmentation patterns for individual components. All direct probe samples were introduced into the source via a small glass capillary tube mounted on the end of a direct insertion probe, which was heated to 180-260°C to assure complete volatilization of

the sample. Preliminary GC/MS analyses on the FBC fly ash extracts were made with this instrument coupled to a Hewlett-Packard Model 5840A gas chromatograph, equipped with a DB-5 fused-silica capillary column (J&W Scientific). The end of the column was inserted through modified transfer lines, maintained at 300°C, to within 5 mm of the source's ionizing electron beam. The experiments were conducted with a dynamic resolution of 1:1500 and a scan rate of 2 sec/decade of mass. All spectra for all experiments were acquired and processed using a VG Data System 2035.

High resolution analyses were carried out on the PCC-derived fractions using the ZAB-1F at a dynamic resolution, as measured by the data system, equal to or greater than 1 part in 10,000 (10% valley definition). Data from selected scans were averaged together to improve the precision and accuracy of the mass assignments. Elemental compositions were assigned to masses in the spectra only if all three studies (GCMS, LRMS, and LV/LRMS) indicated that the mass could be uniquely assigned to a given component.

Unimolecular metastable ion decompositions for ions of interest were monitored for reactions occurring in the second field-free region (between the magnetic and electrostatic sectors) using mass-analyzed ion kinetic energy spectrometry (MIKES). In this technique, the magnet is set to pass ions of a selected mass-to-charge ratio, some of which undergo unimolecular decomposition in the region between the magnet and the electrostatic analyzer (ESA). By leaving the magnet fixed and scanning the voltage applied to the ESA, fragment ions (daughter ions) resulting from the decomposition of the mass-selected ions (parent ions) are brought into focus at the detector and can be recorded. Magnetic sector resolution was nominally 1:1000 for these analyses and the collector slit was set to give a triangular peak shape (approximately 90% transmission) when the electrostatic analyzer was scanned across the primary ion beam. Data were recorded using either the 2035 Data System or a light beam oscillographic recorder (Hewlett-Packard model 5-154).

Mutagenicity Assays: PCC Fly Ash Extracts

Microbial mutagenicity assays were performed on the 12 PCC-derived fractions as described previously with S. typhimurium strains TA1538, TA100, and TA98 (Ames et al. 1975; Wei et al. 1982). Samples of the total extract were

prepared by evaporating an appropriate volume of extract to dryness and dissolving the residue in DMSO to give the desired value of mg of extracted ash per 25 μL of DMSO. In brief, 0.1 mL of overnight cultures of each bacterial strain was mixed with 2 mL of top agar and 25 μL of DMSO containing the extract from increasing weights of fly ash (ranging from 0 to 1D0 mg), and spread on bottom agar. Replicates of four were plated, incubated for 48 hr, and revertants to his^+ were counted with an automatic colony counter (New Brunswick Scientific). The slopes of the plotted numbers of revertants per plate versus ash extract (mg/25 μL) were used to calculate the revertants/mg fly ash. The reversion rate was compared with the activity of 1-nitropyrene. The resin which precipitated during the concentration of the total extract was dissolved in 1 mL DMSD, and 25-μL aliquots were tested in the manner described above.

Fractions collected from the HPLC were evaporated to dryness, and the residues taken up in 700 μL of DMSO. S. typhimurium, TA1538, was used to determine the mutagenicity of 25-μL aliquots of these fractions in experiments conducted both with and without metabolic activation from rat liver extract (S-9).

Mutagenicity Assays: FBC Fly Ash Extracts

The mutagenicity of the ash extract was tested with three strains of histidine-requiring S. typhimurium, TA1538, TA100 and TA98, and three nitroreductase-deficient strains, TA1538nr, TA100nr, and TA98nr1, obtained from Dr. H. S. Rosenkranz, New York Medical College, Valhalla, NY. The assay was conducted essentially as previously described for the PCC fly ash extracts. The S-9 preparation in KCl, from Aroclor-induced rats, was purchased from Litton (Charleston, SC). In brief, the test material was added in 25 μL of DMSO per 2 mL of top agar, 0.1 mL of an overnight culture of each tester strain and 0.1 mL of S-9 preparation or solvent control. The S-9 was omitted with the nitroreductase-deficient strains. After 48 hr, colonies were counted with an automatic colony counter. 2-Nitrofluorene at 1 μg per plate was used as a positive control for nitroreductase-deficient strains (Wei et al. 1982). Characteristics of all strains were checked for each experiment. The nitroreductase-deficient strains were used to determine the role of nitro compounds in the mutagenicity of ash extract.

In each experiment, extracts from three or four different amounts of fly ash were tested with three or four replicate plates for each sample of ash. The initial slope of the line was used to calculate the revertants per mg of initial ash extracted. The Salmonella added per plate was usually between 1.2 and 2.4 x 10^7 cells in 0.1 ml of suspension. The extracts were judged not toxic by the appearance of minute background colonies (Ames et al. 1975). Each HPLC fraction was tested for mutagenicity with TA1538, TA98 and TA98nr1, with and without microsomal (S-9) activation.

RESULTS AND DISCUSSION

MUTAGENICITY OF PCC FLY ASH EXTRACTS

The LC chromatogram of the total extract from 52 g of PCC-derived fly ash is shown in Figure 1. Bacterial mutagenesis assays with TA1538 were performed on all fourteen fractions (of which the first twelve are shown in Figure 1). Fractions 1 and 2 (0 to 10 min) and 12 to 14 (55 to 70 min) were not mutagenic. The intervening fractions, 3 through 11, were mutagenic, as shown by the data in Table 6. The mutagenic activity is reported as revertants/plate and is compared with that of 2-nitrofluorene, which was run simultaneously as a positive control.

TABLE 6. Mutagenicity of HPLC Fractions of the Benzene/Methanol Extract of PCC-Derived Fly Ash in Salmonella typhimurium, TA1538[a]

Fraction	Elution Time, min	+ S-9 rev/plate	- S-9 rev/plate	% of Total
SR[b]	---	14 ± 4	8 ± 1	---
2-nitrofluorene (0.5 μg)	---	197 ± 17	351 ± 40	---
3	10 - 15	371 ± 38	151 ± 18	3.0
4	15 - 20	518 ± 39	779 ± 41	15.3
5	20 - 25	967 ± 93	1032 ± 86	20.3
6	25 - 30	322 ± 2	1083 ± 115	21.3
7	30 - 35	208 ± 12	449 ± 65	8.8
8	35 - 40	208 ± 14	335 ± 27	6.6
9	40 - 45	376 ± 15	653 ± 56	10.8
10	45 - 50	430 ± 36	551 ± 10	10.8
11	50 - 55	125 ± 14	59 ± 8	1.2
		Σ = 3525	Σ = 5092	Σ = 100.1

(a) From Harris et al. (1984).
(b) Spontaneous revertants.

The addition of S-9 liver homogenate decreased the number of revertants of TA1538 per plate for all fractions except 3 and 11. Wei et al. (1982) have reported that the mutagenic activity associated with the total benzene/methanol extract of this ash is greatly decreased in nitroreductase-deficient variants of the S. typhimurium strains TA1538, TA100 and TA98. This decrease in activity upon addition of S-9 appears to be a common, though not universal, feature of nitro-PAH mutagenicity in Salmonella (Tokiwa et al. 1981). The data for the individual fractions indicate that the activity due to organic nitro-containing compounds is widely distributed, with not more than 22% of the total mutagenic activity located in any one fraction. From 10 to 22% is found in each of fractions 4, 5, 6, 9, and 10. Thus, there appears to be a variety of mutagens expressed without metabolic activation in this fly ash extract.

To avoid losing substantial amounts of mutagenic activity when the resin was separated from the more soluble extractable components, we redissolved the resin in DMSO and evaluated for mutagenic activity along with samples of the total extract. The resin was mutagenic in all three strains of S. typhimurium, without activation, but the total resin accounted only for 2 to 3% of the activity of the total extract.

HPLC retention times were measured for 15 nitro-PAH standards. Mononitro-PAH with 2 to 4 rings eluted from the CN-10 column at 10 to 20 min; the corresponding dinitro-PAH eluted at 25 to 45 min. From the distribution of the mutagenic activity in the fractions shown in Figure 1, it is likely that a substantial portion of the total mutagenic activity is due to di- and probably trinitroaromatics or to nitro compounds with other polar substituents.

Dose response curves for direct mutagens in the Ames mutagenesis assay were measured for both the unfractionated fly ash extract and pure 1-nitropyrene (certified >99.7% 1-nitropyrene) in TA1538, TA98, and TA100. The results are shown in Table 7. The 1-nitropyrene activity is similar to literature values from Mermelstein et al. (1981), in which care was taken to eliminate contamination by dinitro compounds. Based on a concentration of 12 ppb of nitropyrene in the coal fly ash (see results from chemical characterization of Fraction B), we calculated that the nitropyrene is responsible for 0.04% to 0.17% of the total mutagenic activity, depending on the particular strain of

TABLE 7. Contribution of Nitropyrene to the Total Mutagenic Activity of PCC-Derived Coal Fly Ash[a]

	Fly Ash[b]	1-Nitropyrene[c]	% (Fly Ash)[d]	% (Diesel)[d,e]
TA98	13.8 ± 1.1	1880 ± 80	0.17 ± 0.01	18 ± 5
TA100	10.5 ± 1.1	730 ± 20	0.083 ± 0.009	5 ± 4
TA1538	11.7 ± 1.1	430 ± 60	0.044 ± 0.004	12 ± 5

(a) From Harris et al. (1984).
(b) Activity reported in rev/mg extracted ash.
(c) Activity reported in rev/μg.
(d) Percent of mutagenic activity due to nitropyrene.
(e) Data on diesel from Salmeen et al. (1982).

S. typhimurium used in the calculation. Because of the extremely high mutagenic activity of the dinitropyrenes, even low levels of contamination can alter the apparent activity of 1-nitropyrene. If one assumes the worst case of 0.3% contamination of the 1-nitropyrene by dinitropyrene, the activity in the total extract due to nitropyrene would decrease to approximately 60 to 85% of the initial calculated values, based on the relative mutagenic activity of mono- and dinitropyrene reported by Mermelstein et al. (1981). Thus, the uncertainty can be accommodated by using 0.02%, as the lower limit, rather than 0.04%.

Most of the activity that eluted in the range of mononitro compounds appeared to be in fraction 4. Therefore, this fraction was concentrated and subfractionated on an MCH-10 analytical HPLC column. The resulting chromatogram is shown in Figure 3. Ten subfractions were collected and evaluated for mutagenic activity. Those compounds responsible for the mutagenic activity eluted at between 13 and 18 min, with the highest activity in subfraction 4.8 (14 to 15 min). Summation of the activity for all the mutagenic subfractions represented only about 34% of the mutagenic activity observed in the original fraction 4. It is not clear whether a substantial amount of material was irreversibly adsorbed or chemically altered during the second HPLC fractionation step, or whether the separation of components reduced cooperative matrix effects that may have enhanced the activity of the total complex extract.

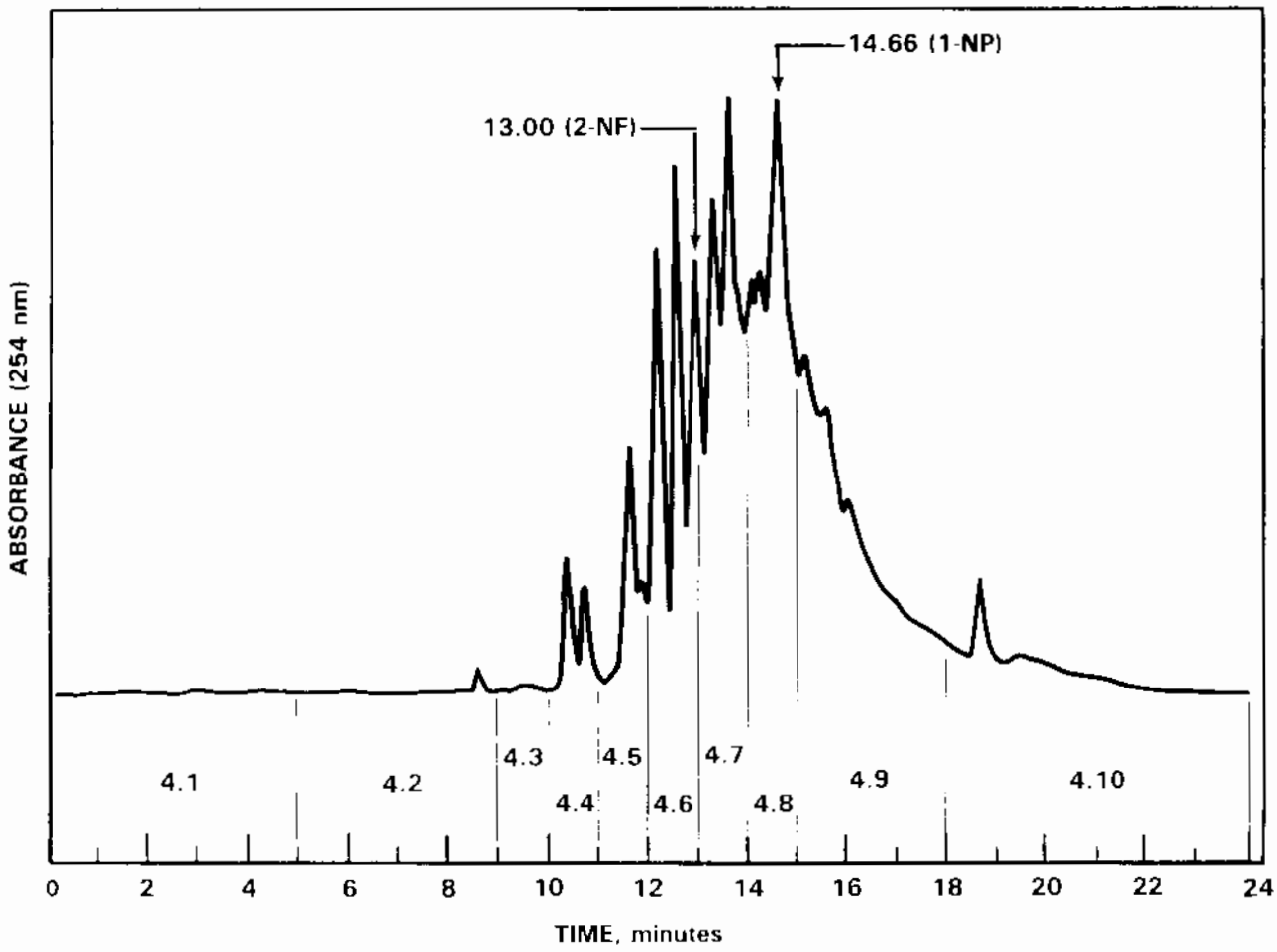

FIGURE 3. HPLC Chromatogram for Subfractionation of Fraction 4 from PCC Fly Ash Extract on an Analytical MCH-10 HPLC Column. Retention times for 2-nitrofluorene (2-NF) and 1-nitropyrene (1-NP) are indicated.

Based on the retention time of the standard compound on the CN-10 column, 1-nitropyrene should have eluted in fraction 4. Furthermore, a sample component of fraction 4 eluted at 14.7 min on the MCH-10 column (Figure 3), corresponding to the correct retention time of 1-nitropyrene. Additionally, GC analyses of subfraction 4.8 produced responses having retention times appropriate for 1-nitropyrene using both universal (flame ionization, FID) and nitrogen- and phosphorus-specific (NPD) detection. Thus, 1-nitropyrene was tentatively identified from its chromatographic behavior on two HPLC columns and by GC detector and retention data.

MUTAGENICITY OF FBC FLY ASH EXTRACTS

All six strains of S. typhimurium (TA1538, TA100, TA98, TA1538nr, TA100nr, and TA98nr) were exposed to increasing amounts of total fly ash extract. Dose response curves were linear for all strains up to 40 mg fly ash, and the general appearances of the curves for the parent strains with the addition of S-9 liver homogenate were very similar to the results obtained without the addition of S-9. However, statistical analysis of the data on the parental strains, with and without the addition of S-9, showed some significant differences. In some cases, moreover, the S-9 appeared to increase the number of revertants; in other cases, the addition of S-9 reduced the activity of the extract. There were also statistically significant differences between the slopes obtained from replicate experiments. The presence of polycyclic organic compounds in this ash has been demonstrated by Sears (1982), but we believe that there is little, if any, actual activation of indirect mutagens by S-9, and that the variability between experiments with and without S-9 is primarily a reflection of the limited reproducibility of the assay.

The initial slopes of the dose response curves (in rev/mg ash) from replicate experiments are summarized in Table 8. The ash extract is clearly

TABLE 8. Activity of Fluidized-Bed Fly Ash in Standard and Nitroreductase-Deficient Strains of Salmonella typhimurium

Strain	Mean Rev/mg	Range of Activity[b], Rev/mg
TA98	4.3[a]	3.4 - 5.3
TA98nr	1.3	1.0 - 1.7
TA100	3.2	2.4 - 4.7
TA100nr	0.3	0.1 - 0.5
TA1538	5.8	4.3 - 6.6
TA1538nr	0.4	0.2 - 0.6

(a) Includes both + and - (S-9).
(b) Range of results from three replicate experiments.

mutagenic in all three standard strains, with specific activities of 3.2 to 5.8 rev mg^{-1}. There was a substantial decrease in the slopes of the nitroreductase strains relative to those of the corresponding standard strain; in all cases, the differences in slopes were statistically significant. Although the activity in the nitroreductase-deficient strains was low, the dose response slopes were significantly different from zero by analysis of variance. Thus, the extract was considered mutagenic in all six strains. The activity of the nitroreductase-deficient strains with 2-nitrofluoranthene was always less than 30% of the standard strain and usually close to the number of spontaneous revertants. (See Table 9.)

The HPLC profile of the FBC extract separated on the CN-10 column is shown in Figure 2. Assay of the 15 fractions from HPLC showed one major peak of activity, using TA1538 and TA98 as tester strains, as shown in Table 10. This peak eluted between 40 and 55 min (fractions 9-11). Activities with and without rat liver microsomal activation were comparable (Table 10). Further confirmation that nitro-substituted compounds were responsible for a significant amount of mutagenic activity is shown in Table 9, where the results of assays using strains TA98 and TA98nr are compared. The TA98nr results showed a peak of activity in the same fractions as the results for TA98, but with only 18 to 19% of the activity. This was confirmed in two experiments. TA98nr demonstrated residual nitroreductase activity to total extract as well. This may be due to the fact that cells contain several nitroreductase enzymes responsible for metabolizing different nitro-compounds (Rosenkranz et al. 1981). The presence of other direct-acting mutagens that do not contain nitro groups may also be responsible for this activity.

Retention times have been measured for 15 mononitro aromatic compounds containing from one to five rings (see Mutagenicity Results for PCC Fly Ash Extracts). The longest retention time associated with a mononitro PAH (3-nitroperylene) was 26 min. Since the peak in mutagenic activity eluted in the 40- to 55-min range, it is highly unlikely that mononitro compounds which are not substituted with additional polar groups represent a significant contribution to the overall mutagenic activity of the FBC extract.

TABLE 9. Comparison of Mutagenicity of FBC Fly Ash HPLC Fractions in TA98 and TA98nr Strains Without S-9 Activation

Fraction	TA98	%	TA98nr	%
1	0[a]	--	0	--
2	0	--	0	--
3	6	1.0	1	--
4	17	2.9	1	--
5	18	3.0	5	3.4
6	7	1.2	2	1.4
7	17	2.9	3	2.1
8	27	4.6	7	4.8
9	125	21.1	40	27.4
10	230	38.8	53	36.3
11	70	11.8	11	7.5
12	29	4.9	11	7.5
13	22	3.7	7	4.8
14	13	2.2	2	1.4
15	12	2.0	3	2.1
Sum	593	100	146	100
Total Extract	528	--	102	--
2NF (1 μg)[b]	226	--	31	--
TA98nr/TA98	--	--	--	19
SR[c]	12		14	

(a) Revertants per plate minus spontaneous revertants.
(b) 2NF = 2-nitrofluoranthene.
(c) SR = spontaneous revertants.

ORGANIC CHEMICAL CHARACTERIZATION OF PCC FLY ASH

A large batch of PCC fly ash (97 g) was extracted with benzene/methanol and fractionated on the CN-10 column. Specific fractions were collected in regions which showed high mutagenic responses and/or well-characterized peaks, as indicated by the shaded, labeled areas in the chromatogram depicted in Figure 1. Most of these fractions were subfractionated on a gel permeation HPLC

TABLE 10. Mutagenicity of Fluidized-Bed Fly Ash Extract in Salmonella typhimurium, TA98 and TA1538 with and Without Microsomal Activation

Fraction	TA98				TA1538			
	+S9	%	-S9	%	+S9	%	-S9	%
1	0[a]	--	0	--	0	--	29	2.9
2	0	--	0	--	0	--	32	3.2
3	0	--	0	--	0	--	14	1.4
4	14	2.5	19	2.8	26	4.3	38	3.8
5	25	4.5	16	2.3	25	4.1	38	3.8
6	10	1.8	9	1.3	21	3.4	42	4.2
7	15	2.7	31	4.5	37	6.1	64	6.4
8	10	1.8	41	6.0	19	3.1	72	7.2
9	90	16.1	134	19.6	93	15.2	143	14.2
10	233	41.8	259	37.9	208	34.0	253	25.2
11	79	14.2	83	12.1	84	13.7	113	11.3
12	29	5.2	40	5.9	60	9.8	76	7.6
13	28	5.0	28	4.1	25	4.1	38	3.8
14	14	2.5	14	2.0	13	2.1	30	3.0
15	11	2.0	9	1.3	0	-	22	2.2
Sum	558	100	683	100	611	100	1004	100
Total Extract	513		564		549		608	
2AF(50μg)[b]	278		80		523		58	
BaP(10μg)[b]	48		.0		65		0	
SR[c]	24		19		66		30	

(a) Revertants per plate mins spontaneous revertants.
(b) 2AF = 2-aminofluorene; BaP = benzo[a]pyrene.
(c) SR = spontaneous revertants.

column, and the largest peak in each chromatogram was collected. (See Experimental Section for details.) These subfractions were then analyzed by GC, using both FID and NPD, and by MS using both GC and direct-probe sample introduction. The results for each fraction will be discussed individually.

Fraction A

Even though the components in this fraction, collected from the gel permeation column, were detected as in a single peak, the gas chromatogram (Figure 4) reveals that it was a complex mixture of numerous compounds. A comparison between the chromatograms depicting FID (Figure 4A) and NPD (Figure 4B) indicated that most of the compounds in this fraction contained nitrogen. LV/LRMS experiments performed with 12-eV ionizing electrons confirmed the NPD/GC results regarding the abundance of nitrogen-containing compounds. The major peaks in the LV/LRMS spectrum (Figure 5), representing molecular ions at m/z 237, 251, and 265, are at odd masses, indicating a series of homologous compounds, each containing an odd number of nitrogen atoms. This spectrum also suggests the presence of at least two other series of homologous components: one series at m/z 230, 244, and the other at m/z 263, 277. LRMS experiments conducted at 70 eV provided a wealth of information regarding the fragments formed from the molecular ions contained in these homologous series (Figure 6). High resolution peak-matching determined exact masses and, therefore, elemental compositions of important ions, some of which are shown in Figure 6. (The exact masses measured and the calculated error from the assigned elemental compositions are listed in Table 11.)

The elemental compositions provided by the HRMS experiments conclusively proved the presence of nitrogen, and the N:O ratio of 1:2 in the 237, 251, 265 series suggested the presence of one nitro group attached to a series of alkyl-substituted phenanthrenes or anthracenes. Peaks corresponding to the anticipated fragment ions of alkylated nitrophenanthrenes/anthracenes were present in the LRMS spectrum, and the measured exact masses were also consistent with this hypothesis of alkylated nitrophenanthrenes/anthracenes. For example, C_1-nitrophenanthrene, $C_{15}H_{11}NO_2$ (m/z 237), should fragment by losing the neutrals NO, NO_2 and (NO + CO), giving rise to fragment ions at m/z 207, 191, and 179, having the elemental compositions $C_{15}H_{11}O$, $C_{15}H_{11}$, and $C_{14}H_{11}$, respectively.

In order to confirm the tentative identifications made on the basis of direct probe mass spectrometry (DPMS), GC/MS was used to acquire separate spectra for individual components of the mixture, thus allowing distinction between the molecular ions and fragment ions observed in the DPMS experiments. The

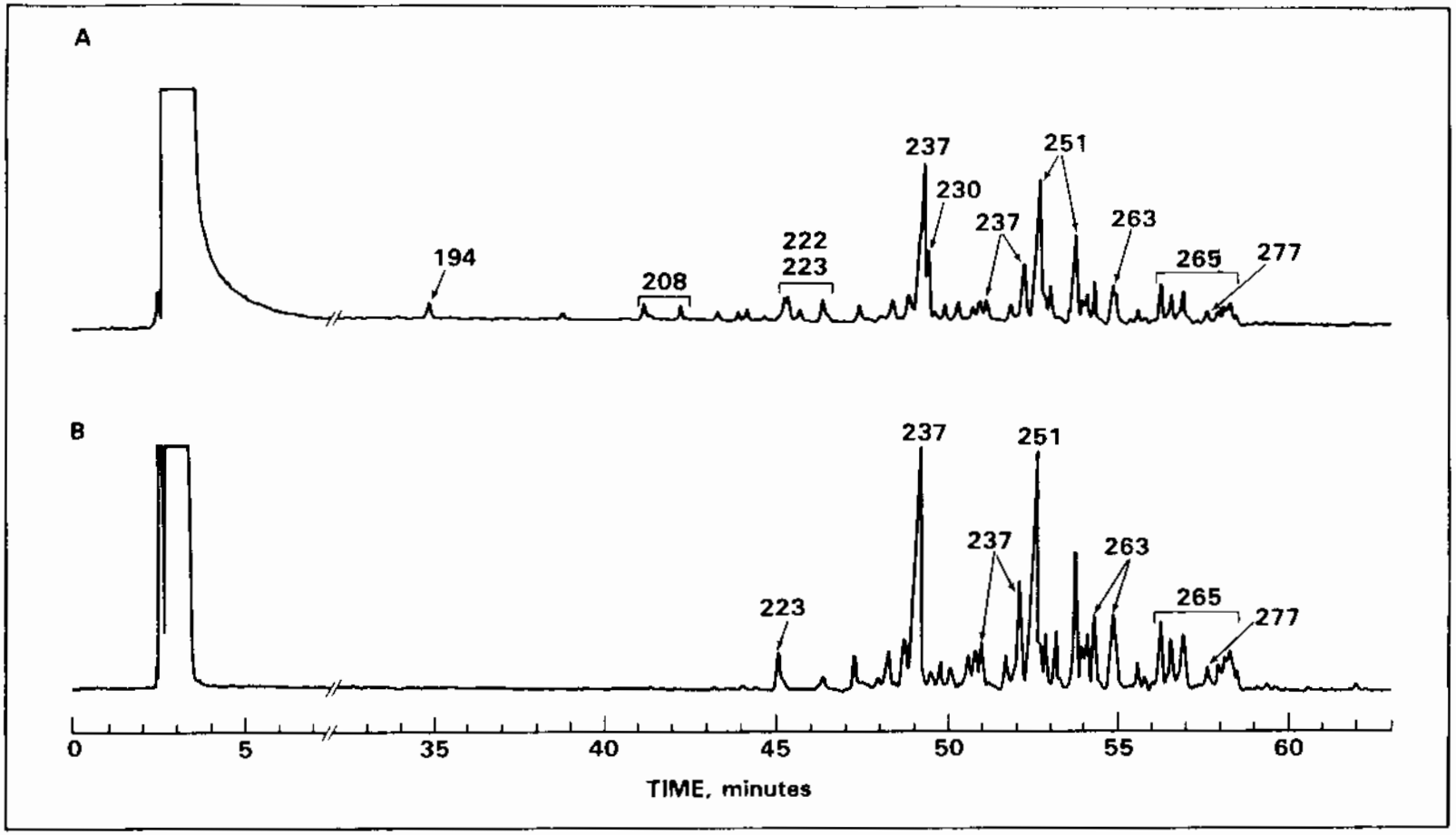

FIGURE 4. Gas Chromatograms for Fraction A, Using A) a Flame Ionization Detector and B) a Nitrogen-Phosphorus Detector. Peaks are labeled with the nominal mass of the molecular ion determined by GC/MS.

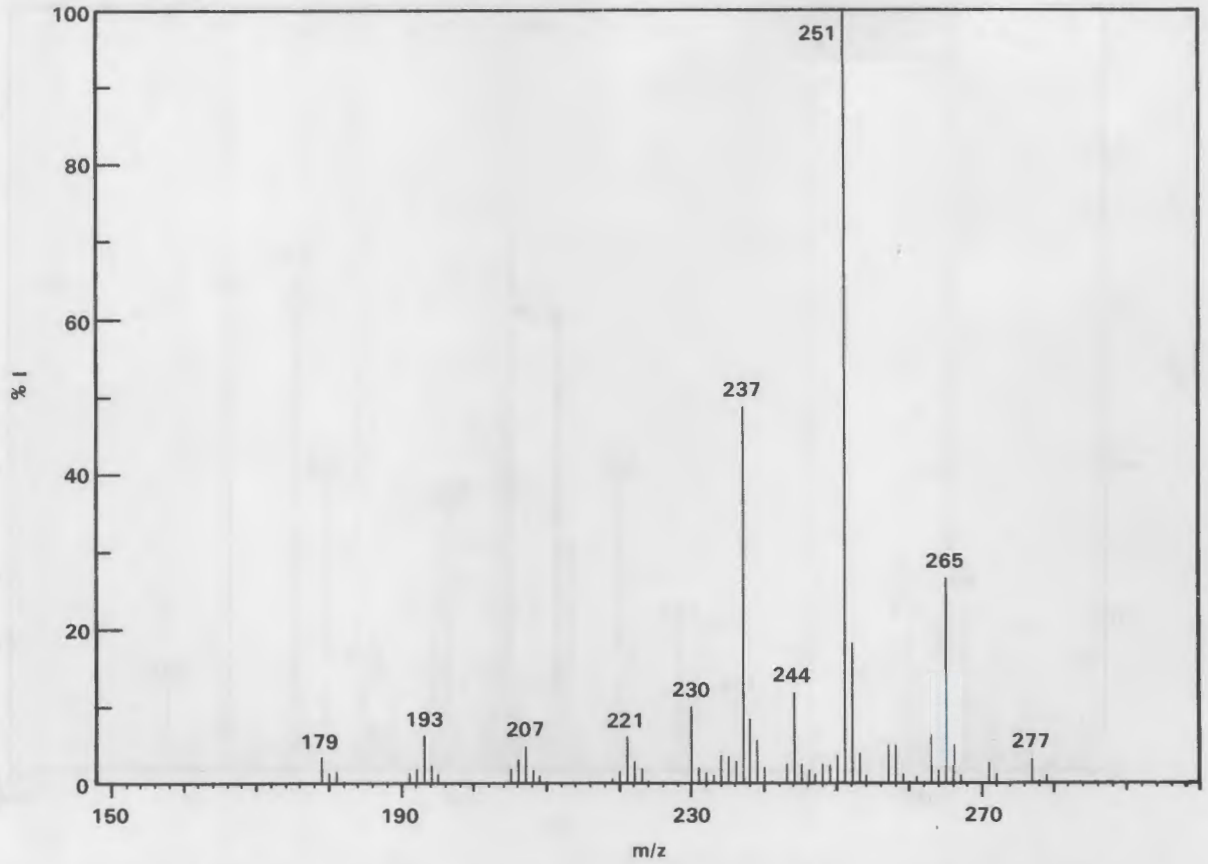

FIGURE 5. 12-eV Low Resolution Mass Spectrum of Fraction A

reconstructed chromatogram plotting total ion current (TIC) versus time (scan number) is shown in Figure 7, along with the selected ion chromatograms for m/z 223, 237, 251, and 265. These masses account for the major peaks observed in the TIC. This is consistent with observation that the corresponding molecular ion clusters account for 61% of the total ion current in the LV/LRMS spectrum (Figure 5).

The individual GC/MS spectra of the members of this homologous series, two of which are shown in Figure 8, have features consistent with the presence of nitro groups but do not match the spectra reported for the homologous series of aklylated nitroanthracenes recently reported by Paputa-Peck et al. (1983). HPLC experiments with nitroanthracene and nitrophenanthrene standards have shown that nitrophenanthrene elutes within the time window of this collected fraction, whereas nitroanthracene does not. GC retention index measurements

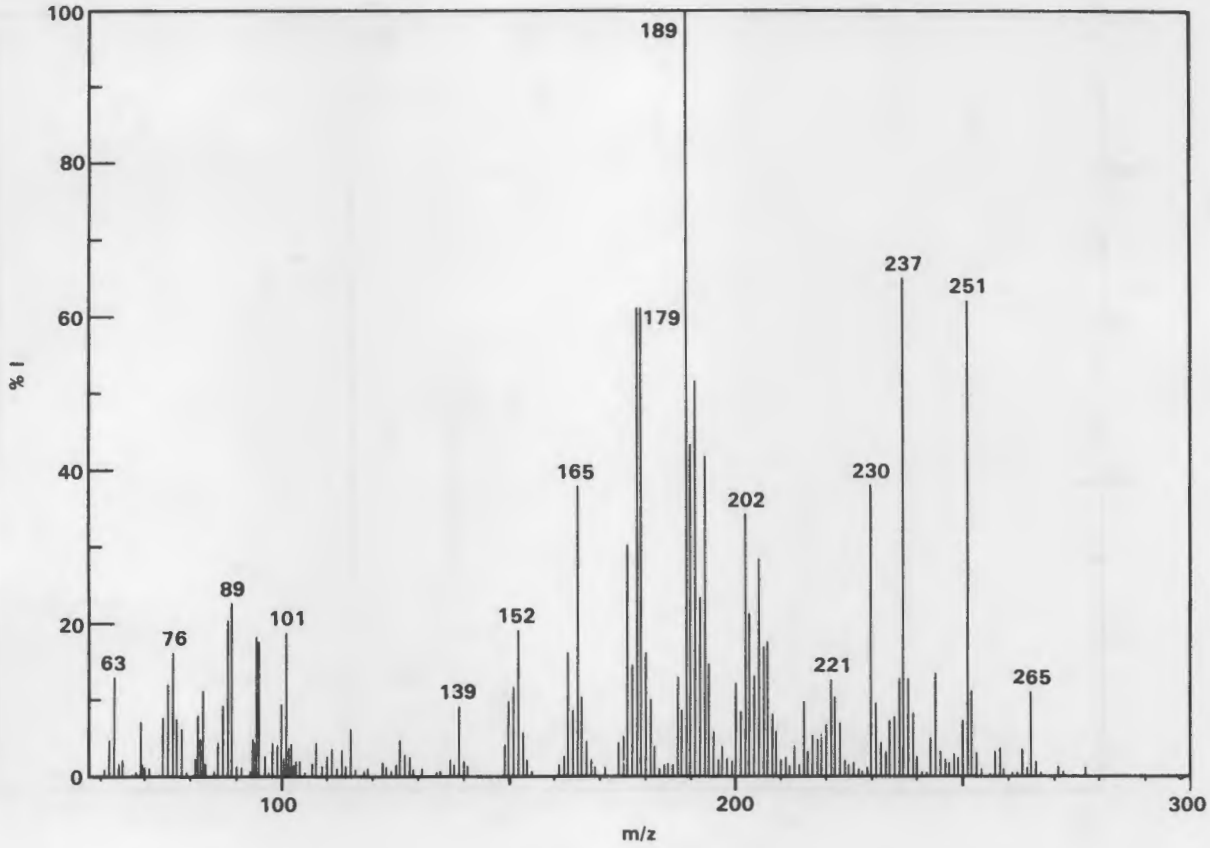

FIGURE 6. 70-eV Low Resolution Mass Spectrum of Fraction A

were made on the mixture products obtained by nitrating phenanthrene and 1-methylphenanthrene, using the procedure outlined by Schuetzle et al. (1982). The retention indices of the three isomers of nitrophenanthrene produced were 367.33, 386.51, and 373.48, respectively; the first is comparable to the measured retention index (367.00) of the mononitro isomer found in Fraction A. The retention indices of the three major nitration products from 1-methylphenanthrene were 379.33, 384.37, and 394.02, respectively. The retention index value of 384.77 measured for the major C_1-isomer in Fraction A (Figure 8A) tentatively identifies it as a nitro-1-methylphenanthrene. On the basis of the HPLC results and preliminary GC retention index measurements, we have tentatively identified this series of homologs as alkylated nitrophenanthrenes. Further experiments will be required to determine the isomeric identities of the members of this series.

TABLE 11. Exact Masses and Elemental Compositions Determined[a] for Selected Ions from the Mass Spectrum of Fraction A. (See also Figure 6)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)[b]
179	179.0854	$C_{14}H_{11}$	(0.8)
189	189.0719	$C_{15}H_{9}$	(-1.4)
191	191.0865	$C_{15}H_{11}$	(-0.4)
202	202.0777	$C_{16}H_{10}$	(0.7)
205	205.0999	$C_{16}H_{13}$	(1.7)
230	230.0732	$C_{17}H_{10}O$	(0.0)
237	237.0786	$C_{15}H_{11}NO_2$	(0.4)
251	251.0945	$C_{16}H_{13}NO_2$	(0.3)
277	277.0744	$C_{17}H_{11}NO_3$	(0.4)

(a) Exact masses measured by 70-eV HRMS experiments.
(b) Difference in mass between experimental value and exact mass calculated for the assigned elemental composition, reported in millimass units (mmu).

The GC/MS spectra for the homologous series at m/z 263 and 277 (Figure 9) clearly indicate that these compounds contain a single nitro group. The characteristic losses of NO and NO_2 are present in both spectra (visible at [M-30] and [M-46]), and the loss of CNO_2 (mechanistically, NO + CO) accounts for the other prominent peak (m/z 219) in the spectrum of the m/z 277 isomer. The one high-resolution measurement that could be associated with this series, resulting in the assignment of $C_{17}H_{11}NO_3$ as the elemental composition of the molecule with molecular weight 277, is consistent with the spectral evidence for the presence of a single nitro group. The functionality of the additional oxygen atom is unknown. If the isomer at m/z 263 (assumed to be $C_{16}H_9NO_3$) represents the parent structure with no alkylation, then we can draw at least three reasonable structures for a combustion product likely to be found in this HPLC fraction: a nitro-benzonaphthofuran, a nitrocyclopentaphenanthrenone, and a nitropyrenone. No standard compounds of these types were available for use in chromatography experiments to make more definitive assignments.

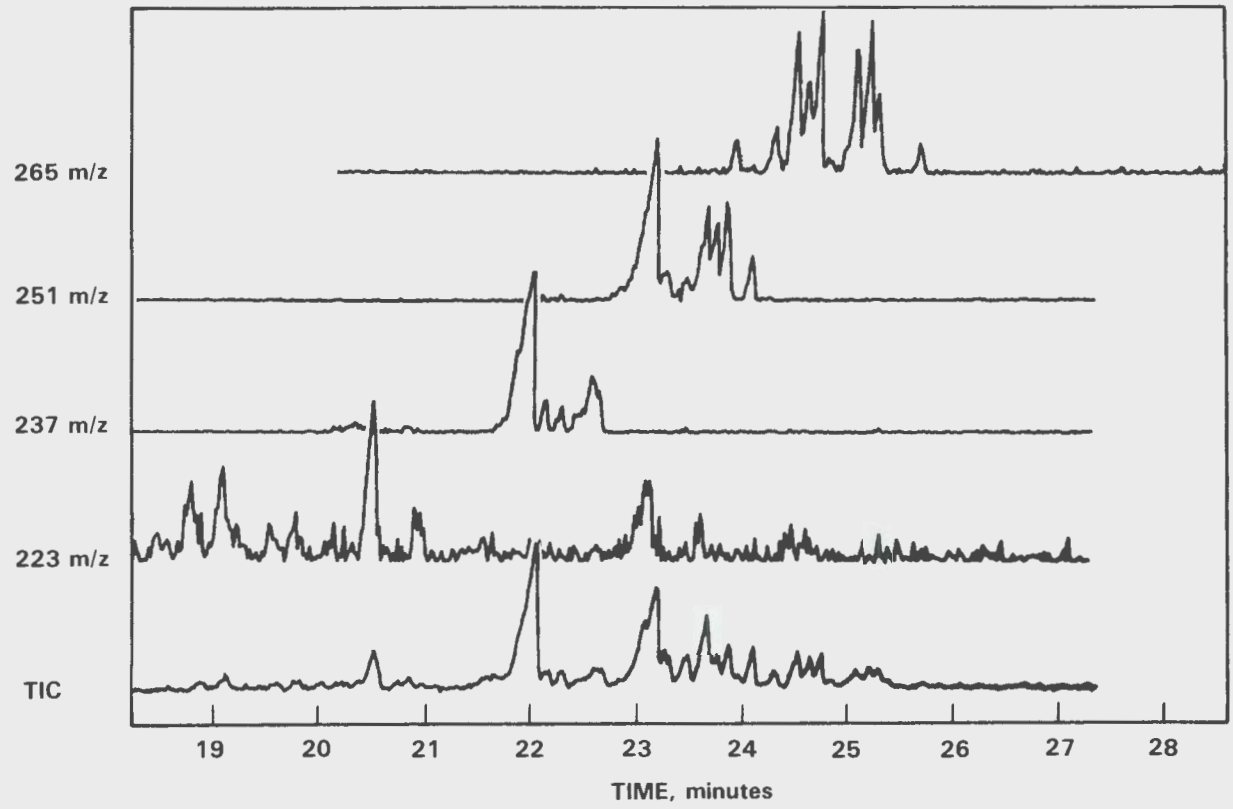

FIGURE 7. Selected Ion Chromatograms for Total Ion Current (TIC) and m/z 223, 237, 251, and 265 Found in GC/MS Analysis of Fraction A

Fraction A also contained several other homologous series, two of which were present in high enough concentrations to allow for some GC/MS characterization. One series was composed of compounds with molecular weights of 194, 208, 222, 236 and 250 and was evident in the selected ion chromatograms depicted in Figure 10. Because no high-resolution data were acquired for any of these molecular ions, the elemental composition of the components are not known. However, the spectrum of the single component with a molecular ion at m/z 194 and a major fragment at m/z 165 (loss of HCO?) is very similar to the spectrum of 2-methylfluorenone (Yu and Hites 1981) and not greatly different from the mass spectrum of anthrone. The spectra of the higher molecular weight members of this series show similar losses of HCO, or loss of methyl followed by loss of CO and HCO. These spectra appear to be consistent with either of the proposed structures. Even though no evidence for the nonalkylated parent

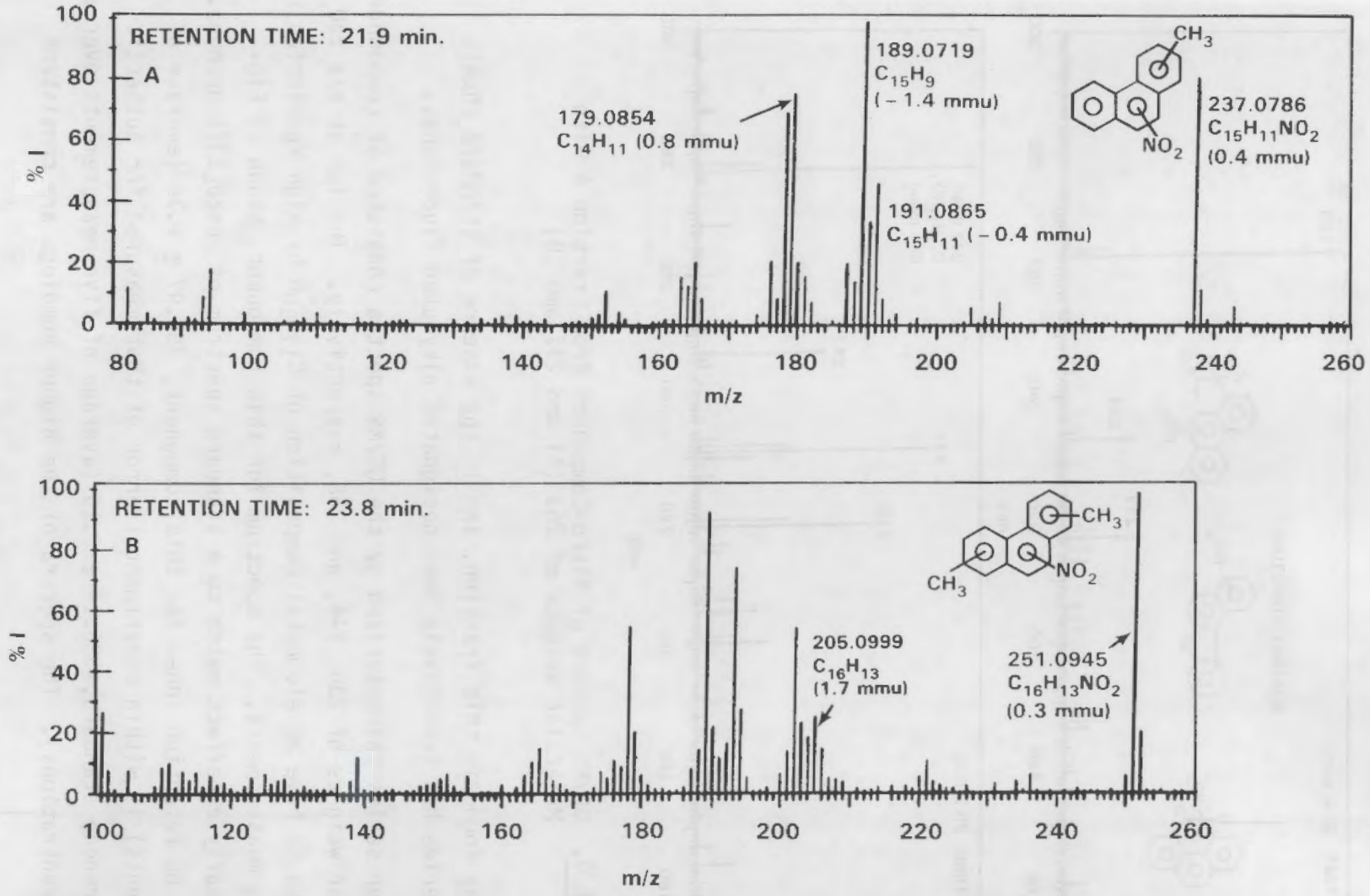

FIGURE 8. GC/MS Spectra of Possible Alkylated Nitrophenanthrenes Found in Fraction A. The C_1-analog (A) has tentatively been identified as a 1-methyl-x-nitrophenanthrene. The isomeric identity of the C_2-analog (B) has not been elucidated

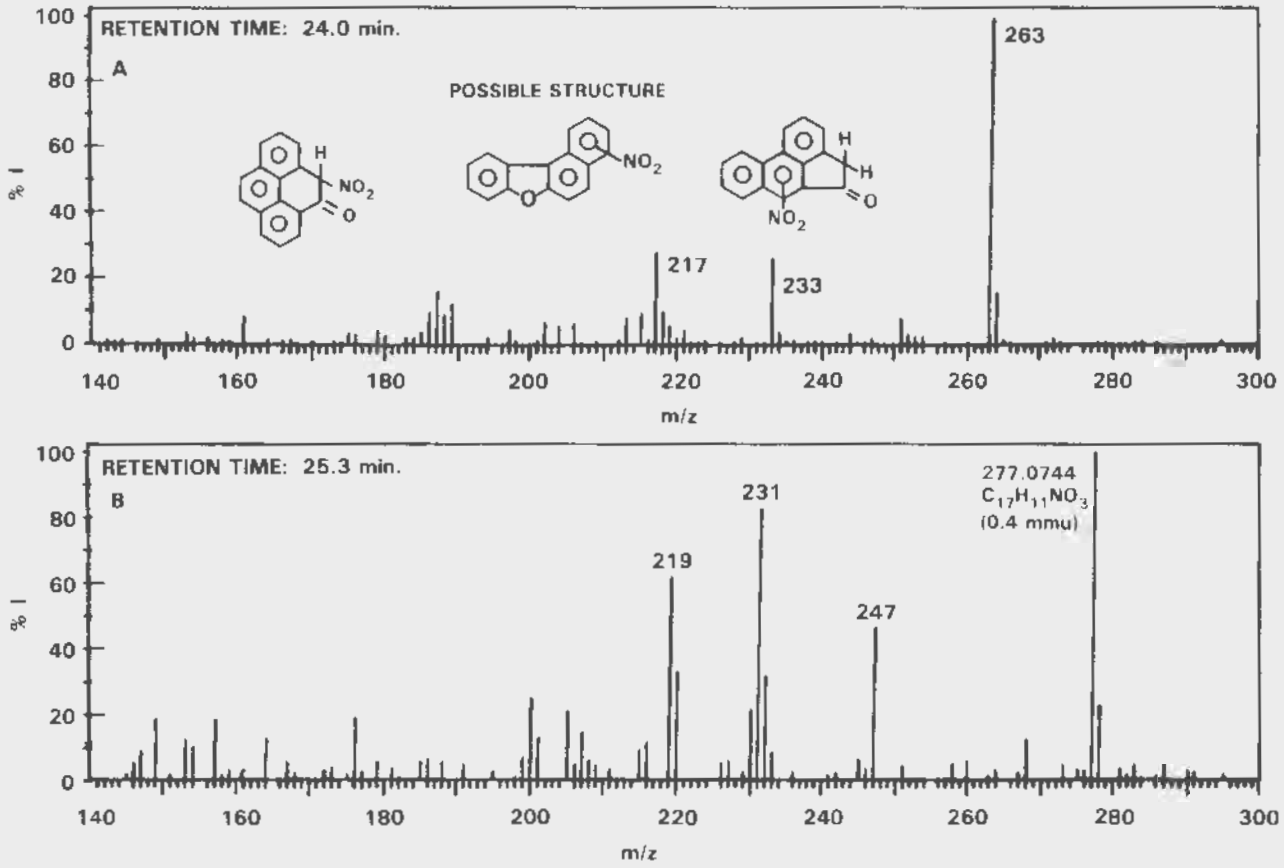

FIGURE 9. GC/MS Spectra of Nitro-Compounds from Fraction A with Molecular Weights of 263 (A) and 277 amu (B)

fluorenone was found in this fraction, and in the absence of standard chemicals, this series has tentatively been designated alkylated fluorenones.

The other series characterized by the GC/MS spectra consisted of compounds with molecular weights of 230, 244, and 258, respectively. The ion at m/z 230 was determined to have an elemental composition of $C_{17}H_{10}O$ by high resolution peak-matching measurements. The spectrum for this component, shown in Figure 11, is nearly a perfect match to a standard spectrum of benzo[a]fluorenone. The measured GC retention index for this component, 385.97 ± 0.36 (average of two measurements) is within experimental error of that measured for authentic benzo[a]fluorenone standard, 386.17 ± 0.16 (average of five measurements over a range of concentrations). The spectra of the higher homologs are consistent

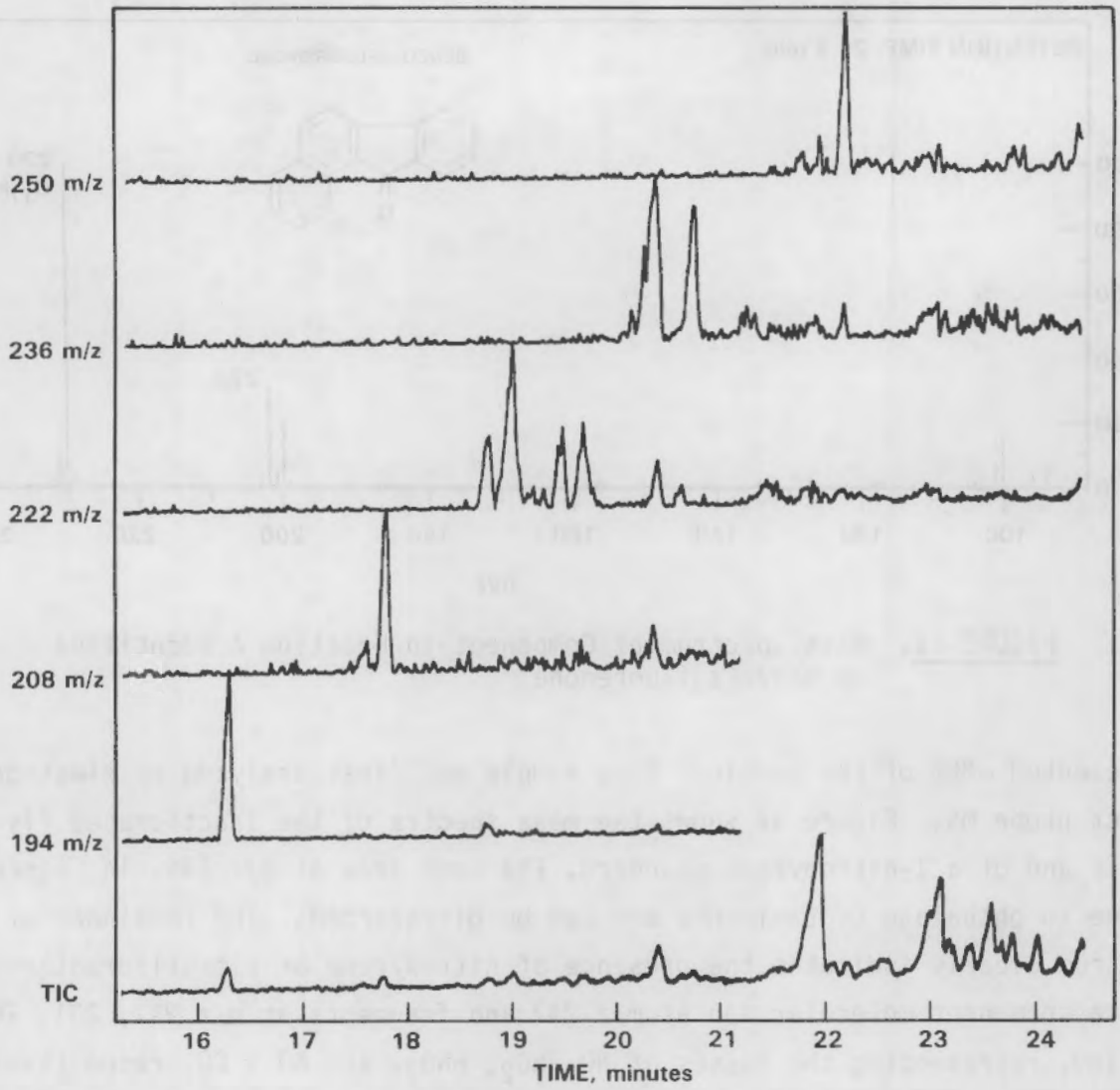

FIGURE 10. Selected Ion Chromatograms for Total Ion Current (TIC) and m/z 194, 208, 222, 236, and 250 Found in GC/MS Analysis of Fraction A

with the expected spectra for alkylated benzo[a]fluorenones. Thus, this series tentatively has been identified as alkylated benzo[a]fluorenones.

Fraction B

The gel permeation column chromatogram of this fraction showed a single component, but three components were detected by GC of this HPLC fraction, which was collected because it had the same HPLC retention time as 1-nitropyrene. Based on a simple GC peak area percent calculation, nitropyrene

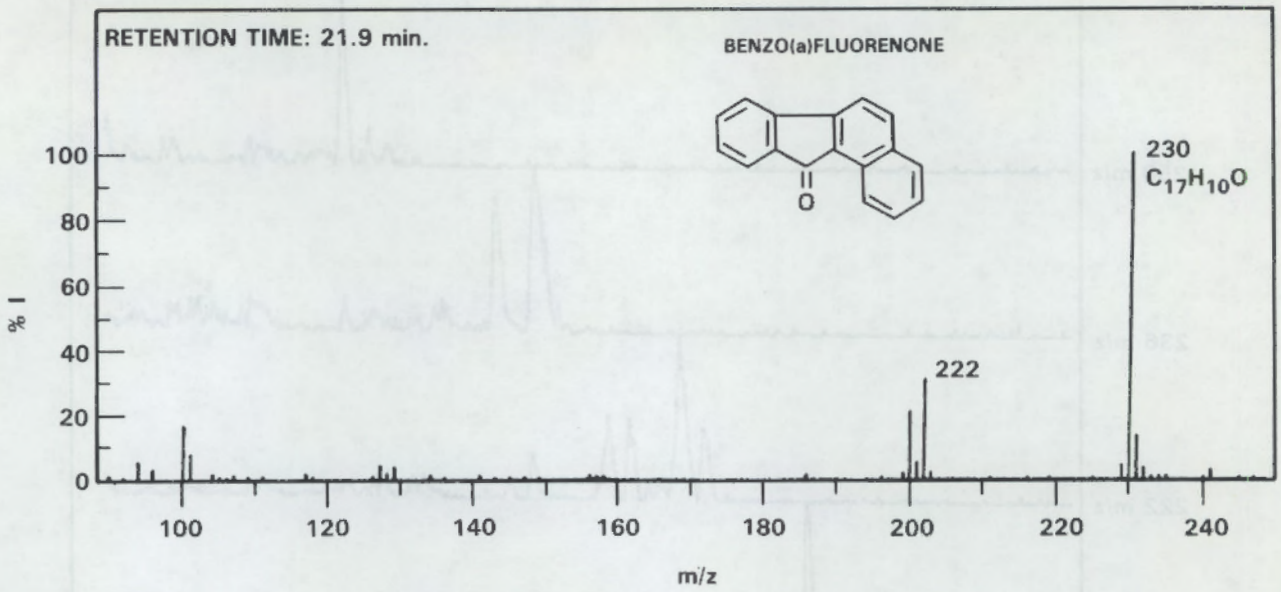

FIGURE 11. Mass Spectrum of Component in Fraction A Identified as Benzo[a]fluorenone

represented ~80% of the sample. This sample was first analyzed by electron impact probe MS. Figure 12 shows the mass spectra of the fractionated fly ash sample and of a 1-nitropyrene standard. The base peak at m/z 149, in Figure 12A, is due to phthalate contaminants and can be disregarded. The remainder of the spectrum clearly indicates the presence of nitropyrene or nitrofluoranthene, with a prominent molecular ion at m/z 247 and fragments at m/z 217, 201, 200, and 189, representing the losses of NO, NO_2, HNO_2, and NO + CO, respectively. The sample spectrum is similar to that of pure 1-nitropyrene, shown in Figure 12B, although the molecular ion is of lower intensity in the spectrum of the fly ash extract. The similarities include the presence of doubly charged ions at m/z 100 and 100.5, which are found in both spectra.

The spectrum in Figure 12A also has a peak at m/z 261, which is evidence of a methylated nitropyrene/fluoranthene. This interpretation is confirmed by the relatively large signal at m/z 231 due to the loss of NO from the molecular ion; this signal is too intense to represent only the loss of O from nitropyrene. In addition, the expected signals for the loss of NO_2 and HNO_2 from the molecular ion are present at m/z 215 and 214, respectively. This spectrum contains no discernible information on which isomers, or mixture of isomers,

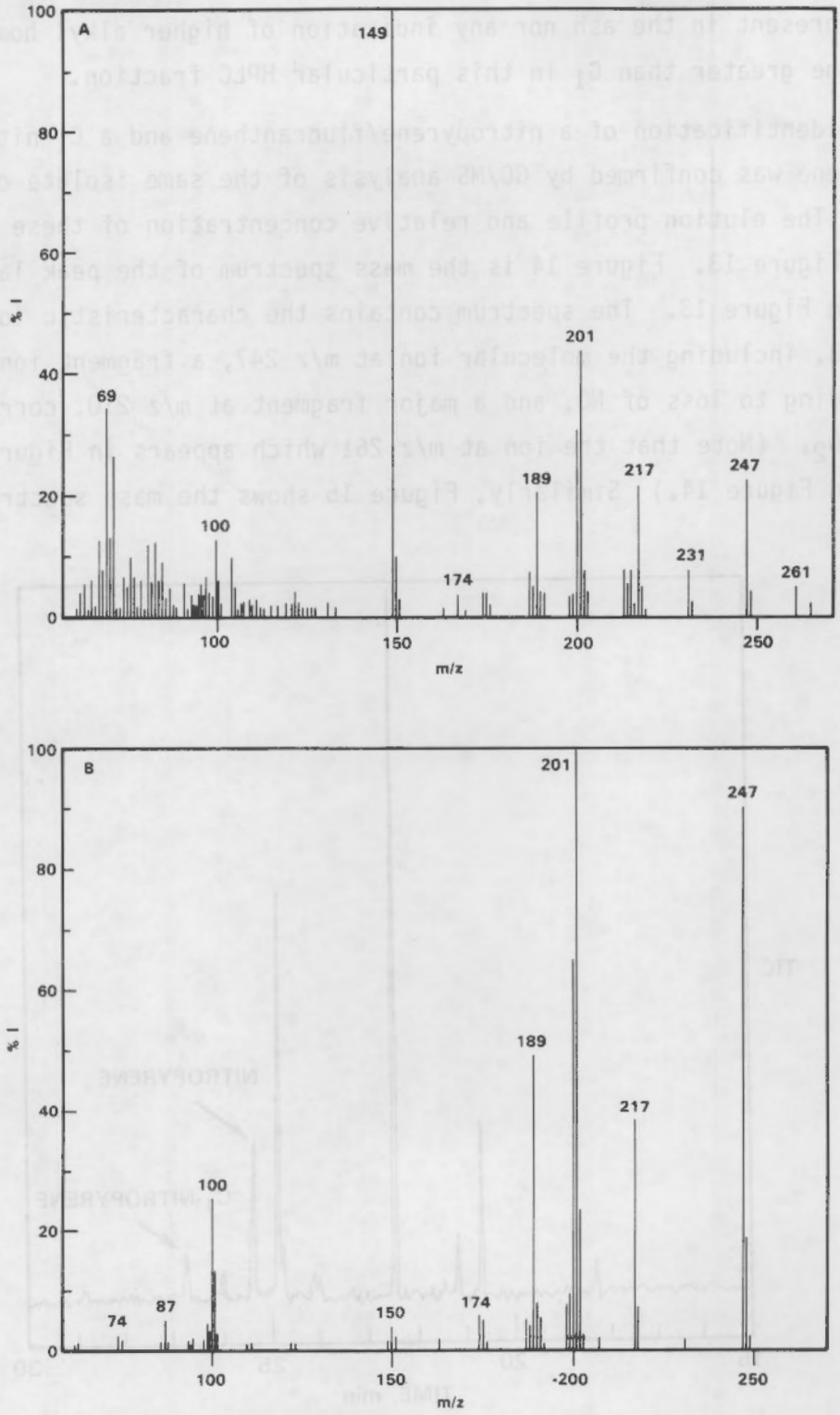

FIGURE 12. 70-eV Mass Spectra of A) Fraction B and B) Authentic 1-Nitropyrene

might be present in the ash nor any indication of higher alkyl homologs of nitropyrene greater than C_1 in this particular HPLC fraction.

The identification of a nitropyrene/fluoranthene and a C_1-nitropyrene/fluoranthene was confirmed by GC/MS analysis of the same isolate of fly ash extract. The elution profile and relative concentration of these compounds are shown in Figure 13. Figure 14 is the mass spectrum of the peak labeled "nitropyrene" in Figure 13. The spectrum contains the characteristic ions shown in Figure 12B, including the molecular ion at m/z 247, a fragment ion at m/z 217 corresponding to loss of NO, and a major fragment at m/z 210, corresponding to loss of NO_2. (Note that the ion at m/z 261 which appears in Figure 12A is not present in Figure 14.) Similarly, Figure 15 shows the mass spectrum of the

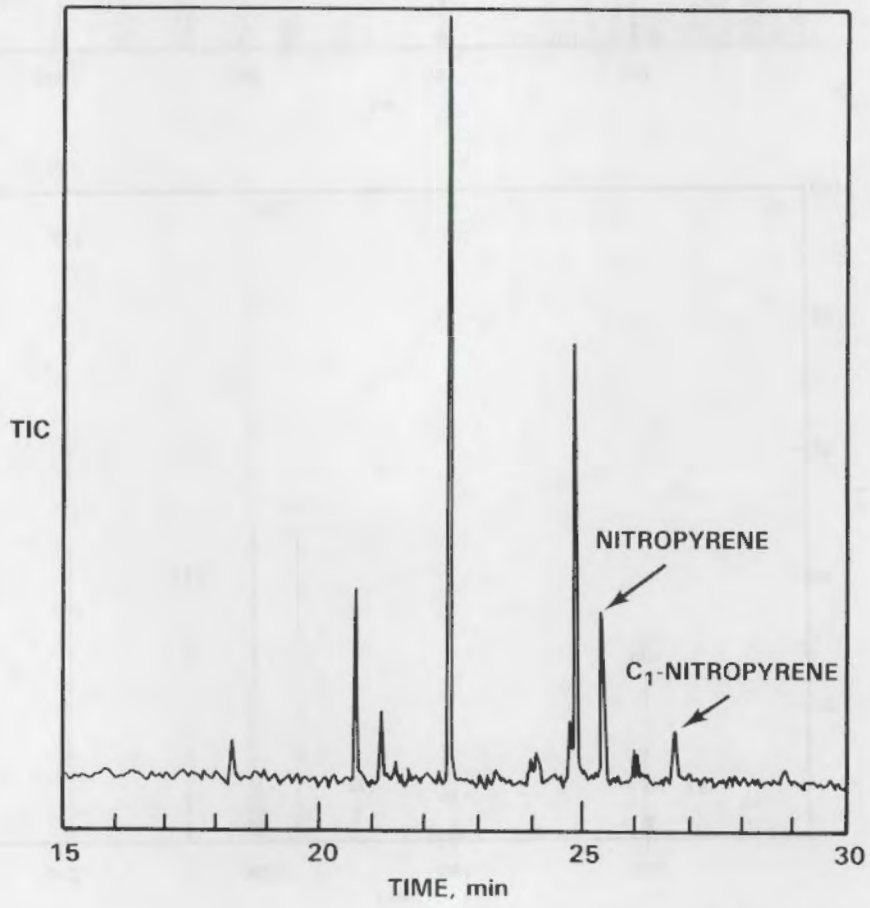

FIGURE 13. Total Ion Current Chromatogram from the GC/MS Analysis of Fraction B. The large peaks at 22.5 and 24.9 min have been identified as phthalate esters.

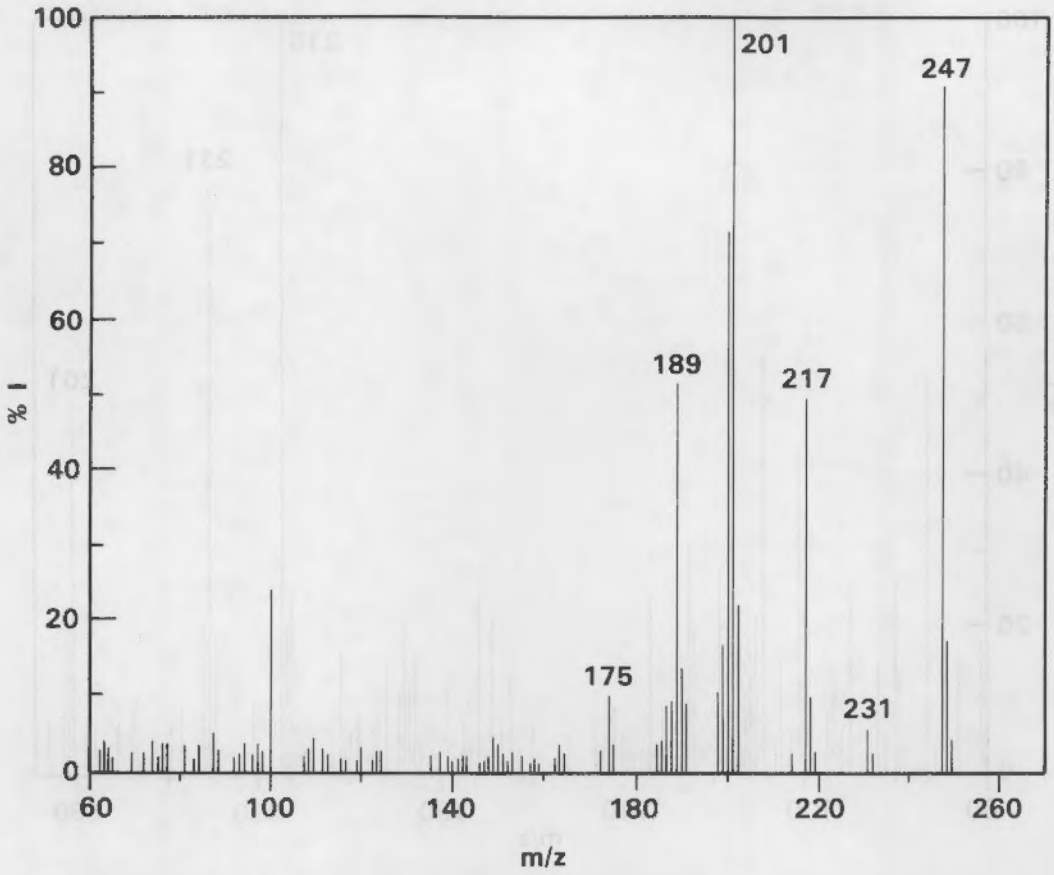

FIGURE 14. Mass Spectrum of the Peak Labeled "Nitropyrene" in Figure 13

peak in Figure 13 identified as methylnitropyrene/fluoranthene. Although the spectrum is contaminated with background ions, the molecular ion (m/z 261) and the two characteristic fragment ions corresponding to loss of NO (m/z 231) and NO_2 (m/z 215) are clearly present. Higher alkyl homologs of nitropyrene were not detected in this fraction.

In an attempt to obtain absolute isomeric identification of the nitropyrene/fluoranthene isomer found in the fly ash extract, the GC retention indices of all the available isomers of nitropyrene and nitrofluoranthene were measured and compared against the retention index value of 421.12 measured for the extract isomer. The results are listed in Table 12. The retention indices for 1-, 2-, 3-, 7-, and 8-nitrofluoranthenes ranged from 407.45 to 420.55. These values do not adequately match that measured for the isomer found in the

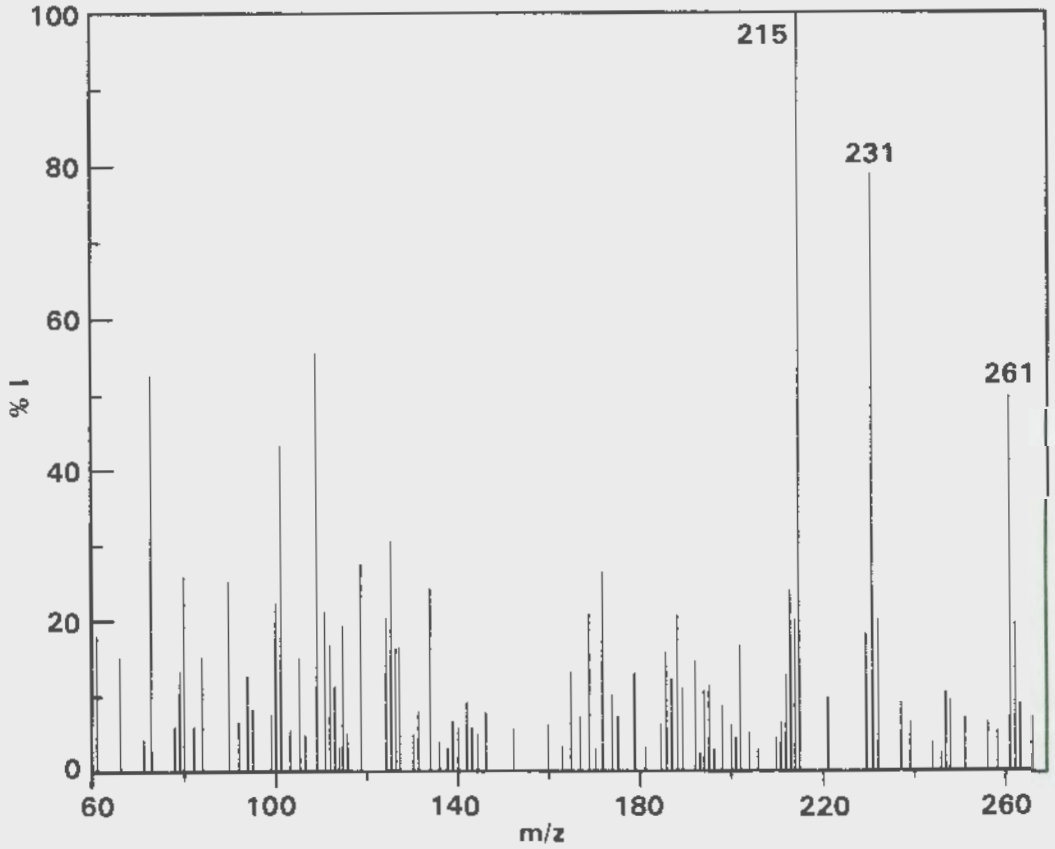

FIGURE 15. Mass Spectrum of the Peak Labeled "C_1-Nitropyrene" in Figure 13

fly ash isolate; thus the extracted compound is clearly not a nitrofluoranthene. Of the three nitropyrene isomers, 2- and 4-nitropyrene had retention indices of 424.38 and 415.52, respectively, and 1-nitropyrene had a retention index of 421.26. The 1-nitropyrene value closely matched the value of 421.12 (within the standard deviation limits of the two measurements) determined for the isolated nitropyrene. Furthermore, testing of the 1-nitropyrene standard at concentrations similar to that of the fly-ash-derived nitropyrene resulted in reproducible retention index values of 421.15 to 421.16.

The total amount (in mg) of nitropyrene from the 97-g sample of fly ash was calculated from the peak area in an MCH-10 chromatogram of the subfractionated extract. Assuming that no nitropyrene was lost in the initial fractionation of the CN-10 preparative column, the concentration of nitropyrene in the original fly ash particles is 12 ppb (12 ng nitropyrene/g ash). This value

TABLE 12. Retention Index Measurements[a] for All Mononitro Isomers of Pyrene and Fluoranthene

Compound	Retention Index
1-nitropyrene	421.26 ± 0.10 (4)
2-nitropyrene	424.38 ± 0.01 (3)
4-nitropyrene	415.52 ± 0.01 (2)
1-nitrofluoranthene	407.95 ± 0.07 (6)
2-nitrofluoranthene	412.59 ± 0.01 (4)
3-nitrofluoranthene	413.24 ± 0.03 (4)
7-nitrofluoranthene	407.45 ± 0.03 (6)
8-nitrofluoranthene	420.55 ± 0.03 (3)
nitropyrene/fluoranthene isolated from fly ash	421.12 ± 0.03 (2)

(a) Average of the number of measurements indicated in parentheses.

is in the range between 0.1 ppb and 130 ppb, reported by Mumford and Lewtas (1982) for conventional and fluidized-bed coal fly ash, respectively. Since 1.9 mg of soluble organic material is extracted per g fly ash, the concentration of nitropyrene is 6.3 ng per mg of extract, or 6.3 ppm.

Fraction C

Gas chromatograms depicting FID and NPD detection of this fraction are shown in Figure 16. It is clear that most of the components contain nitrogen. Because the sample size was very small, no direct probe LRMS, LV/LRMS, or HRMS experiments were performed, allowing the bulk of the sample to be used in a single GC/MS experiment, where the maximum amount of information about the individual components could be obtained. Despite the general success of this approach, not enough information was obtained on the components to allow for any positive identifications. Evidence for at least two homologous series, one at m/z 267, 281, 295 and 309 and the other at m/z 263, 277, 291 was uncovered. These components, along with smaller amounts of compounds with molecular weights of 251, 270, and 286, comprise the bulk of this fraction. The GC/MS data were consistent with the FID/NPD data in that each peak found in both GC

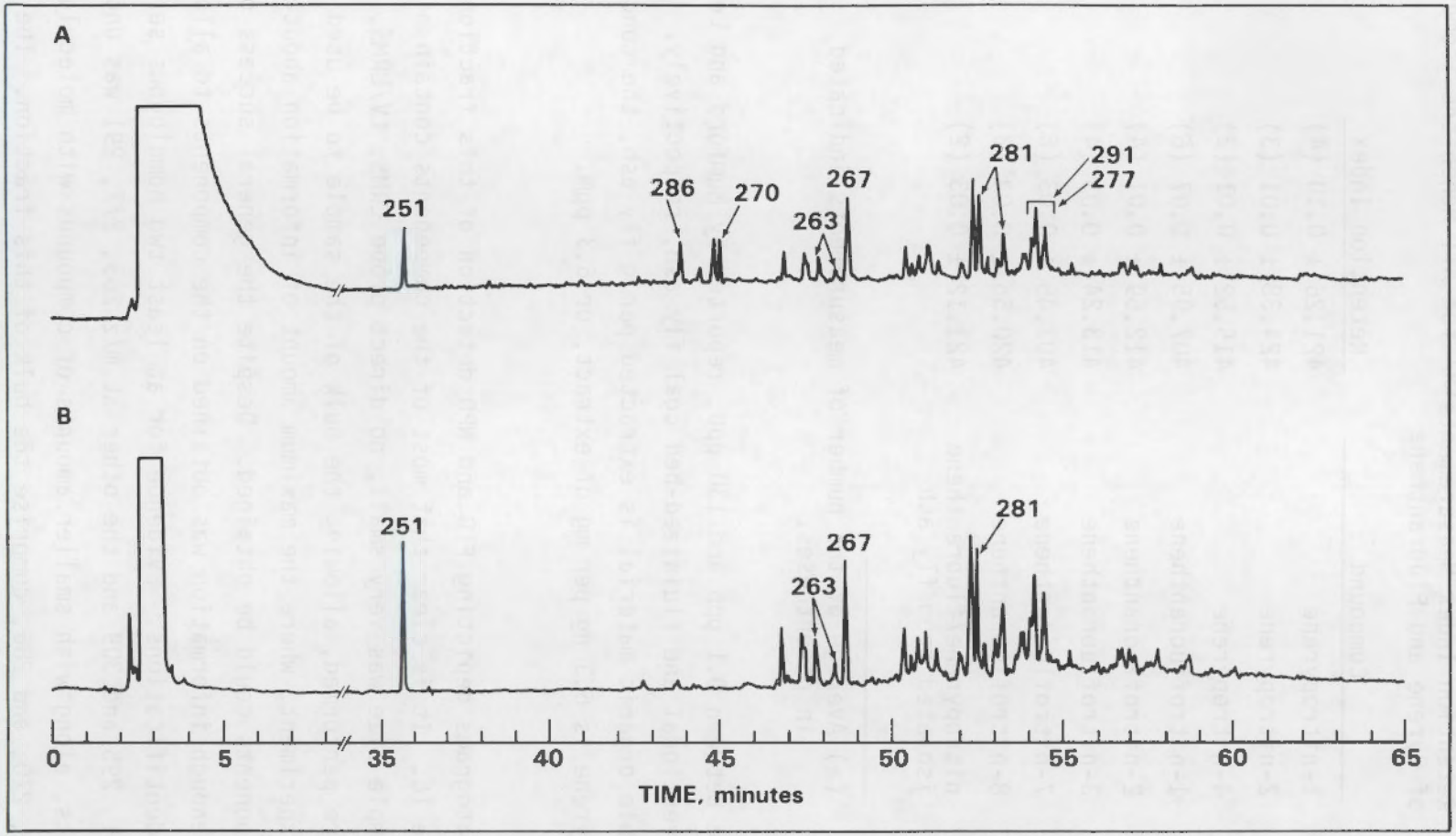

FIGURE 16. Gas Chromatograms of Fraction C Using A) a Flame-Ionization Detector and B) a Nitrogen-Phosphorus Detector. Peaks are labeled with the nominal mass of the molecular ion determined by GC/MS.

traces was found to be associated with a molecular ion of odd mass (indicating an odd number of nitrogen atoms), whereas the cluster of peaks found only in the FID trace (43 to 45 min) was associated with even mass molecular ions, indicating the absence (or presence) of an even number of nitrogen atoms.

The mass spectrum of the component eluting at 35.7 min is shown in Figure 17. The spectrum indicates the losses of 15 amu (CH_3), 31 amu (OCH_3 or HNO), 43 amu (C_2H_3O or C_3H_7), and 59 amu ($C_2H_3O_2$, C_3H_7O or $CHNO_2$) from an assumed molecular ion at m/z 251. Without elemental compositions, and in the absence of a suitable match in compilations of reference spectra, we have not been able to deduce the structure of this molecule from the mass spectrum.

The mass spectrum of the m/z 267 isomer which eluted at 48.7 min is shown in Figure 18. It displays fragmentation features consistent with the presence

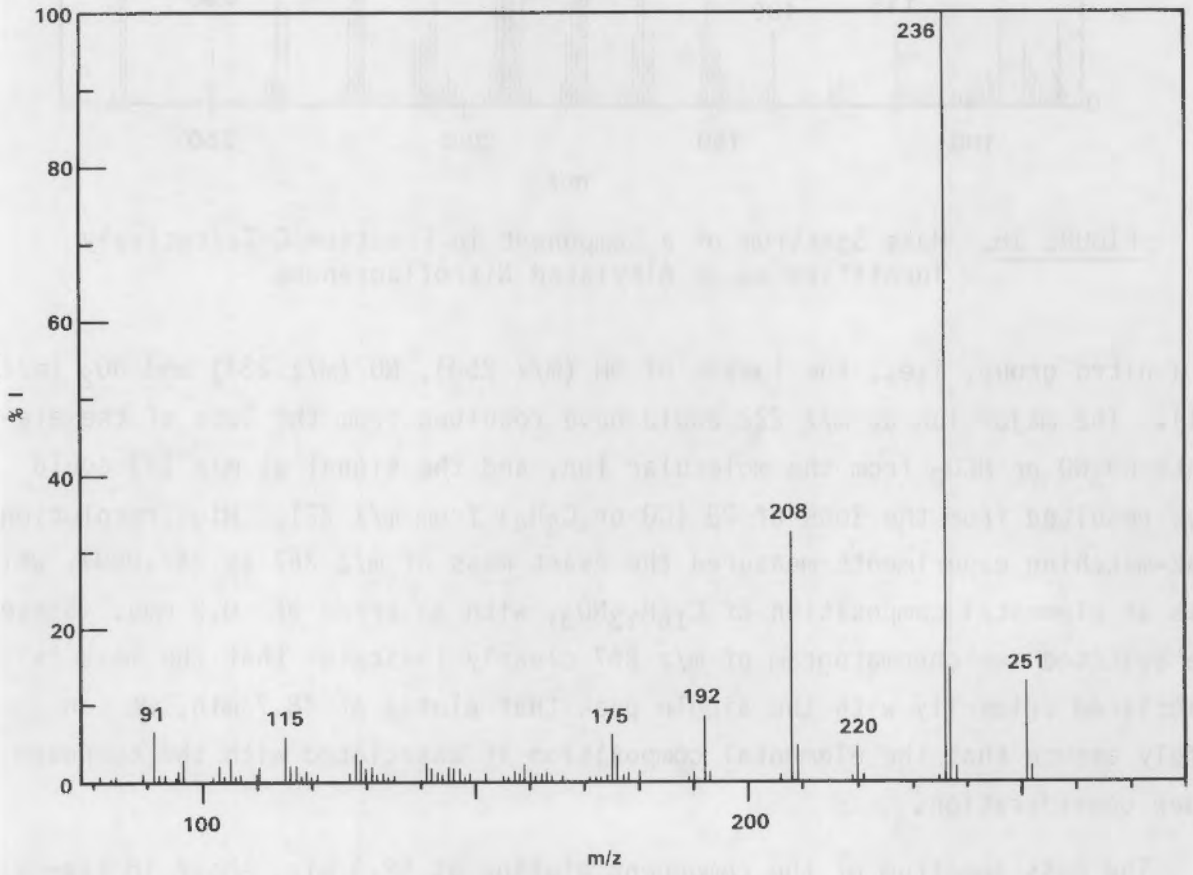

FIGURE 17. Mass Spectrum of a Component in Fraction C with an Apparent Molecular Weight of 251 amu

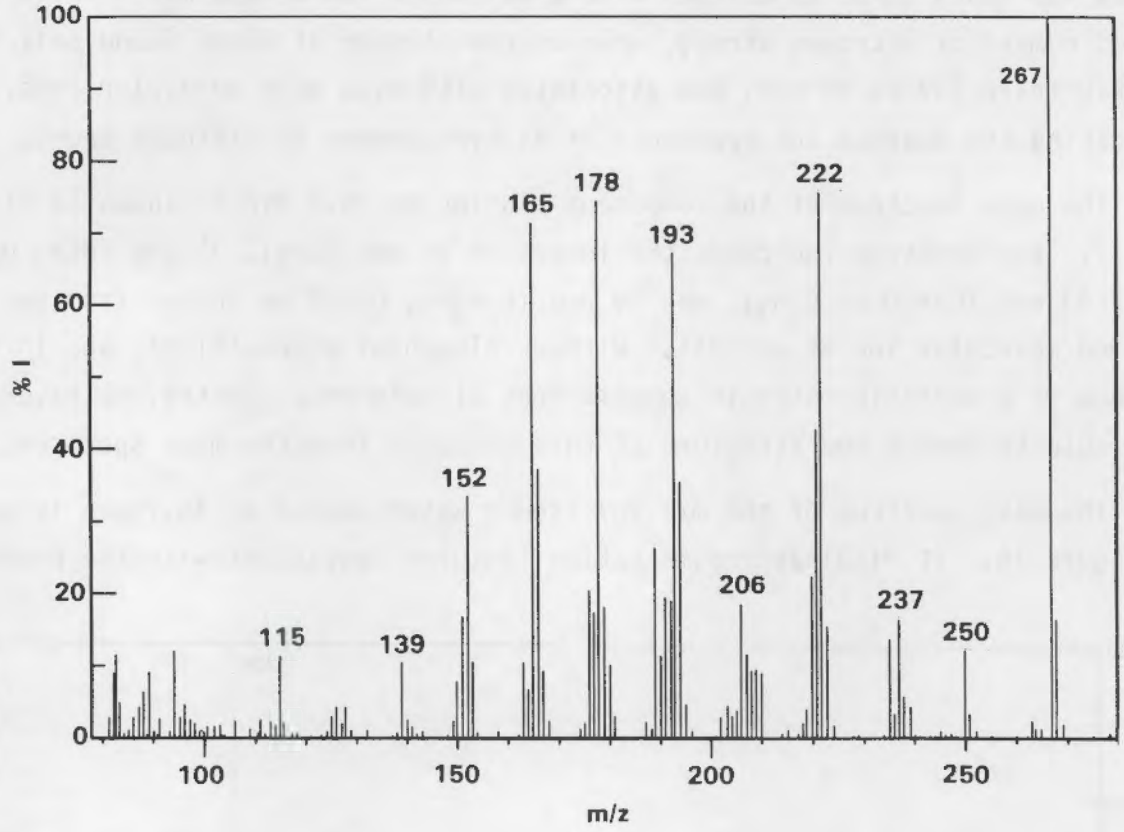

FIGURE 18. Mass Spectrum of a Component in Fraction C Tentatively Identified as an Alkylated Nitrofluorenone

of a nitro group, i.e., the losses of OH (m/z 250), NO (m/z 237) and NO_2 (m/z 221). The major ion at m/z 222 could have resulted from the loss of the elements CH_3NO or HCO_2 from the molecular ion, and the signal at m/z 193 could have resulted from the loss of 28 (CO or C_2H_4) from m/z 221. High resolution peak-matching experiments measured the exact mass of m/z 267 as 267.0897, which fits an elemental composition of $C_{16}H_{13}NO_3$, with an error of -0.2 mmu. Since the selected ion chromatogram of m/z 267 clearly indicates that the mass is associated primarily with the single peak that elutes at 48.7 min, we can safely assume that the elemental composition is associated with the compound under consideration.

The mass spectrum of the component eluting at 52.3 min, shown in Figure 19, is clearly that of an alkyl homolog of the $C_{16}H_{13}NO_3$ compound, and evidence was found for the higher alkyl homologs of m/z 295 and 309. The mass

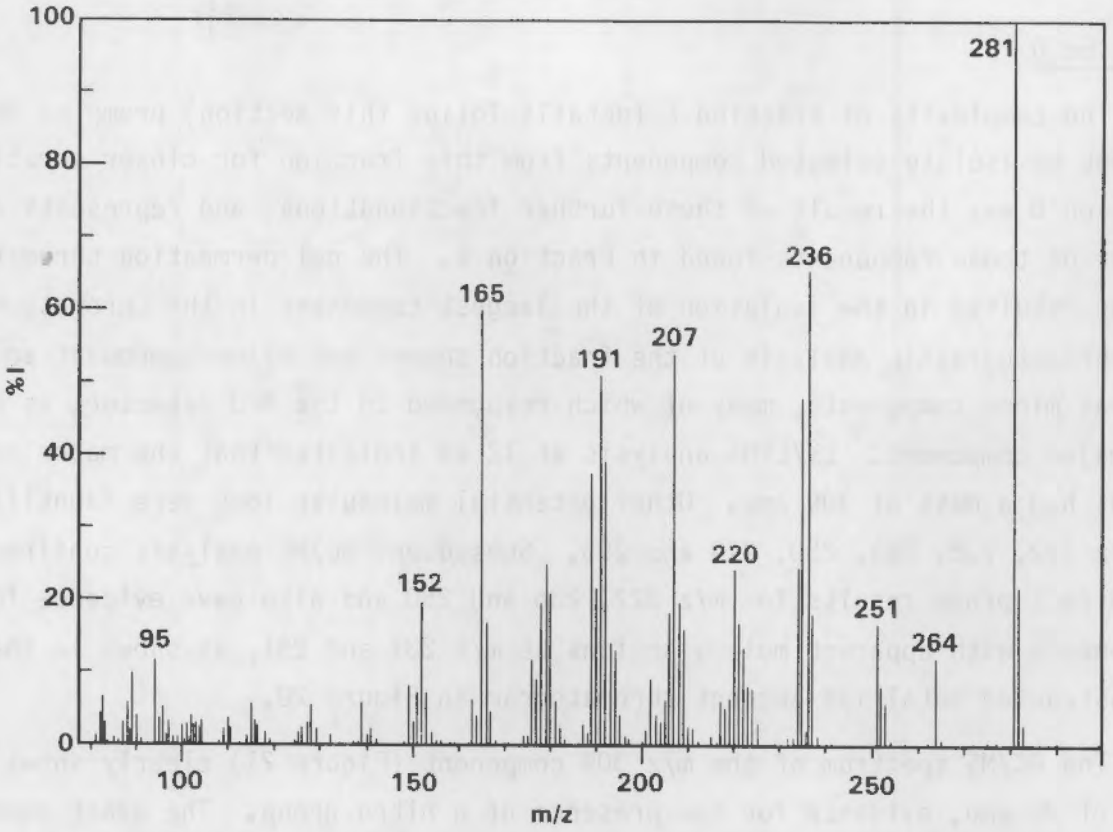

FIGURE 19. Mass Spectrum of a Component in Fraction C Tentatively Identified as an Alkyl Homolog of the Component Detailed in Figure 18

spectra are consistent with the tentative assignment of this series as the mononitro analogs of the alkylated fluorenones found in Fraction A, even though the nitro analogs for the first members of the fluorenone series were not found in Fraction C (i.e., compounds with molecular weights of 239 and 253, corresponding to the fluorenones with weights of 194 and 208). Lack of suitable standards did not allow any further structure elucidation.

The other recognizable series of homologous compounds in this fraction had molecular ions at m/z 263, 277 and 291, respectively. The mass spectra for these compounds showed losses of 17, 29 and 45 amu, but the quality of the data was not sufficient to allow further interpretation. No tentative assignments as to the structure of the series have been made. Similarly, a set of isomers eluting between 43 and 45 min, with apparent molecular ions at m/z 286, has not been identified.

Fraction D

The complexity of Fraction E (details follow this section) prompted an attempt to isolate selected components from this fraction for closer scrutiny. Fraction D was the result of these further fractionations, and represents a subset of those components found in Fraction E. The gel permeation chromatography resulted in the isolation of the largest component in the chromatogram. Gas chromatographic analysis of the fraction showed one major component and several minor components, many of which responded to the NPD detector, as did the major component. LV/LRMS analysis at 12 eV indicated that the major component had a mass of 309 amu. Other potential molecular ions were identified at m/z 222, 236, 245, 250, 279 and 295. Subsequent GC/MS analysis confirmed the direct probe results for m/z 222, 236 and 250 and also gave evidence for components with apparent molecular ions at m/z 237 and 251, as shown in the reconstructed total ion current chromatogram in Figure 20.

The GC/MS spectrum of the m/z 309 component (Figure 21) clearly shows the loss of 30 amu, evidence for the presence of a nitro group. The exact mass of 309.0753 amu, assigned by high resolution peak-matching experiments, fits an elemental composition of $C_{16}H_{11}N_3O_4$ with an error of -0.4 mmu. The abundant fragment ion at m/z 216 can easily be interpreted as arising from the sequential losses of NO_2 and HNO_2 from the molecular ion. Thus, there is ample

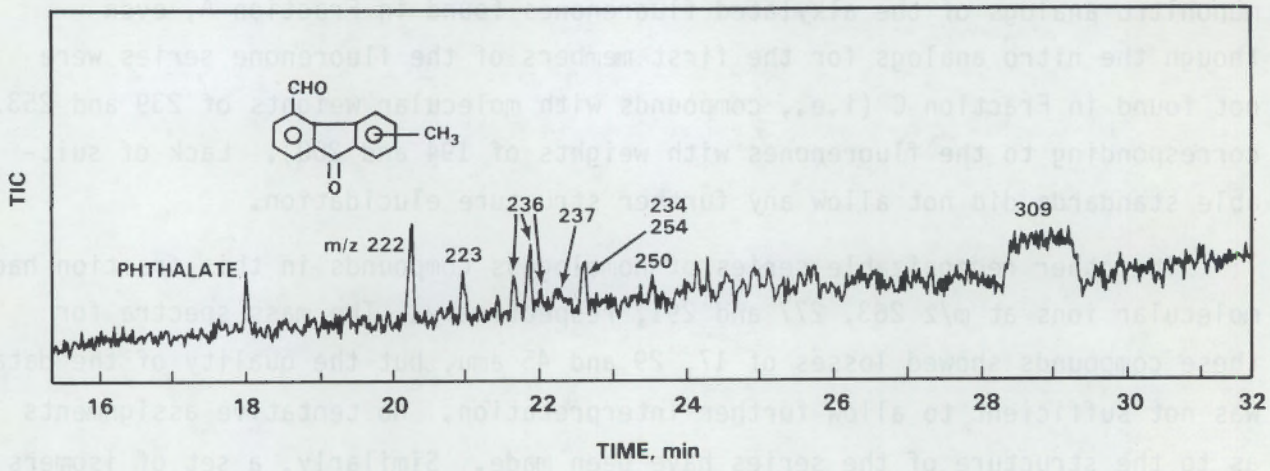

FIGURE 20. Reconstructed Total Ion Current Chromatogram of Fraction D. Peaks are labeled with the nominal mass of the molecular ion determined by GC/MS.

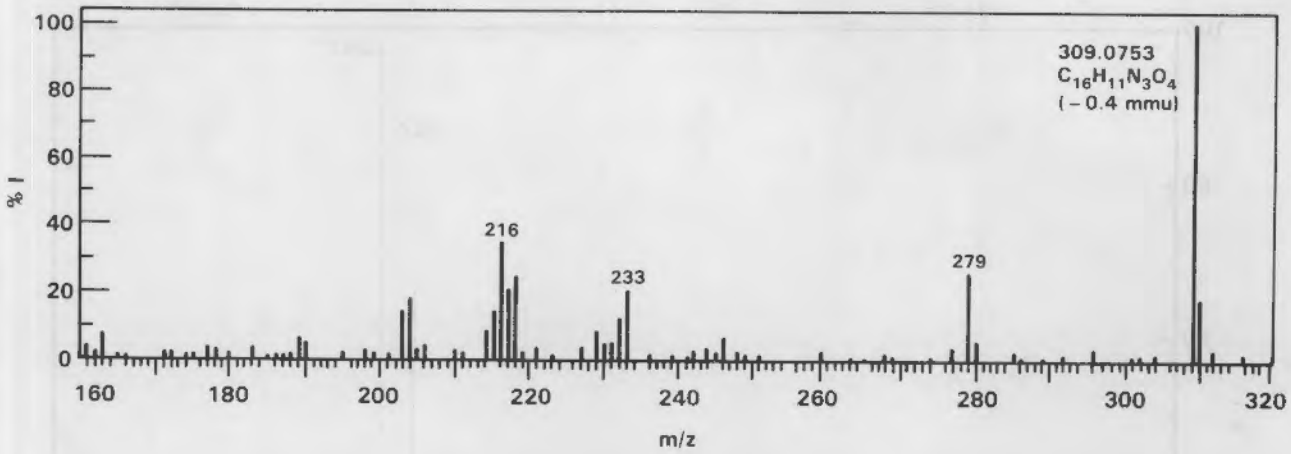

FIGURE 21. Mass Spectrum of Molecular Weight 309 Component in Fraction D

evidence to indicate that this compound is a dinitro-substituted derivative of the general formula $C_{16}H_{13}N$, whose basic structure has not yet been determined.

The spectra for the series of components with molecular weights of 222, 236 and 250 amu clearly showed the compounds to be members of the same homologous series found in Fraction E, tentatively identified as alkylated fluoren-9-one-x-carboxaldehydes. (See Results, Fraction E.) There was evidence from the GC/MS data for another homologous series with molecular ions at m/z 223, 237 and 251. These spectra all showed losses of 15, 16, and 72 amu from the apparent molecular species, but no reasonable interpretation of these losses has been made and no tentative identifications have been assigned. This latter series, which was not found among the major components in Fraction E, must be present in very small amounts which were concentrated in this subfraction.

Fraction E

Gas chromatographic analysis of this fraction indicated three major and numerous minor components. Low resolution direct probe MS revealed spectra that were surprisingly complex, even at an ionizing electron energy of 14 eV (Figure 22). Further analysis by GC/MS greatly simplified the complexity of the mixture, although complete resolution of all components was not achieved. From the GC/MS analysis (the reconstructed chromatogram is shown in Figure 23), a homologous series of components with apparent molecular ions at m/z 208, 222, 236, and 250 was identified. These spectra were characterized by a strong

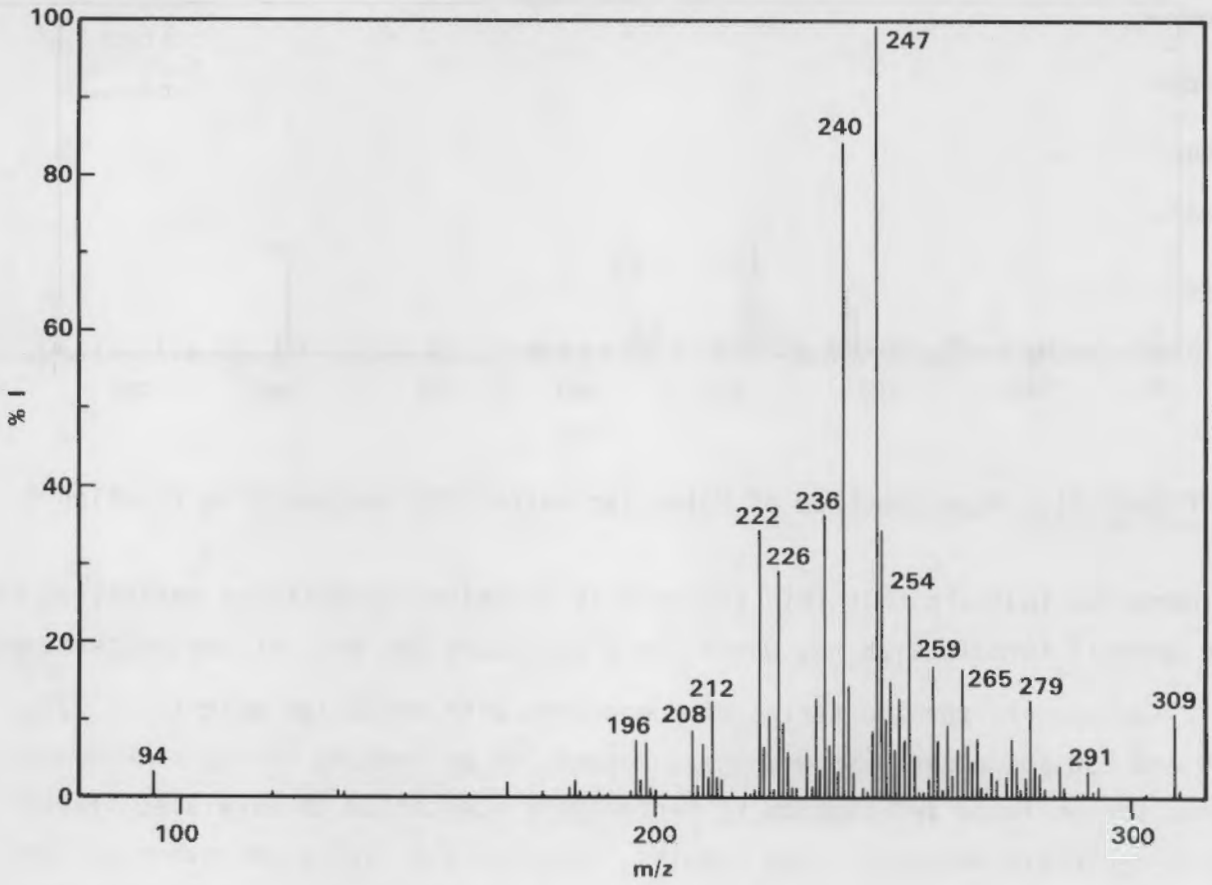

FIGURE 22. 14-eV Probe Mass Spectrum of Fraction E

molecular ion, a large loss of H and smaller losses of 28, 29, 57 and 58 amu, probably resulting from the losses of multiples of CO and HCO. High resolution mass measurements acquired on the total mixture (Table 13) are consistent with the presence of a homologous series beginning with the elemental composition of $C_{14}H_8O_2$ at m/z 208. The mass spectrum for the mass 208 member of the series (Figure 24) does not agree with the spectrum of either 9,10-anthracenedione or 9,10-phenanthrenedione, or with the spectrum of any isomer in the literature. The large loss of H indicates the presence of a very labile hydrogen atom on the molecular ion such as would be present with an aldehyde functionality. Therefore, this series, $C_{14}H_8O_2$, $C_{15}H_{10}O_2$, and $C_{16}H_{12}O_2$ tentatively been identified as alkylated fluoren-9-one-x-carboxaldehydes.

Another homologous series was identified by GC/MS at m/z 226, 240 and 254. The mass spectra of these components were characterized by the minor loss of 28

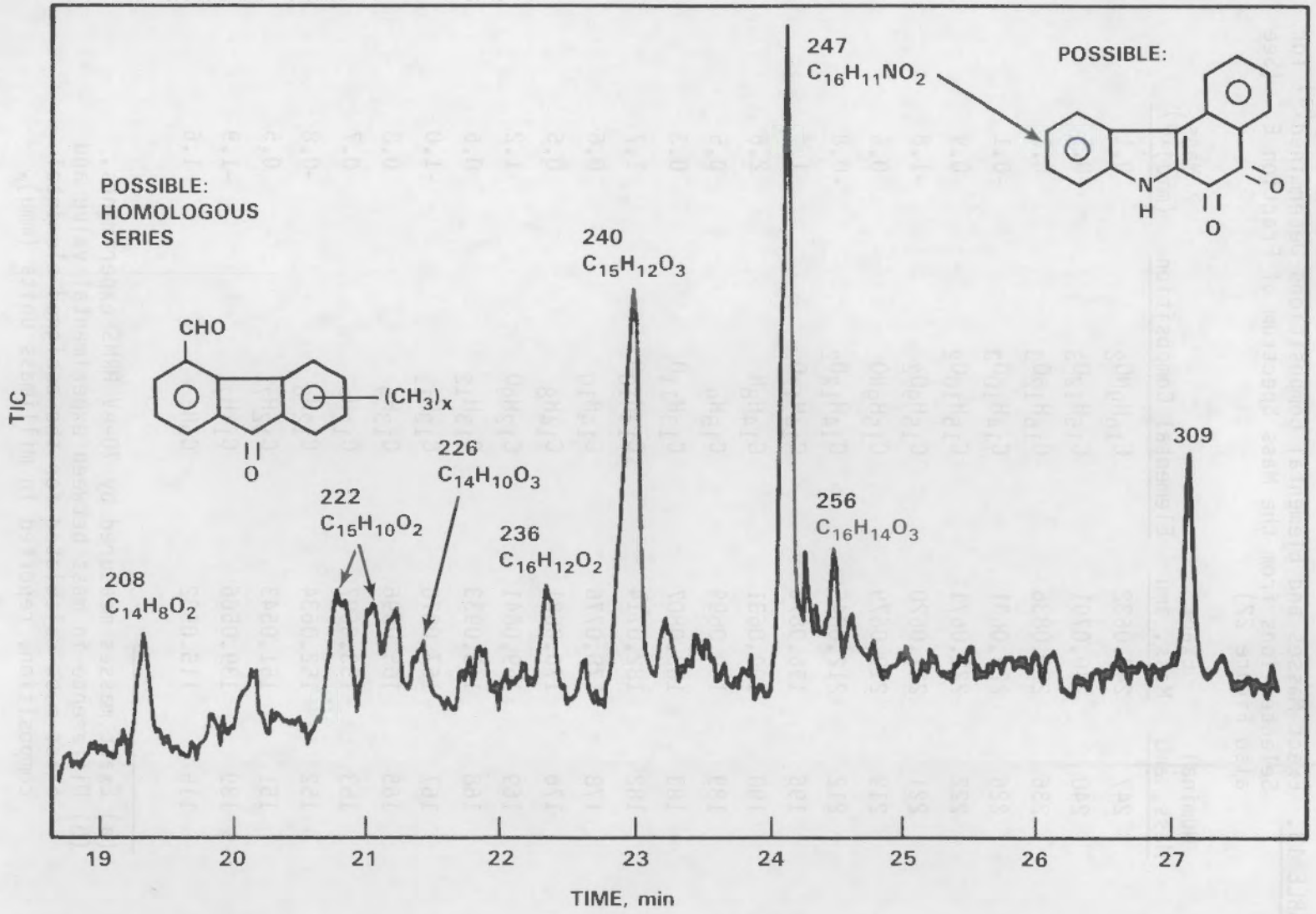

FIGURE 23. Reconstructed Total Ion Current Chromatogram of Fraction E

TABLE 13. Exact Masses and Elemental Compositions Determined[a] for Selected Ions from the Mass Spectrum of Fraction E. (See also Figure 22)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)[b]
247	247.0632	$C_{16}H_9NO_2$	0.1
240	240.0791	$C_{15}H_{12}O_3$	-0.5
236	236.0836	$C_{16}H_{12}O_3$	0.1
226	226.0631	$C_{14}H_{10}O_3$	-0.1
222	222.0671	$C_{15}H_{10}O_2$	0.9
221	221.0620	$C_{15}H_9O_2$	-1.8
219	219.0679	$C_{15}H_9NO$	0.5
212	212.0845	$C_{14}H_{12}O_2$	-0.8
196	196.0876	$C_{14}H_{12}O$	1.2
190	190.0631	$C_{14}H_8N$	2.6
189	189.0699	$C_{15}H_9$	0.5
183	183.0807	$C_{13}H_{11}O$	0.3
182	182.0714	$C_{13}H_{10}O$	1.7
178	178.0776	$C_{14}H_{10}$	0.6
176	176.0621	$C_{14}H_8$	0.5
169	169.0641	$C_{12}H_9O$	1.2
168	168.0933	$C_{13}H_{12}$	0.6
167	167.0870	$C_{13}H_{11}$	-1.0
165	165.0696	$C_{13}H_9$	0.8
153	153.0697	$C_{12}H_9$	0.7
152	152.0634	$C_{12}H_8$	-0.8
151	151.0543	$C_{12}H_7$	0.5
139	139.0566	$C_{11}H_7$	-1.9
115	115.0532	C_9H_7	1.6

(a) Exact masses measured by 70-eV HRMS experiments.
(b) Difference in mass between experimental value and exact mass calculated for the assigned elemental composition, reported in millimass units (mmu).

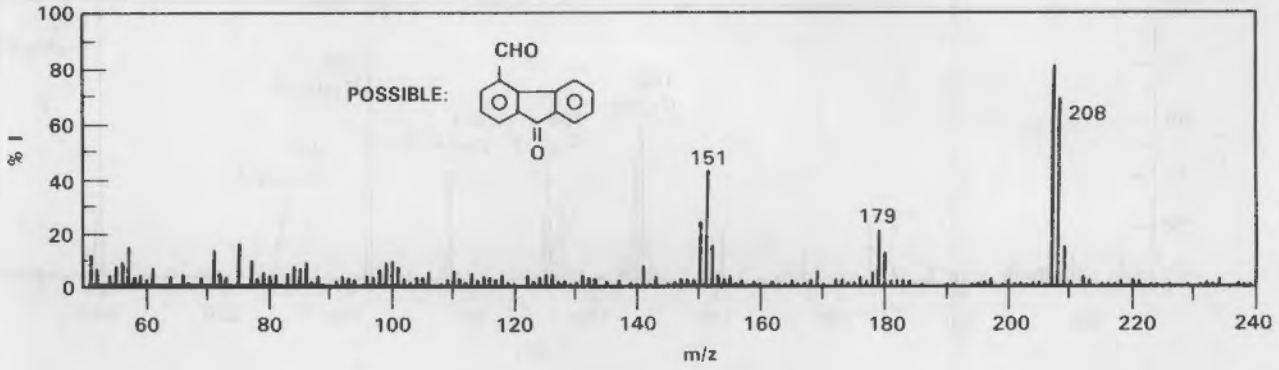

FIGURE 24. Mass Spectrum of Component from Fraction E with Molecular Weight 208

and the prominent losses of 44 and 57, which can be interpreted as the losses of CO, CO_2, and CO + HCO, respectively, from the molecular ions. Because the homolog of molecular weight 240 was a major component in the mixture, the assignments of elemental compositions to the molecular ion and major fragment ions of this compound were based on the high resolution data obtained on the mixture. (See Figure 25.) The assigned elemental compositions are internally consistent and may represent the correct elemental formulas for this mass spectrum.

The loss of CO_2, the sequential losses of CO and HCO, and the presence of three oxygen atoms in the molecular species are good evidence for the tentative identification of an aromatic anhydride. There was some GC/MS evidence that this series also include homologs of molecular weight 212, which would correspond to $C_{13}H_8O_3$, but the data were not conclusive. No tentative structure has been assigned to this series of suspected anhydrides because the starting point of the homologous series is not clear. For example, if the series actually started at molecular weight 198, then a possible parent structure would be naphthalene-1,8-dicarboxylic acid anhydride. However, if the series actually started at mass 212 or 226, entirely different parent structures would be required.

The mass spectrum of the most abundant major component in Fraction E is shown in Figure 26. Elemental compositions have been assigned to the three largest ions in the spectrum. There was no GC/MS evidence for the existence of

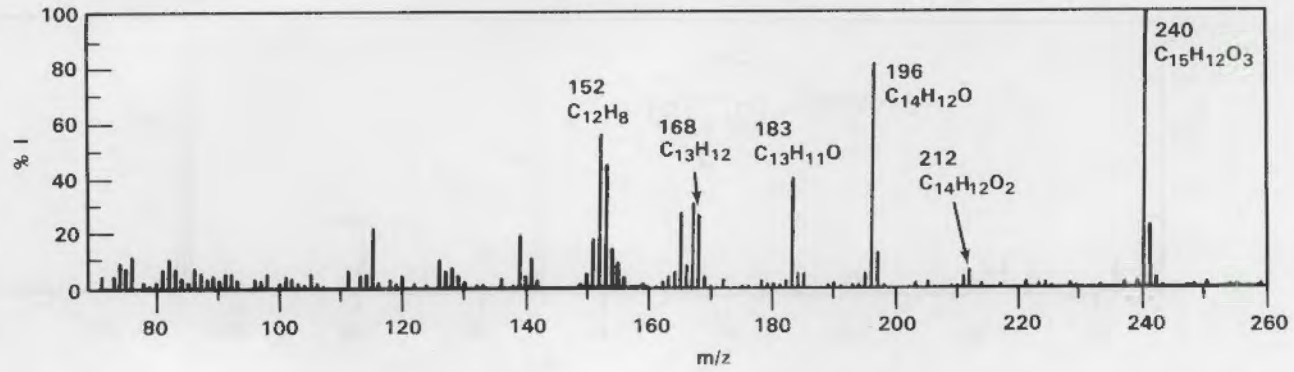

FIGURE 25. Mass Spectrum of Component from Fraction E with Molecular Weight 240

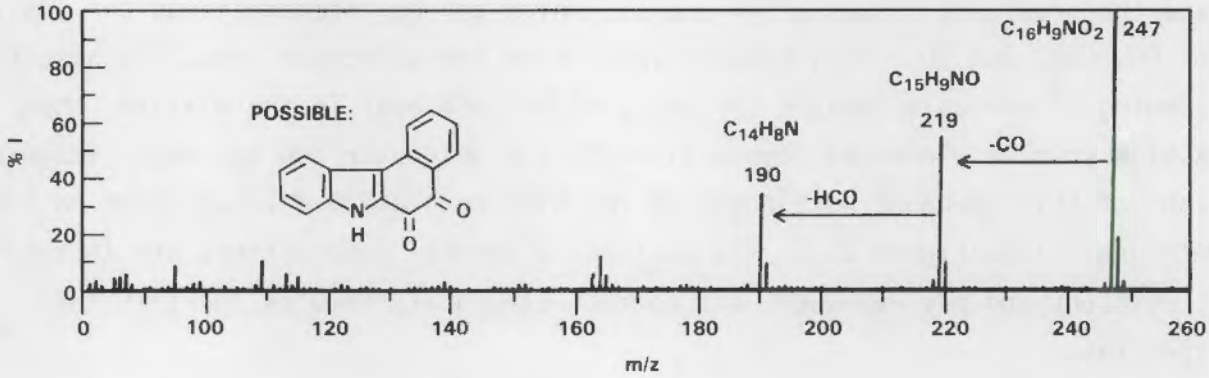

FIGURE 26. Mass Spectrum of Component from Fraction E with Molecular Weight 247

isomeric or homologous compounds of this component in this fraction. Even though this compound is isomeric with nitropyrene, the spectrum clearly rules out the presence of a nitro group and suggests the presence of a nitrogen-containing quinone or lactone. A reasonable structure, that of 7H-benzo[c]-carbazol-5,6-dione, is depicted in Figure 26. Positive identification has not been accomplished.

The mass spectrum of the other major component in Fraction E (Figure 27) indicates a molecular ion at m/z 309, with losses of 30 and 47, which can be interpreted as the losses of NO and HNO_2, and a series of fragment ions 16 amu apart. Unequivocal elemental composition data could not be assigned to the fragments, and no HRMS exact mass measurements were acquired for the molecular

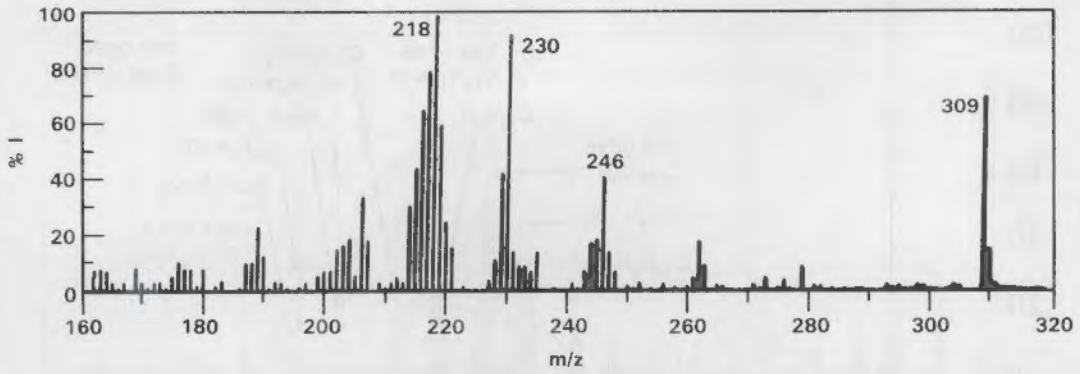

FIGURE 27. Mass Spectrum of Component from Fraction E with Molecular Weight 309

ion. A desire to further investigate this component led to subfractionation and collection of Fraction D (see prior section, Fraction D). Although Fraction D contained fewer components and was enriched in a component with molecular weight 309, for which an elemental composition of $C_{16}H_{11}N_3O_4$ was measured, the GC/MS spectra of the molecular weight 309 components found in Fractions D and E are clearly not identical. There is also some question as to whether Fraction D actually represents an enrichment of the major 309 isomer found in Fraction E. Further work will be required to establish the identities of these molecular weight 309 components and to determine whether they represent other members of the series of dinitro-substituted $C_{16}H_{13}N$ isomers which were found in Fraction F and I.

Fraction F

Purification of this fraction by gel permeation chromatography resulted in the collection of the largest peak in the chromatogram, which appeared to be isolated as a single, pure component. No gas chromatographic analysis of this sample was attempted due to the anticipated high molecular weight and polarity. The 70-eV direct probe mass spectrum of this sample (Figure 28) indicated a molecular weight for the major component of 354 amu. This assignment was confirmed by LV/LRMS analysis, which clearly indicated that m/z 354 was the molecular ion in greatest abundance, but which also uncovered the presence of a minor component of molecular weight 309. The lower molecular weight component was isolated by GC/MS, using a short capillary column (Figure 29), but the

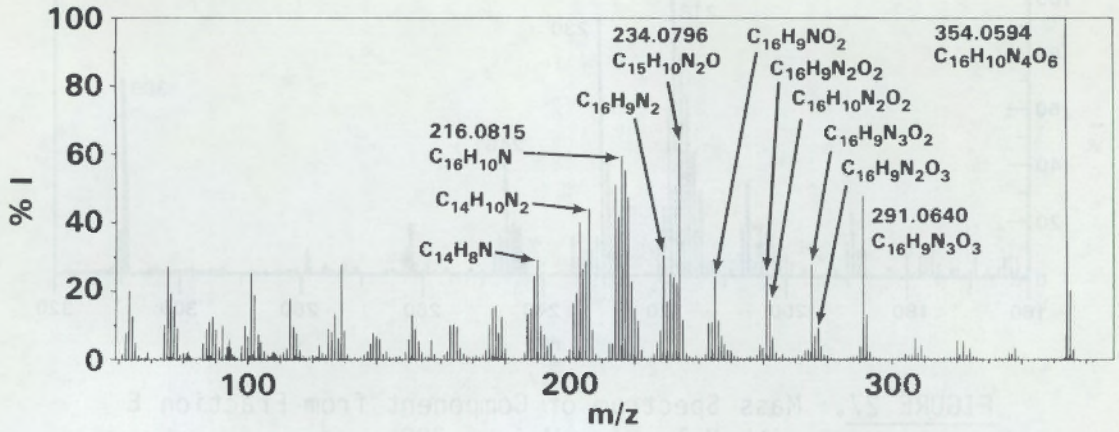

FIGURE 28. 70-eV Probe Mass Spectrum of Fraction F

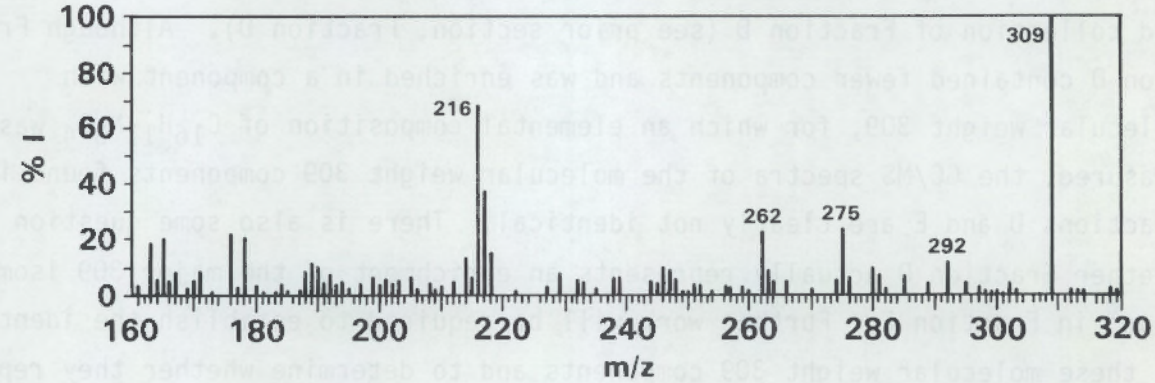

FIGURE 29. Mass Spectrum of Component from Fraction F with Molecular Weight 309

higher molecular weight component eluted as a broad, poorly defined peak which did not permit representative spectra to be acquired.

The molecular ions at m/z 309 and 354 have been assigned elemental compositions of $C_{16}H_{11}N_3O_4$ and $C_{16}H_{10}N_4O_6$, based on direct probe HRMS experiments. The GC/MS spectrum of the component of molecular weight 309 (Figure 29) shows evidence for the loss of 17, 47, and 93 amu, which can be interpreted as the loss of OH, HNO_2, and (HNO_2 + NO_2) from the molecular ion. Thus, the molecular weight 309 species appears to contain two nitro groups. The elemental composition assigned to the major fragment ions (Figure 28) are consistent with the presence of three nitro groups in the component of molecular weight 354 (see Table 14). The major fragment ions at m/z 291 ($C_{16}H_9N_3O_3$) and

TABLE 14. Exact Masses and Elemental Compositions Determined[a] for Selected Ions from the Mass Spectrum of Fraction F. (See also Figure 28)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)[b]
355	355.0607	$^{13}CC_{15}H_{10}N_4O_6$	2.6
354	354.0594	$C_{16}H_{10}N_4O_6$	0.5
338	338.0662	$C_{16}H_{10}N_4O_5$	-1.1
336	336.0511	$C_{16}H_8N_4O_5$	-1.7
309	309.0721	$C_{16}H_{11}N_3O_4$	2.8
307	307.0586	$C_{16}H_9N_3O_4$	0.7
291	291.0640	$C_{16}H_9N_3O_3$	0.4
278	278.0690	$C_{16}H_{10}N_2O_3$	0.2
277	277.0640	$C_{16}H_9N_2O_3$	-2.7
275	275.0688	$C_{16}H_9N_3O_2$	0.6
262	262.0731	$C_{16}H_{10}N_2O_2$	1.1
261	261.0670	$C_{16}H_9N_2O_2$	-0.6
259	259.0752	$C_{16}H_9N_3O$	-0.7
247	247.0731	$C_{15}H_9N_3O$	1.4
246	246.0779	$C_{16}H_{10}N_2O$	1.4
245	245.0719	$C_{16}H_9N_2O$	-0.4
244	244.0663	$C_{16}H_8N_2O$	-2.6
235	235.0806	$^{13}CC_{14}H_{10}N_2O$	2.1
234	234.0796	$C_{15}H_{10}N_2O$	-0.3
233	233.0715	$C_{15}H_9N_2O$	0.0
231	231.0693	$C_{16}H_9NO$	-0.9
229	229.0760	$C_{16}H_9N_2$	0.5
218	218.0830	$C_{15}H_{10}N_2$	1.4
217	217.0804	$C_{15}H_9N_2$	-3.9
216	216.0815	$C_{16}H_{10}N$	-0.2
215	215.0702	$C_{16}H_9N$	3.3
214	214.0658	$C_{16}H_8N$	-0.1
206	206.0833	$C_{14}H_{10}N_2$	1.1
205	205.0757	$C_{14}H_9N_2$	0.9
204	204.0771	$C_{15}H_{10}N$	4.2

TABLE 14. (contd)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)(b)
203	203.0725	$C_{15}H_9N$	1.0
190	190.0676	$C_{14}H_8N$	-2.0
189	189.0696	$C_{15}H_9$	0.8
	189.0559	$C_{14}H_7N$	1.9
188	188.0612	$C_{15}H_8$	1.4
	188.0492	$C_{14}H_6N$	0.8
187	187.0537	$C_{15}H_7$	1.1

(a) Exact masses measured by 70-eV HRMS experiments.
(b) Difference in mass between experimental value and exact mass calculated for the assigned elemental composition, reported in millimass unit.

234 ($C_{15}H_{10}N_2O$) are formed from the 354 molecular ion as a result of the losses of the elements HNO_3 and $CO(NO_2)_2$, respectively. These are unusual losses for multinitro compounds and are most certainly clues to the structure of this compound. The negative ion chemical ionization (NICI) mass spectrum of Fraction F (Figure 30) does not display the molecular ion at m/z 354, but strong signals are present for most of the high molecular weight fragment ions found in the positive ion 70-eV mass spectrum. The lack of significant fragmentation below m/z 254 and the abundance of the ion at m/z 275 (assumed to be $C_{16}H_9N_3O_2$) are also clues to the molecular structure. Once the structure of the nitrogen-substituted parent PAH has been determined, the many unique fragmentation characteristics displayed by this isomer may allow the positions of the nitro groups to be deduced.

Fraction G

As seen from Figure 1, Fraction G contains the peak of greatest intensity among the well-defined peaks in the 45 to 55-min region of the chromatogram. Because of the anticipated high molecular weight and polarity of the component, no GC analyses were initially performed. Low resolution direct probe MS analyses, acquired at both 70- and 14-eV electron energies, indicated that this

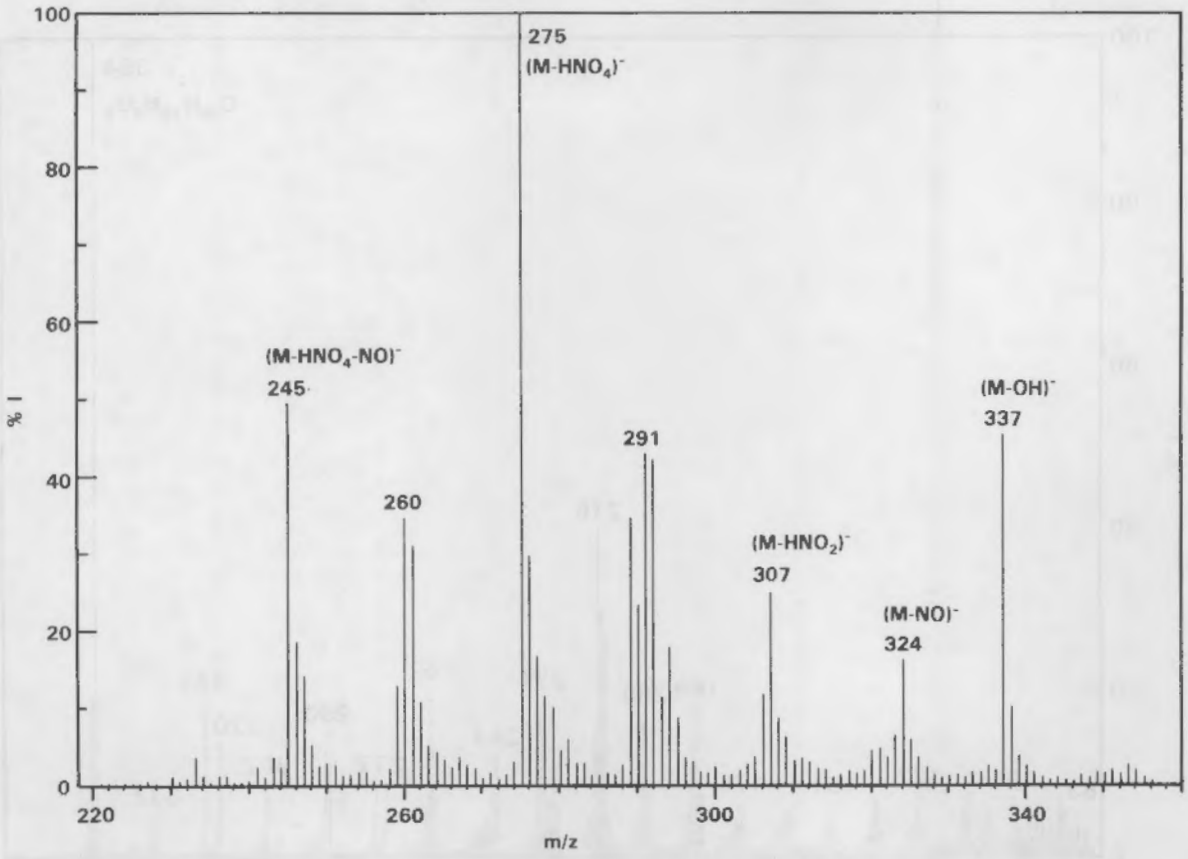

FIGURE 30. Negative Ion Chemical Ionization Mass Spectrum of Fraction F

fraction was composed primarily of a single component (molecular weight, 354 amu) that had a fragmentation pattern somewhat different from the component found in Fraction F with the same nominal molecular weight. (See Figure 31.) Although many of the major fragments in both spectra were similar (compare Figure 31 with Figure 28), the fragments at m/z 291 and 234, so prominent in the spectrum of Fraction F, were not nearly so important in Fraction G. HRMS experiments (see Table 15) confirmed that the molecular ion at m/z 354 had an elemental composition of $C_{16}H_{10}N_4O_6$ and was, as anticipated, isomeric with that compound found in Fraction F. As for the compound in Fraction F, the fragment ions were all consistent with the presence of three nitro groups attached to an aromatic nucleus. NICI experiments (see spectrum in Figure 32) yielded results comparable to those found for Fraction F: 1) no molecular ion is present, 2) the highest mass in the spectrum corresponds to the loss of OH from the

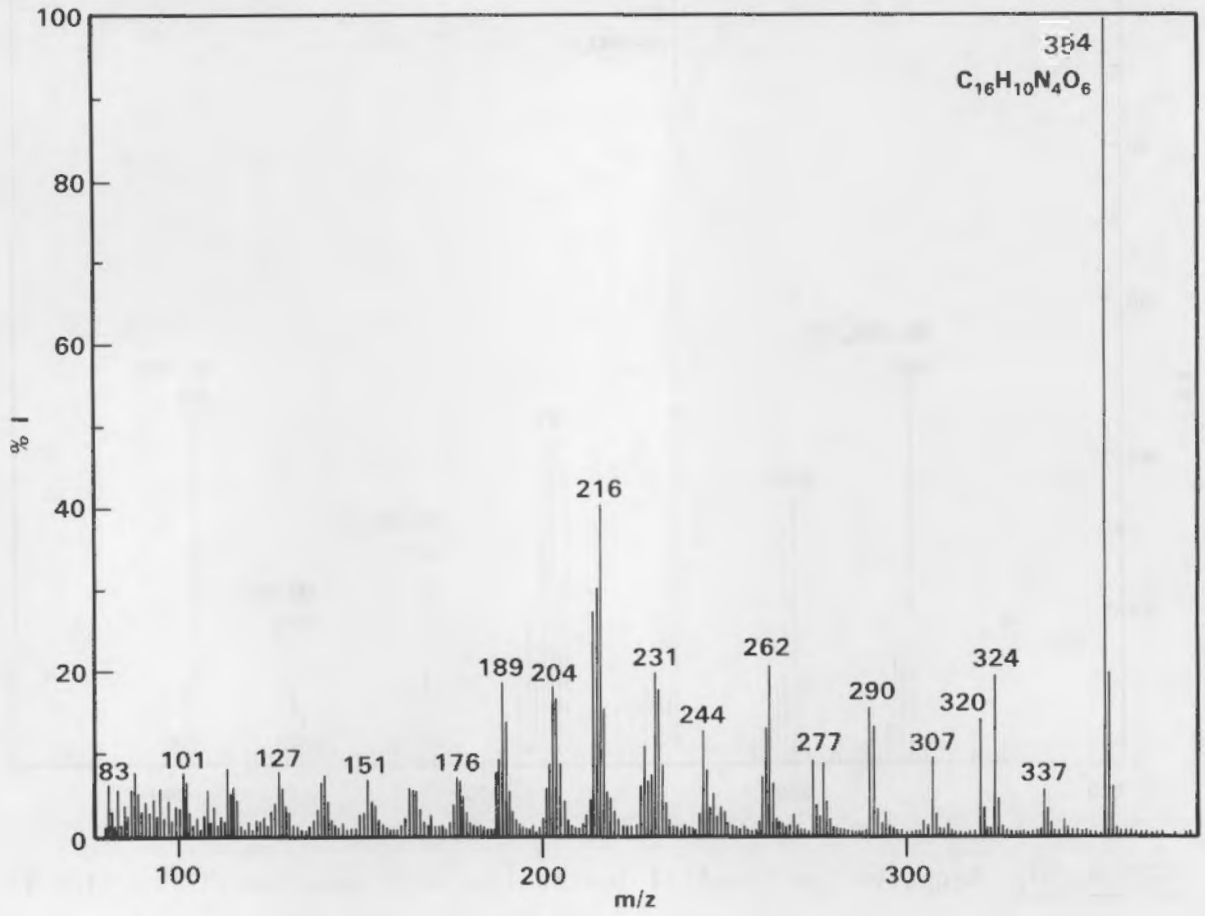

FIGURE 31. 70-eV Probe Mass Spectrum of Fraction G

unstable parent ion, and 3) many of the same fragment ions are found as in the postive-ion EI spectrum. However, a comparison of the two spectra (Figures 30 and 32) reveals significant differences in the intensities of the various fragment ions, probably resulting from the different locations of the nitro groups on the isomeric forms.

In an attempt to confirm the presence of only one isomer at m/z 354 in Fraction G, the sample was analyzed by GC/MS using on-column injection, a short column and linear programming to a reasonably high final temperature. Although the isomer at m/z 354 failed to emerge as a well-characterized peak, a component with a molecular ion at m/z 320 eluted. (See Figure 33.) The mass spectrum of this minor componerit was readily interpreted as that of an aromatic nucleus substituted with two nitro groups: m/z 320, $M^{\ddagger}$; m/z 290, $(M-NO)^{\ddagger}$;

TABLE 15. Exact Masses and Elemental Compositions Determined[a] for Selected Ions from the Mass Spectrum of Fraction G. (See also Figure 31)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)[b]
355	355.0630	$^{13}CC_{15}H_{10}N_4O_6$	0.3
354	354.0597	$C_{16}H_{10}N_4O_6$	0.3
338	338.0647	$C_{16}H_{10}N_4O_5$	0.4
337	337.0580	$C_{16}H_9N_4O_5$	-0.7
324	324.0641	$C_{16}H_{10}N_3O_5$	-2.1
320	320.0544	$C_{16}H_8N_4O_4$	0.1
307	307.0580	$C_{16}H_9N_3O_4$	1.3
291	291.0643	$C_{16}H_9N_3O_3$	0.0
290	290.0579	$C_{16}H_8N_3O_3$	-1.3
277	277.0627	$C_{16}H_9N_2O_3$	-1.4
274	274.0618	$C_{16}H_8N_3O_2$	-0.5
262	262.0670		
261	261.0657	$C_{16}H_9N_2O_2$	0.7
245	245.0723	$C_{16}H_9N_2O$	-0.8
244	244.0632	$C_{16}H_8N_2O$	0.4
232	232.0737	$C_{16}H_{10}NO$	2.5
231	231.0686	$C_{16}H_9NO$	-0.2
228	228.0680	$C_{16}H_8N_2$	0.7
216	216.800	$C_{16}H_{10}N$	1.4
215	215.0726	$C_{16}H_9N$	0.9
214	214.0659	$C_{16}H_8N$	-0.2
213	213.0583	$C_{16}H_7N$	-0.5
205	205.0772	$C_{14}H_9N_2$	-0.7
204	204.0797	$C_{15}H_{10}N$	1.6
203	203.0745	$C_{15}H_9N$	-1.0
202	202.0662	$C_{15}H_8N$	-0.5
190	190.0638	$C_{14}H_8N$	1.8
189	189.0696	$C_{15}H_9$	0.8
177	177.0580	$C_{13}H_7N$	-0.1
165	165.0702	$C_{13}H_9$	0.2

TABLE 15. (contd)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)(b)
163	163.0554	$C_{13}H_7$	-0.6
151	151.0535	$C_{12}H_7$	1.3
139	139.0538	$C_{11}H_7$	0.9
115	115.0541	C_9H_7	0.7

(a) Exact masses measured by 70-eV HRMS experiments.
(b) Difference in mass between experimental value and exact mass calculated for the assigned elemental composition, reported on millimass units (mmu).

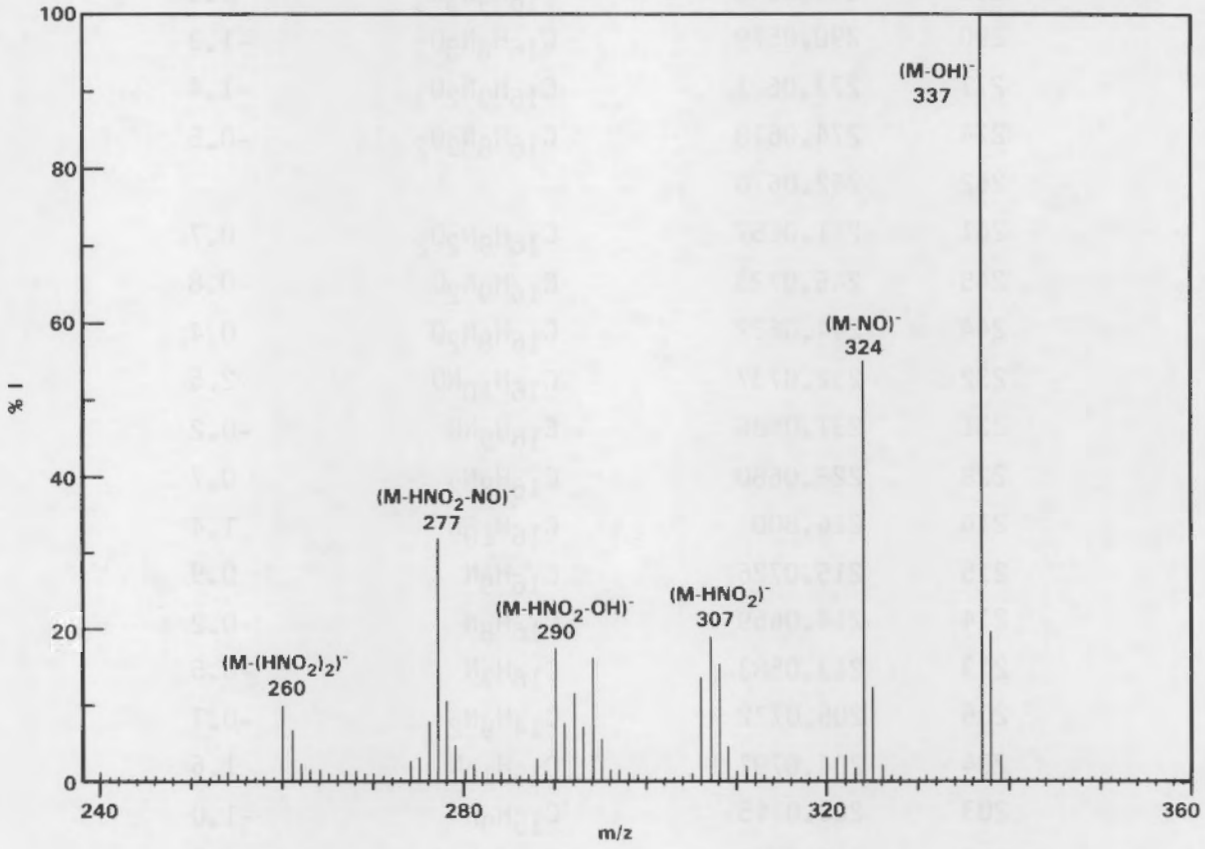

FIGURE 32. Negative Ion Chemical Ionization Mass Spectrum of Fraction G

m/z 274, $(M-NO_2)^{\ddagger}$; m/z 262, $(M-NO-CO)^{\ddagger}$; m/z 228, $(M-(NO_2)_2)^{\ddagger}$; and m/z 216, $(M-NO_2-NO-CO)^{\ddagger}$. With the exception of the composition of ion at m/z 216, all of the elemental compositions assigned by high resolution measurements on the mixture of components in Fraction G are consistent with this interpretation of the spectrum. Based on the spectrum derived from GC/MS analysis of the component and on the elemental composition of the molecular ion from the high resolution measurements made on the mixture, the minor component has been tentatively identified as an isomer of the general form represented by a dinitrodiazachrysene, as depicted in Figure 33.

In order to gain information regarding the fragmentation pathways of the component of m/z 354, and to demonstrate that all of the ions in the relatively rich spectrum could be identified as ultimately originating with the molecular ions at m/z 354 and 320, the unimolecular metastable ion decompositions occurring in the 2nd field-free region of the mass spectrometer were examined using mass-analyzed ion kinetic energy spectrometry (MIKES). These studies provided direct evidence for the association of parent ions with their corresponding daughter ions, thus indicating how the ionized molecules fragment. These data were used to help confirm whether the elemental compositions assigned to the fragment ions by high resolution experiments are consistent with the total loss of mass from the molecular ion. The MIKES spectrum for m/z 354 is shown in Figure 34; MIKES spectra were also acquired for the species at m/z 354, 324, 320, 307, 291, 290, and 277, and the results are illustrated

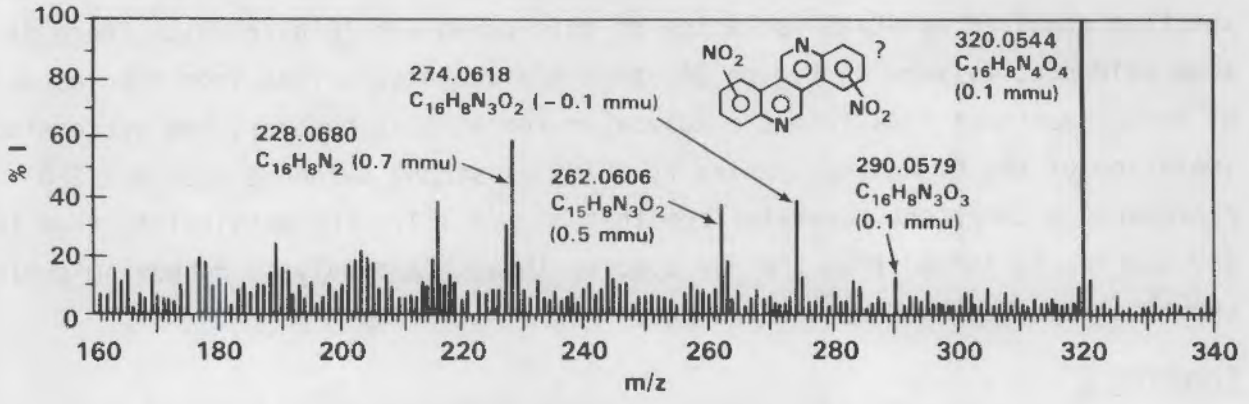

FIGURE 33. Mass Spectrum of Component from Fraction G with Molecular Weight 320

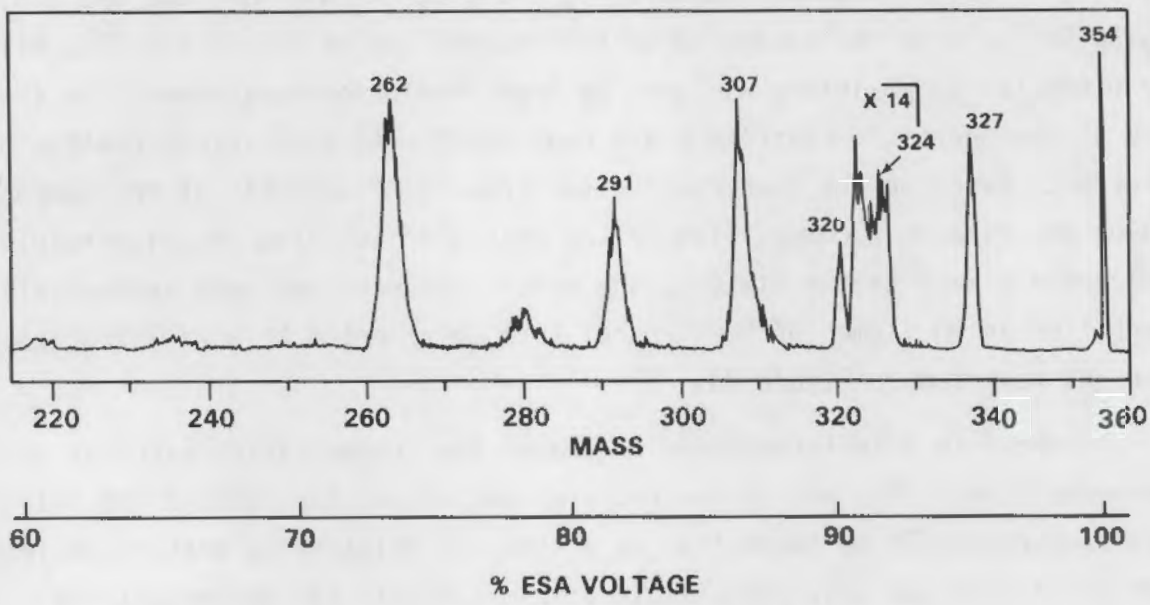

FIGURE 34. Unimolecular MIKES Spectrum for m/z 354 from Fraction G

schematically in Figure 35. All the losses listed were consistent with the high resolution data, with the exception of the few losses which appeared to involve CO. These discrepancies are not serious, as the mass assignment could be one or two mass units in error for wide composite metastable ion signals such as those to which CO loss was assigned. (Typical signal widths are seen in Figure 34.) Overall, the MIKES data confirmed the HRMS mass assignments and demonstrated the presence of three nitro groups in the $C_{16}H_{10}N_4O_6$ species. Furthermore, the MIKES data for the ion of m/z 320 are consistent with the mass spectrum acquired by GC/MS isolation of this component (Figure 33). There is some evidence, as seen in Figure 34, that m/z 320 also arises from the losses of the elements of H_2O_2 from the molecular ion at 354; however, the successful isolation of the $C_{16}H_8N_4O_4$ species by GC/MS is strong evidence that m/z 320 represents a component separate from that at m/z 354. The possibility that the 320 species is formed from the 354 species through a pyrolysis mechanism during chromatography has not been ruled out.

Fraction H

Gel permeation chromatography of Fraction H resulted in the collection of a single chromatographic peak. However, when this fraction was gas

chromatographed using vaporization injection, several peaks eluted at very long (>60 min) retention times. These peaks had very strong responses to the NPD detector, indicating the presence of nitrogen atoms. The peak shapes were badly distorted, and it was not clear whether the compounds eluting represented original components of the mixture, or whether they were pyrolysis products from the vaporization injection. Additional evidence for the possibility of pyrolysis came from gas chromatographic experiments using cool, on-column injection, which resulted in an absence of peaks eluting at the long retention times which corresponded to the vaporization injection retention times.

Low resolution direct probe MS experiments conducted with both 70- and 14-eV ionization energies indicated that Fraction H contained a major component with a molecular weight of 399, a minor component of mass 354, and a large amount of extraneous, hydrocarbon-like material which was probably stationary phase from the LC column. As seen in the selected ion chromatograms in Figure 36, the ion currents for molecular ions at m/z 399 and 354, along with their corresponding fragment ions (such as m/z 290) maximize between scans 60 and 65, whereas the extraneous hydrocarbon material desorbs from the probe at a lower temperature, represented in the TIC chromatogram by the first broad hump in the desorption profile. The 70-eV mass spectrum, shown in Figure 37, is the data from scan 62 and thus represents a composite spectrum, composed primarily of the mass spectra of the two components with molecular weights 399 and 354, the vast majority of the signals arising from the mass 399 compound.

If a fourth nitro group were added to one of the trinitro-containing species found in Fraction F or G, the resulting molecular weight would be 399 amu. The HRMS results (Table 16) confirmed the possibility of the fourth nitro group, and the fragmentation pathways determined by MIKES analysis (Figure 38) conclusively demonstrated the presence of four nitro groups. This mass 399 component represents the third and largest member yet found of a series of related compounds which probably differ only in the location and number of nitro groups. Additional evidence that the mass 399 component is related to the mass 354 components is seen in its NICI spectrum (Figure 39), which shows the characteristic absence of a molecular ion and the presence of a (M-OH) pseudomolecular ion at m/z 381.

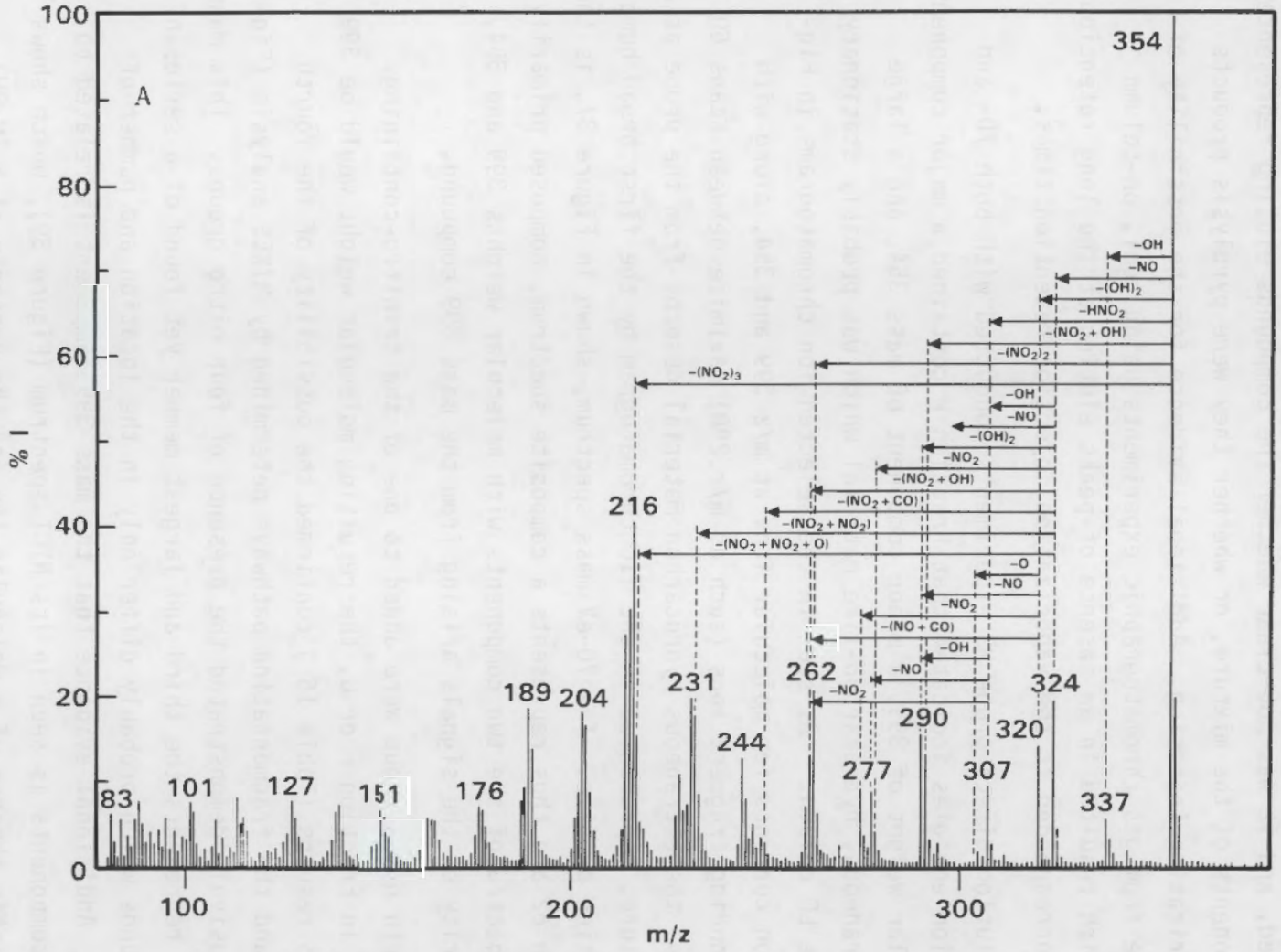

FIGURE 35. Summary of Fragmentation Pathways Demonstrated Through the Observation of Metastable Ion Signals in the MIKES Spectra of Selected Ions from Fraction G, Displayed as as Follows: A) m/z 354, 324, 320 and 307, and B) m/z 291, 290 and 277

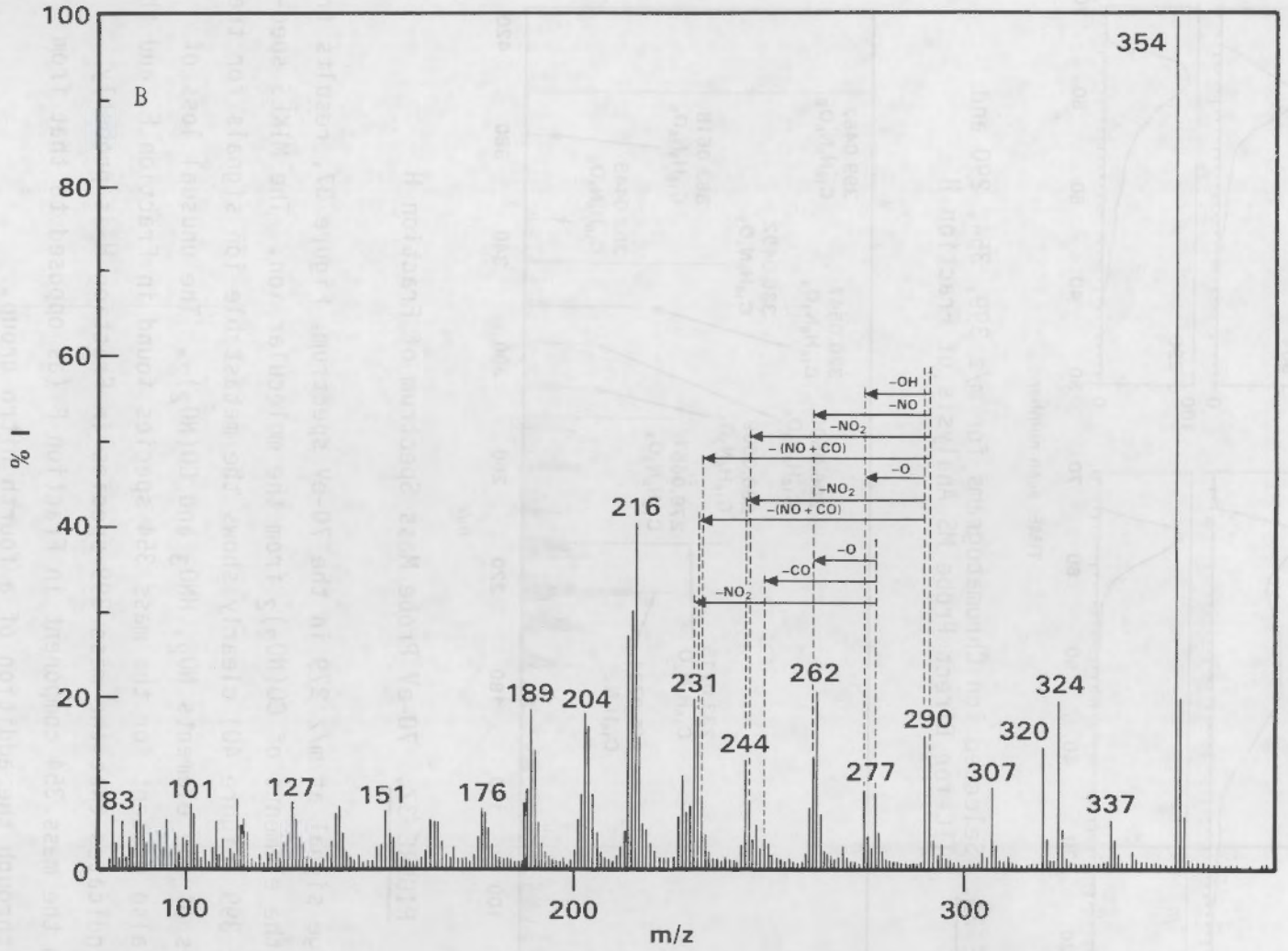

FIGURE 35. (contd)

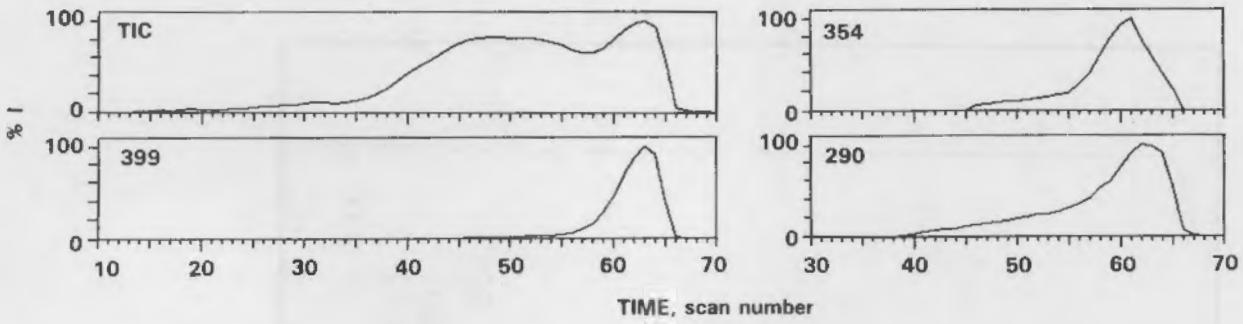

FIGURE 36. Selected Ion Chromatograms for m/z 399, 354, 290 and TIC for Direct Probe MS Analysis of Fraction H

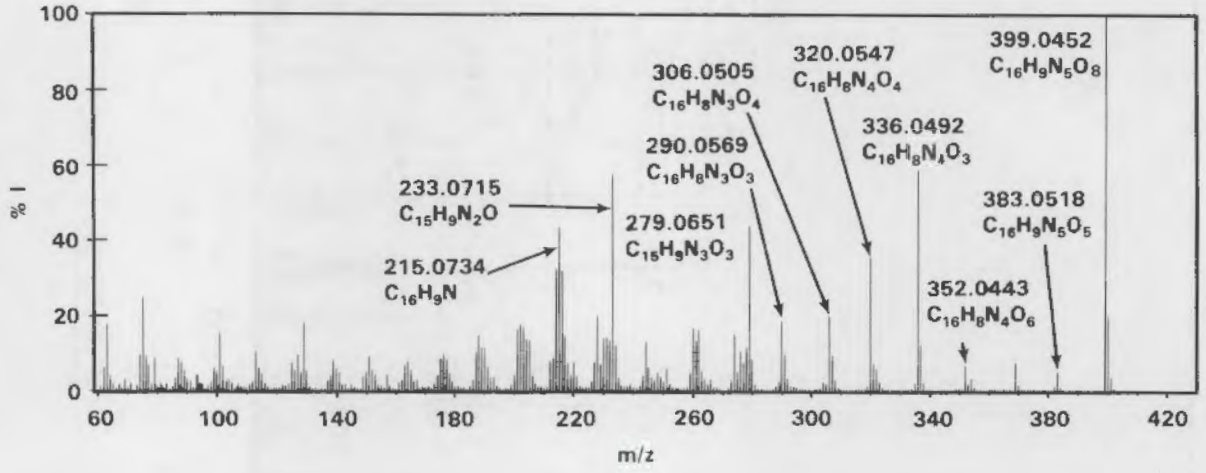

FIGURE 37. 70-eV Probe Mass Spectrum of Fraction H

The large signal at m/z 279 in the 70-eV spectrum, Figure 37, results from the loss of the elements of $CO(NO_2)_2$ from the molecular ion. The MIKES spectrum for m/z 399 (Figure 40) clearly shows the metastable ion signals for the direct losses of the elements NO_2, HNO_3 and $CO(NO_2)_2$. The unusual loss of $CO(NO_2)_2$ is also present for the mass 354 species found in Fraction F, and this similarity indicates that the mass 399 species in Fraction H is probably derived from the mass 354 component in Fraction F (as opposed to that from Fraction G) through the addition of a fourth nitro group.

At the present time, no conclusive structure has been assigned to the hydrocarbon nucleus from which the series of di-, tri-, and tetra-nitro isomers

TABLE 16. Exact Masses and Elemental Compositions Determined[a] for Selected Ions in the Mass Spectrum of Fraction H. (See also Figure 37)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)[b]
400	400.0471	$^{13}CC_{15}H_9N_5O_8$	1.3
399	399.0452	$C_{16}H_9N_5O_8$	-0.1
383	383.0492	$C_{16}H_9N_5O_7$	0.9
354	354.0598	$C_{16}H_{10}N_4O_6$	0.2
352	352.0443	$C_{16}H_8N_4O_6$	0.0
337	337.0559	$^{13}CC_{15}H_8N_4O_5$	0.7
336	336.0492	$C_{16}H_8N_4O_5$	0.6
322	322.0482	$C_{16}H_8N_3O_5$	-1.9
321	321.0575	$^{13}CC_{15}H_8N_4O_4$	0.4
320	320.0546	$C_{16}H_8N_4O_4$	0.0
307	307.0554	$^{13}CC_{15}H_8N_3O_4$	-0.6
306	306.0510	$C_{16}H_8N_3O_4$	0.4
291	291.0634	$C_{16}H_9N_3O_3$	1.0
290	290.0569	$C_{16}H_8N_3O_3$	-0.3
280	280.0680	$^{13}CC_{15}H_9N_3O_3$	-0.3
279	279.0651	$C_{15}H_9N_3O_3$	-0.7
278	278.0572	$C_{15}H_8N_3O_3$	-0.7
277	277.0593	$C_{16}H_9N_2O_3$	2.0
276	276.0540	$C_{16}H_8N_2O_3$	-0.5
275	275.0643	$^{13}CC_{15}H_8N_3O_2$	0.6
274	274.0610	$C_{16}H_8N_3O_2$	0.6
262	262.0649	$C_{15}H_8N_3O_2$	-3.3
261	261.0658	$C_{16}H_9N_2O_2$	0.6
260	260.0576	$C_{16}H_8N_2O_2$	0.9
251	251.0691	$C_{14}H_9N_3O_2$	0.4
244	244.0627	$C_{16}H_8N_2O$	0.9
234	234.0738	$^{13}CC_{14}H_9N_2O$	0.9
233	233.0715	$C_{15}H_9N_2O$	-0.1
232	232.0647	$C_{15}H_8N_2O$	-1.0
231	231.0670	$C_{16}H_9NO$	1.4

TABLE 16. (contd)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)(b)
230	230.0615	$C_{16}H_8NO$	-0.9
228	228.0685	$C_{16}H_8N_2$	0.3
227	227.0614	$C_{16}H_7N_2$	-1.0
220	220.0647	$C_{14}H_8NO_2$	-0.1
217	217.0767	$C_{15}H_9N_2$	-0.1
216	216.0693	$C_{15}H_8N_2$	-0.6
215	215.0725	$C_{16}H_9N$	1.0
214	214.0653	$C_{16}H_8N$	0.4
213	213.0574	$C_{16}H_7N$	0.5
212	212.0502	$C_{16}H_6N$	-0.2
205	205.0757	$C_{14}H_9N_2$	0.9
204	204.0674	$C_{15}H_8N_2$	1.4
203	203.0735	$C_{15}H_9N$	0.0
202	202.0664	$C_{15}H_8N$	-0.7
201	201.0581	$C_{15}H_7N$	-0.3
190	190.0649	$C_{14}H_8N$	0.7
189	189.0579	$C_{14}H_7N$	0.0
188	188.0510	$C_{14}N_6N$	-1.0
187	187.0551	$C_{15}H_7$	-0.3
176	176.0621	$C_{14}H_8$	0.5
175	175.0538	$C_{14}H_7$	0.9
151	151.0533	$C_{12}H_7$	1.4
129	129.0339	C_9H_5O	0.1
113	113.0392	C_9H_5	-0.1
101	101.0383	C_8H_5	0.8

(a) Exact masses measured by 70-eV HRMS experiments.
(b) Difference in mass between experimental value and exact mass calculated for the assigned elemental composition reported in millimass units (mmu).

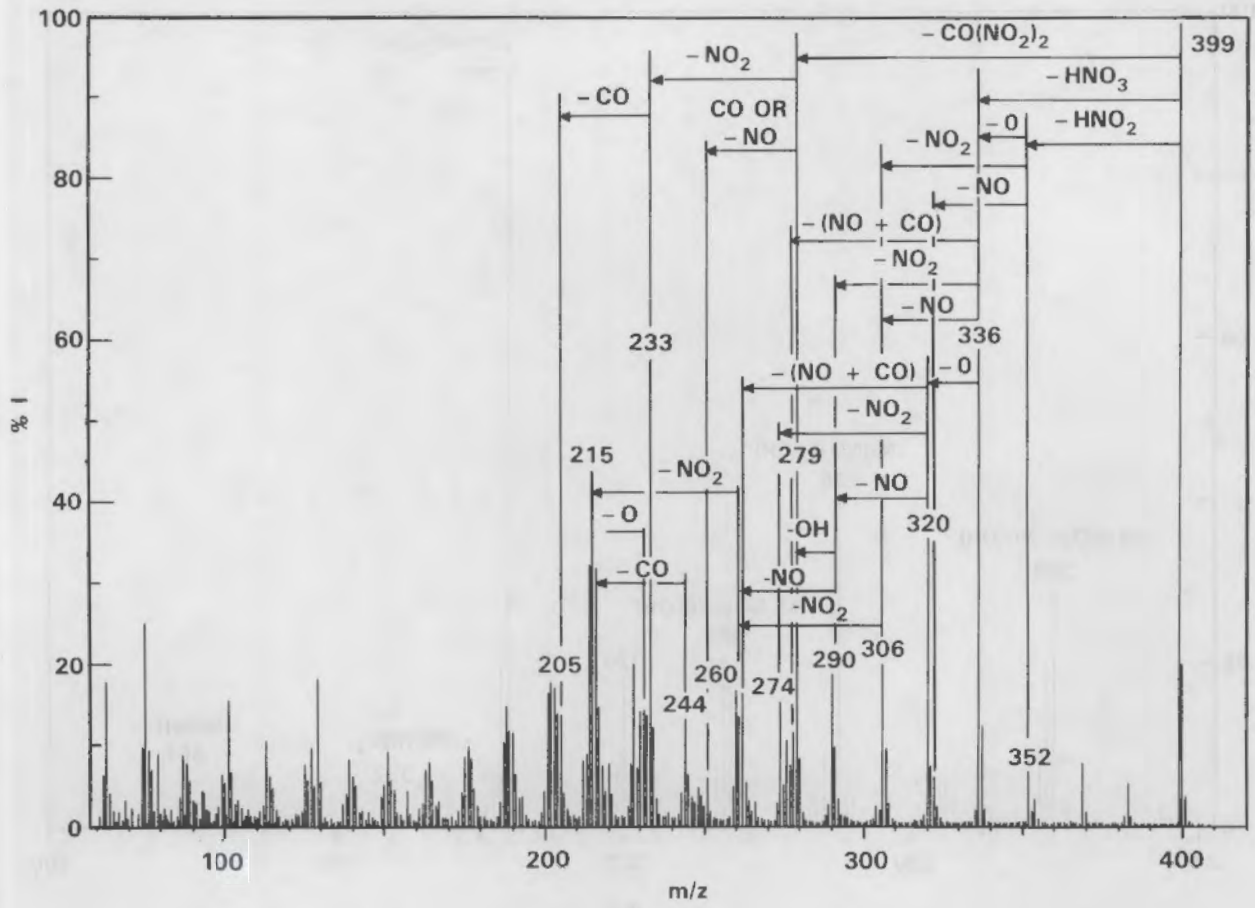

FIGURE 38. Summary of Fragmentation Pathways Demonstrated Through the Observation of Metastable Ion Signals in the MIKES Spectra of Selected Ions from Fraction H

(with nominal molecular weights of 309, 354 and 399, respectively) has been derived. Possible structures for the $C_{16}H_{13}N$ nucleus include aminophenylnaphthalene, methylphenylquinoline, and a dihydrobenzocarbazole. Of these choices, the phenylnaphthalene nucleus seems the most reasonable.

Fraction I

The final clean-up of this fraction using the reverse-phase MCH-10 preparatory column resulted in the collection of what appeared to be a single component of very high purity. Because the molecular weight of this component was expected to be at least 399 (based on the retention time from the CN-10 column chromatography), no gas chromatographic analysis was conducted. It was anticipated that this component would have a molecular weight of 399, and would be

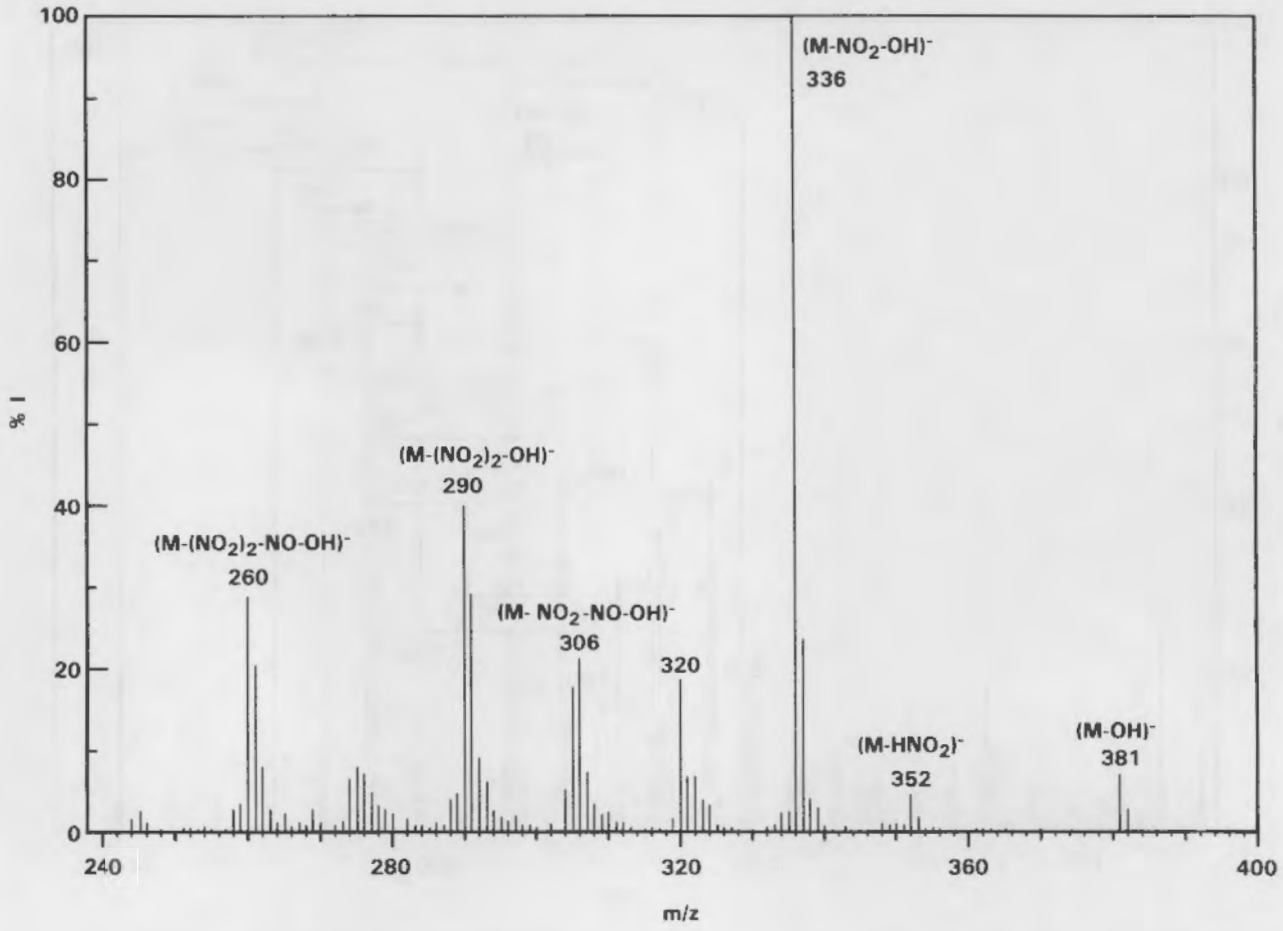

FIGURE 39. Negative Ion Chemical Ionization Mass Spectrum of Fraction H

the tetranitro isomer formed from adding a fourth nitro group to the major trinitro isomer found in Fraction G. Low resolution direct probe 70-eV MS analysis clearly indicated that Fraction I was composed of a single component with a molecular weight of 309. (See Figure 41.) We hypothesized that all of the major fragment ions above m/z 216 arise, as expected, from the loss of the small neutral molecules from an aromatic nucleus substituted with two nitro groups. The possibility of two nitro groups was confirmed by the HRMS data (Table 17), which assigned the elemental composition of $C_{16}H_{11}N_3O_4$ to the molecular ion at m/z 309. Furthermore, the HRMS data were consistent with the fragmentation pathways illustrated in Figure 41, which are, in turn, consistent with the presence of two nitro groups. MIKES experiments to confirm these proposed fragmentation pathways have not yet been conducted.

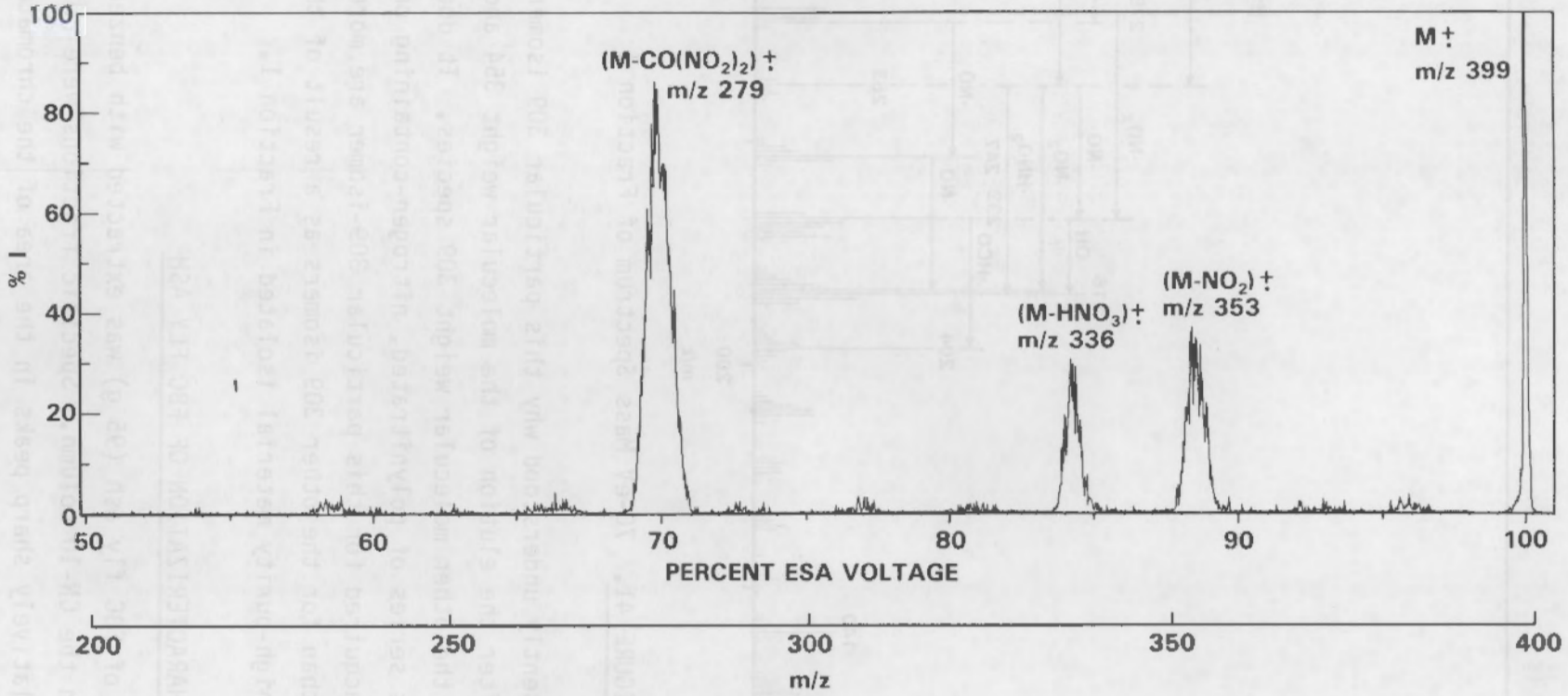

FIGURE 40. Unimolecular MIKES Spectrum of m/z 399 from Fraction H

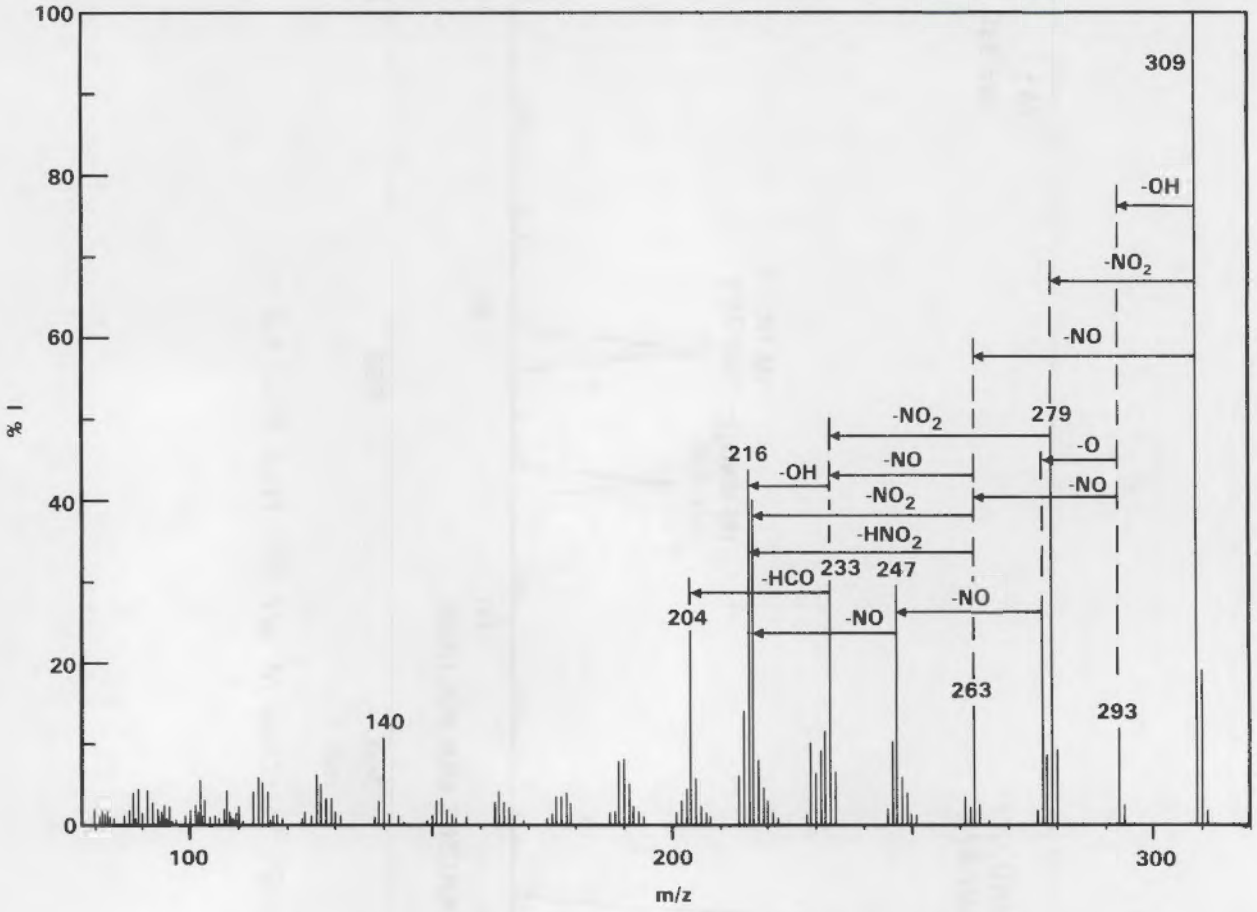

FIGURE 41. 70-eV Mass Spectrum of Fraction I

It is not presently understood why this particular 309 isomer elutes from the CN-10 column after the elution of the molecular weight 354 and 399 species, so much later than the other molecular weight 309 species. It does appear to be a member of this series of polynitrated, nitrogen-containing organic compounds. The data acquired for this particular 309-isomer are more detailed and of higher quality than for the other 309 isomers as a result of the relatively large quantity of high-purity material isolated in Fraction I.

ORGANIC CHEMICAL CHARACTERIZATION OF FBC FLY ASH

A large batch of FBC fly ash (95 g) was extracted with benzene/methanol and fractionated on the CN-10 column. Specific fractions were collected which corresponded to relatively sharp peaks in the area of the chromatogram

TABLE 17. Exact Masses and Elemental Compositions Determined[a] for Selected Ions in the Mass Spectrum of Fraction I. (See also Figure 41)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)[b]
310	310.0790	$^{13}CC_{15}H_{11}N_3O_4$	-0.7
309	309.0756	$C_{16}H_{11}N_3O_4$	-0.6
293	293.0811	$C_{16}H_{11}N_3O_3$	-1.1
280	280.0815	$^{13}CC_{15}H_{11}N_2O_3$	-1.3
279	279.0779	$C_{16}H_{11}N_2O_3$	-0.9
278	278.0859	$^{13}CC_{15}H_{11}N_3O_2$	2.5
277	277.0845	$C_{16}H_{11}N_3O_2$	0.6
263	263.0817	$C_{16}H_{11}N_2O_2$	0.4
247	247.0887	$C_{16}H_{11}N_2O$	-1.6
234	234.0875	$^{13}CC_{15}H_{11}NO$	-0.1
233	233.0846	$C_{16}H_{11}NO$	-0.5
232	232.0749	$C_{16}H_{10}NO$	1.3
231	231.0889	$C_{16}H_{11}N_2$	3.3
230	230.0817	$C_{16}H_{10}N_2$	2.7
229	229.0770	$C_{16}H_9N_2$	-0.5
219	219.0916	$C_{15}H_{11}N_2$	0.7
218	218.0906	$^{13}CC_{15}H_{11}N$	1.9
217	217.0887	$C_{16}H_{11}N$	0.4
216	216.0817	$C_{16}H_{10}N$	-0.6
215	215.0736	$C_{16}H_9N$	-0.1
214	214.0673	$C_{16}H_8N$	-1.6
205	205.0840	$^{13}CC_{14}H_{10}N$	0.7
204	204.0803	$C_{15}H_{10}N$	1.0
203	203.0752	$C_{15}H_9N$	1.0
190	190.0677	$C_{14}H_8N$	-2.1
189	189.0686	$C_{15}H_9$	1.8
141	141.0529	$^{13}CC_9H_6N$	0.5
140	140.0500	$C_{10}H_6N$	0.0

TABLE 17. (contd)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)(b)
126	126.0474	$C_{10}H_6$	-0.4
114	114.0454	C_9H_6	1.5
113	113.0391	C_9H_5	0.1
76	76.0315	C_6H_4	-0.2

(a) Exact mass measured by 70-eV HRMS experiments.
(b) Difference in mass between experimental value and exact mass calculated for the assigned elemental composition, reported in millimass units (mmu).

(40 to 55 min elution time) which showed the highest mutagenic activity. These fractions are depicted as shaded areas in the chromatogram in Figure 2. All these fractions were rechromatographed using the MCH-10 analytical column, and the largest peak or peaks in each chromatogram were collected. (See Table 5 for retention times of the various subfractions.) These seven subfractions were then submitted to preliminary GC/MS and direct probe MS. Although information for the FBC materials is not yet as complete as for the PCC materials, the preliminary results for each subfraction will be discussed individually.

Fraction 1

The fraction collected from the MCH-10 analytical column was composed of a single, well-defined peak in the chromatogram. Direct probe MS and GC/MS analyses confirmed the presence of only one major component in this fraction. The compound (see Figure 42) has an apparent molecular weight of 254 amu and displays losses of 28 and 30 mass units. High resolution mass measurements (Table 18) indicate that the ion at m/z 254 has an elemental composition of $C_{19}H_{10}O$, that m/z 226 is $C_{18}H_{10}$, and that m/z 224 is $C_{18}H_8$. Thus, the HRMS data are consistent with the mass spectrum and indicate the loss of the elements CO and H_2CO from the molecular ion. Although the actual identity of this compound has not yet been determined, it can be tentatively identified as the ketone which results from the oxidation of the methylene carbon contained in

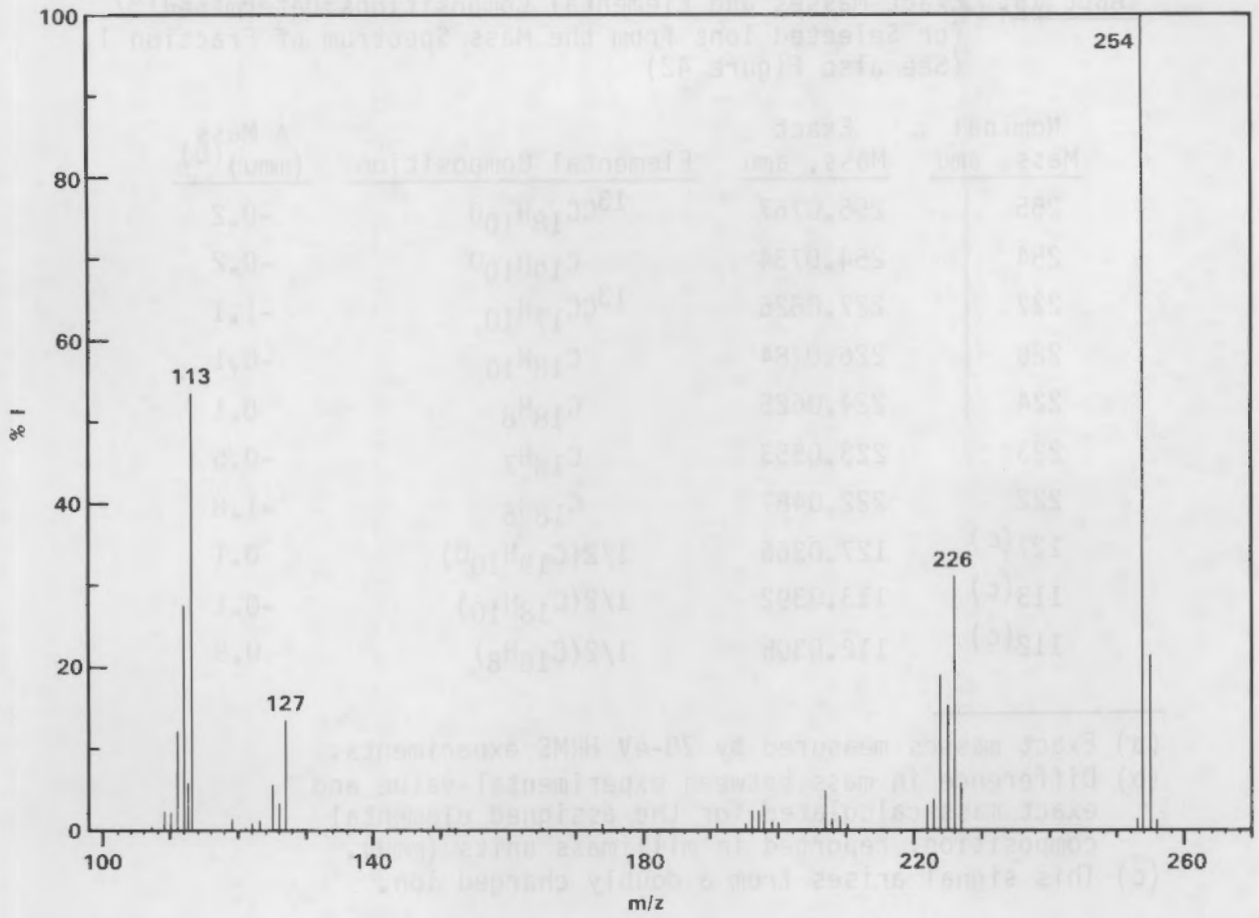

FIGURE 42. Mass Spectrum of Major Component in Fraction 1

the five-member ring of a $C_{19}H_{12}$ hydrocarbon such as 11H-benz[bc]aceanthrylene, 4H-cyclopenta[def]chrysene, or 4H-cyclopenta[def]triphenylene.

Fraction 2

Mass spectrometric analyses of this fraction, using both GC and direct probe introduction, indicated the presence of two major components and a large amount of hydrocarbon-like material which has tentatively been identified as column bleed from the MCH-10 analytical column. The hydrocarbon background was large enough, in both direct probe MS and GC/MS analyses, to obscure any fragmentation information regarding the two major components, which had apparent molecular ions at m/z 219 and 236. The small sample size precluded HRMS

TABLE 18. Exact Masses and Elemental Compositions Determined[a] for Selected Ions from the Mass Spectrum of Fraction 1. (See also Figure 42)

Nominal Mass, amu	Exact Mass, amu	Elemental Composition	Δ Mass (mmu)[b]
255	255.0767	$^{13}CC_{18}H_{10}O$	-0.2
254	254.0734	$C_{19}H_{10}O$	-0.2
227	227.0826	$^{13}CC_{17}H_{10}$	-1.1
226	226.0784	$C_{18}H_{10}$	-0.1
224	224.0625	$C_{18}H_8$	0.1
223	223.0553	$C_{18}H_7$	-0.5
222	222.0487	$C_{18}H_6$	-1.8
127[c]	127.0366	$1/2(C_{19}H_{10}O)$	0.1
113[c]	113.0392	$1/2(C_{18}H_{10})$	-0.1
112[c]	112.0305	$1/2(C_{18}H_8)$	0.8

(a) Exact masses measured by 70-eV HRMS experiments.
(b) Difference in mass between experimental value and exact mass calculated for the assigned elemental composition, reported in millimass units (mmu).
(c) This signal arises from a doubly charged ion.

analysis of the fraction, therefore no elemental composition data are available for these apparent molecular ions. No tentative identifications have been made at the present time.

Fraction 3

Even though the fraction collected from the analytical reverse-phase column appeared to be a well-resolved, single chromatographic peak, GC/MS analysis revealed a mixture of one major and two minor components. The major component has an apparent molecular weight of 180 amu (see Figure 43) and a prominent fragment at m/z 152. This spectrum is similar to those of 9-fluorenone, benzo[c]-cinnoline, and phenalen-1-one, and positive identification could not be made based on the mass spectrum alone. The GC retention indices for the three compounds in question were measured, in replicates, over a range of concentrations and compared to the value of 319.86 measured in a

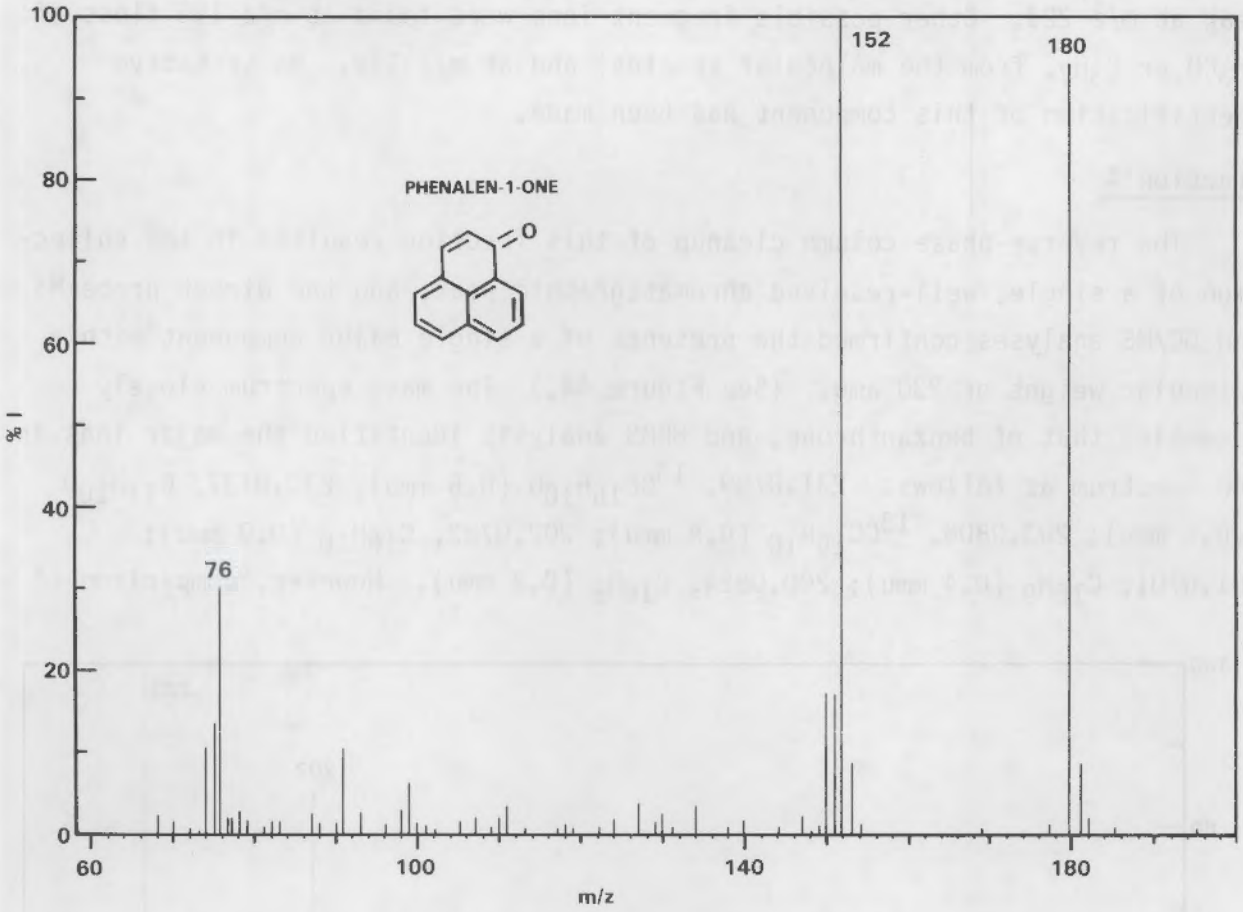

FIGURE 43. Mass Spectrum of Major Component in Fraction 3 Identified as Phenalen-1-one

single experiment for the component in Fraction 3. The retention index for phenalen-1-one (319.87 ± 0.17, six measurements) was identical with that of the unknown and was very close to the value of 320.52 ± 0.07 recently reported by Leary et al. (1983). The retention indices for the other compounds were also comparable to the literature values: 9-fluorenone, 294.09 ± 0.20 from four measurements (294.20 ± 0.02, Leary et al. 1983); benzo(c)cinnoline, 322.35 ± 0.24 from six measurements (323.50 ± 0.10, Leary et al. 1983). Thus, the major component in Fraction 3 has been unambiguously identified as phenalen-1-one.

The two minor components have apparent molecular weights of 218 and 238 amu. The spectrum of the 218 component has no discernible fragmentation patterns, and no tentative identification has been made. The spectrum of the molecular weight 238 component displayed a large loss of CH_3 to give a base

peak at m/z 223. Other possible fragment ions were found at m/z 195 (loss of CH_3CO or C_3H_7, from the molecular species) and at m/z 139. No tentative identification of this component has been made.

Fraction 4

The reverse-phase column cleanup of this fraction resulted in the collection of a single, well-resolved chromatographic peak, and the direct probe MS and GC/MS analyses confirmed the presence of a single major component with a molecular weight of 230 amu. (See Figure 44.) The mass spectrum closely resembled that of benzanthrone, and HRMS analysis identified the major ions in the spectrum as follows: 231.0759, $^{13}CC_{16}H_{10}O$ (0.6 mmu); 230.0737, $C_{17}H_{10}O$ (-0.6 mmu); 203.0808, $^{13}CC_{15}H_{10}$ (0.8 mmu); 202.0782, $C_{16}H_{10}$ (0.0 mmu); 201.0701, $C_{16}H_9$ (0.4 mmu); 200.0624, $C_{16}H_8$ (0.2 mmu). However, comparison of

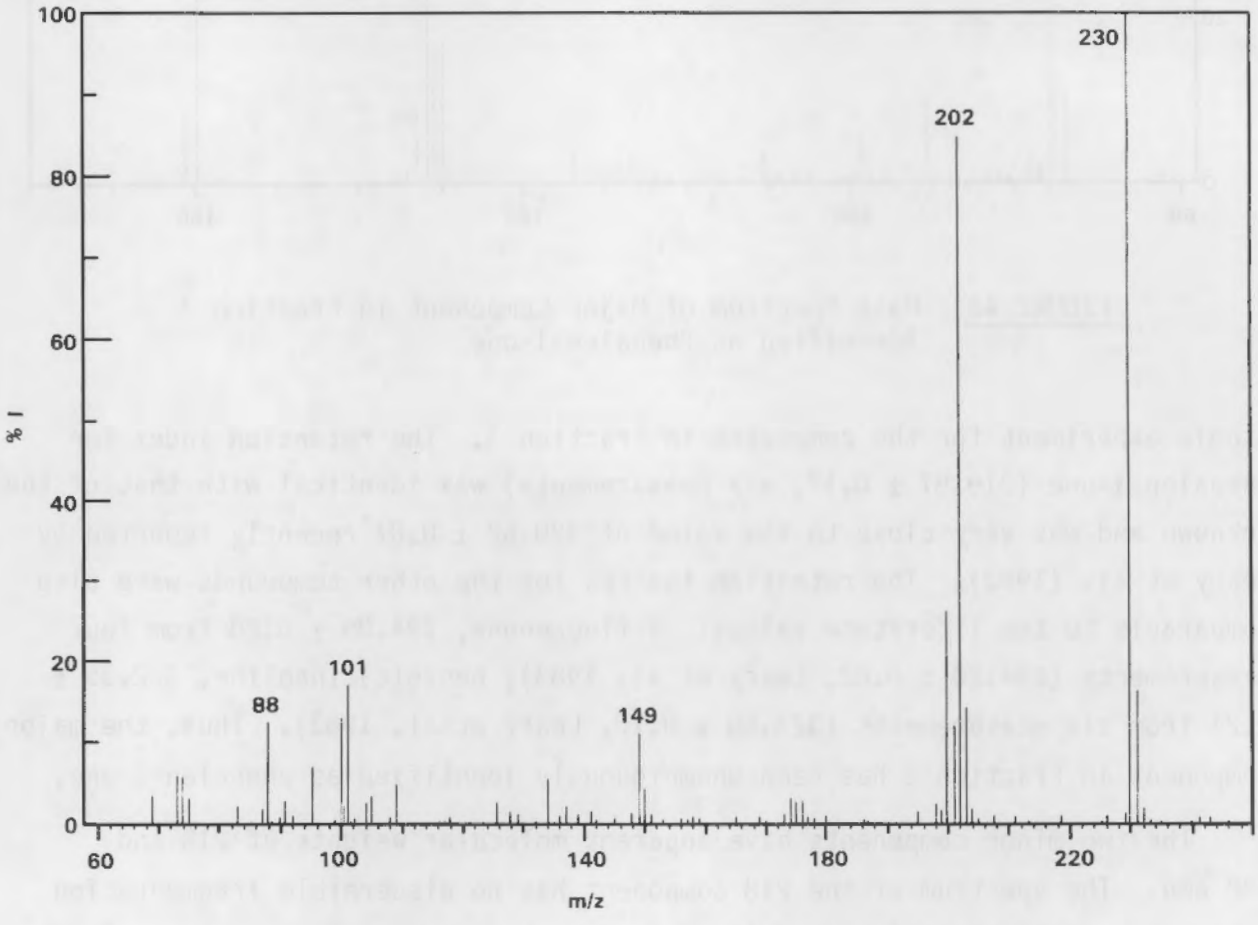

FIGURE 44. Mass Spectrum of the Major Component of Fraction 4

the GC retention index of 418.35 determined for this component with those of benzanthrone (404.99 ± 0.09) and benzo[a]fluorenone (386.17 ± 0.16) clearly rules out these two possibilities. It is possible that this presently unidentified component is another benzofluorenone isomer.

Fraction 5a

Fraction 5a was shown to be a mixture of at least seven components by direct probe MS and GC/MS analyses. Apparent molecular ions were found at m/z 247 and 259, but no fragmentation information could be discerned. Several other components were found for which the associated fragment ions could not be adequately confirmed: m/z 223, with fragments at m/z 168, 166; m/z 220, with fragments at m/z 164, 163; m/z 264, with fragments at m/z 221, 249. Only two compounds were present in large enough quantities to unequivocally determine the major ions in their mass spectra: one with a molecular ion at m/z 231 and fragment ions for the losses of 28, 29, and 30 at m/z 203, 202, and 201; the other with a molecular ion at m/z 212, which lost 30, 46, and 73 mass units, giving rise to fragment ions at m/z 182, 166, and 139, respectively. The latter compound shows clear evidence for the presence of a nitro group and has been tentatively identified as a nitrocarbazole or a nitroazafluorene. No other preliminary identifications have been made.

Fraction 5b

Direct probe MS and GC/MS analyses revealed the presence in this fraction of three major components, of apparent molecular weights 220, 222, and 256 amu. The mass spectrum of the molecular weight 220 component indicated the losses of 28 and 57, which could be interpreted as the losses of CO and CO+HCO from m/z 220. These losses are consistent with a quinone or a lactone formed from the oxidation of a molecule of the general formula $C_{15}H_{10}$ (molecular weight 190). However, in the absence of HRMS data and suitable standards, no tentative structural assignment has been made.

The interpretation of the molecular weight 222 mass spectrum followed a similar line of reasoning, as major fragment ions were found at m/z 194 (loss of 28) and m/z 165 (loss of 57). In addition, this spectrum also showed a substantial loss of H (m/z 221), and fragment ions at m/z 120 and 92. A

quinone or lactone of a molecular weight 192 PAH species is considered a possible structure, along with that of a carboxaldehyde derivative of a ketone, such as methylfluorenone.

The mass spectrum for the component of molecular weight 256 indicated fragment ions at m/z 234, 228, and 201. The loss of 28 (CO) from m/z 256 (giving m/z 228) is reasonable, but the ion at m/z 234 clearly cannot be a fragment from m/z 256. Therefore, two possibilities must be considered: (1) that m/z 234 is a coeluting component, and (2) that m/z 234 and 256 are both fragment ions from a common unstable molecular ion that does not appear in the mass spectrum. No tentative identifications have been made.

Fraction 6

Direct probe MS analysis of Fraction 6 revealed possible molecular ions at m/z 254, 226, 219, 217, and 203. Further analysis by GC/MS confirmed the presence of components with molecular ions at m/z 226, 219, 217 and 203, but the component at m/z 254 (the most abundant species in the probe spectrum) did not elute from the chromatograph. Due to the small sample quantity, the signal-to-noise ratio for the GC/MS data was so small that fragmentation information from each component could not be discerned. Thus, no tentative assignments regarding the identities of any of the components in Fraction 6 were possible.

COMPARISON OF ORGANIC CONSTITUENTS FOUND IN PCC AND FBC FLY ASH EXTRACTS

This report has presented: (1) the results from mutagenesis assays on whole and HPLC-fractionated extracts of fly ash originating from both FBC and PCC technologies, and (2) the data and results from instrumental chemical characterization of selected subfractions from each of these fly ash extracts. From this data base, direct comparisons can be made regarding the relative mutagenic activities of the two fly ashes. However, the specific identities of individual organic components from each fly ash cannot be directly compared because of the highly subjective selection procedure used to generate subfractions for chemical characterization. Thus, only general conclusions, based on an overview of all the analytical chemistry data, can be drawn.

A comparison of the relative mutagenic activities of the FBC fly ash (Table 8) with those of the PCC fly ash in the nitroreductase-deficient strains (Wei et al. 1982) shows that the FBC fly ash has a consistently lower total activity. However, there are strong similarities in the ratio of the mutagenic activity of the nitroreductase-deficient strains to those of the corresponding parental strains, as shown in Table 19. The nitroreductase-deficient variants of TA100 and TA1538 show less than 10% of the activity of the parental strains (Table 19). This sharp decrease in activity is a strong indication that much of the activity in both PCC and FBC fly ash extracts is due to the presence of nitro-organic compounds.

When the whole extracts were separated by HPLC, and individual fractions were assayed for mutagenic activity, the PCC extract showed a bimodal distribution of activity, with the major maximum at a time (20 to 30 min, see Table 6) corresponding to the longest period within the range of elution times characteristic of simple nitro organic compounds (10 to 26 min). The minor maximum from the PCC fly ash (40 to 50 min) corresponded very closely to the single maximum of mutagenic activity displayed by the HPLC fractions from the FBC fly ash (40 to 50 min; see Tables 9 and 10). Retention times for simple dinitroaromatic compounds were measured in the range of 25 to 40 min. Thus, there is strong evidence, based on mutagenicity assays using nitroreductase-deficient strains of S. typhimurium, that the mutagenic activities of both the PCC and FBC fly ashes studied here are due primarily to nitro organic compounds. While simple mononitroaromatics are implicated for much of the activity

TABLE 19. Activity in Nitroreductase-Deficient Bacteria as a Percent of that of the Standard Strain for Fluidized-Bed and Conventional Coal Fly Ash

Strain	FBC Fly Ash	PCC Fly Ash[a]
TA98	30	24
TA100	9	3
TA1538	7	4

(a) Data from Wei et al. (1982).

of the PCC fly ash, the HPLC retention times indicate that such components are unlikely to be major contributors to the mutagenic activity of the FBC fly ash.

Chemical characterization of HPLC subfractions from PCC fly ash extract revealed the expected presence of simple mononitroaromatic compounds, such as alkylated series of nitrophenanthrenes and nitropyrenes, in the subfractions eluting slightly prior to those exhibiting maximum mutagenic activity. Although the fraction of greatest mutagenic activity (fraction 5, Figure 1) has not yet been fully characterized, preliminary data indicate the presence of mononitroaromatic ketones, tentatively identified as nitrofluorenones. (See Results and Discussion, Fraction C.) Similarly, nitro-keto-compounds were recently reported as constituents of FBC ash (Li et al. 1983). A series of di-, tri-, and tetranitro derivatives of a parent compound or a series of parent compounds with a molecular formula of $C_{16}H_{13}N$ (see Figure 45) were identified as the major components in the HPLC fractions of the PCC fly ash extract which exhibit the mutagenic activity in the bimodal mutagenicity distribution. To our knowledge, this series of components has not been reported previously. Further study is presently being conducted to elucidate the structures of these components and to determine whether this series constitutes the major source of mutagenic activity in these fractions.

Although 1-nitropyrene has been reported in the organic extracts of FBC fly ash (Mumford and Lewtas 1982), it is presently not known whether it is a component of the FBC fly ash in this study because no chemical characterization was made of the appropriate HPLC fractions because of the lack of significant mutagenic activity of these fractions. The FBC fly ash HPLC fractions that were examined corresponded closely in retention times to those of the polynitrated $C_{16}H_{13}N$ compounds found in the PCC fly ash extract. (See Figure 45.) However, no evidence of these polynitro compounds was found in the FBC extract. Even though the mutagenesis assays conducted with nitroreductase-deficient strains of S. typhimurium strongly indicated the presence of nitro organic compounds, only one component was tentatively identified as a nitro-compound (a nitrocarbazole or nitroazafluorene), and the majority, which did not appear to contain nitrogen, could be generally classified as oxygenated PAH. Although these results appear to be in conflict with those from the

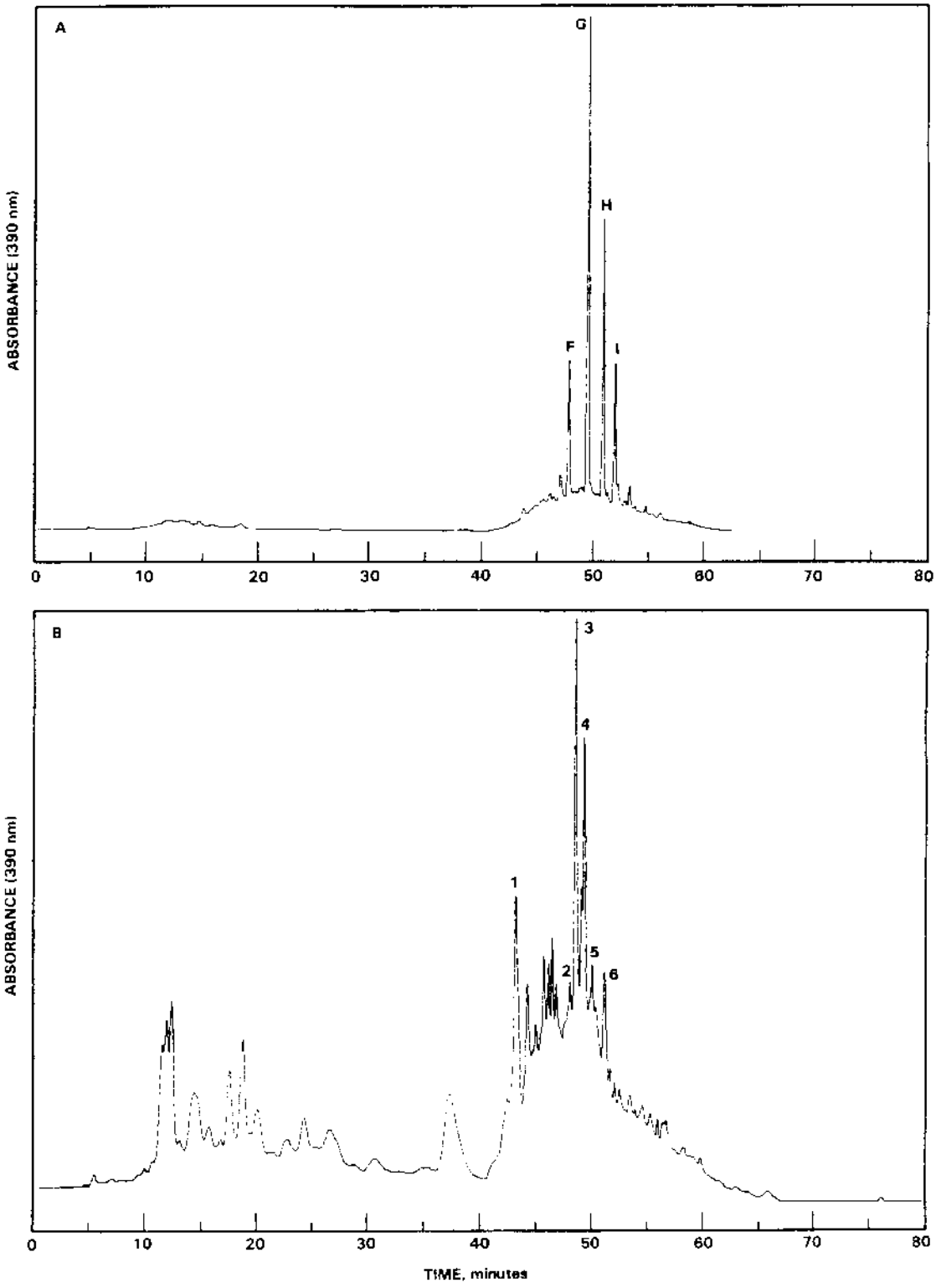

FIGURE 45. Chromatograms for the CN-10 Column HPLC Separations of A) PCC Fly Ash Extract and B) FBC Fly Ash Extract. The peaks indicated by letters and numbers correspond to fractions collected and characterized for organic constituents.

mutagenesis assays, the chemical characterization of the FBC fly ash extract was not a comprehensive assessment and nitro organic constituents may have been present in the fractions that were not examined.

The tentative identifications of a dinitrodiazachrysene in the PCC fly ash extract (see Results and Discussion, Fraction H) and of a nitrocarbazole (or nitroazafluorene) in the FBC fly ash extract (see Results and Discussion, Fraction 5a) prompt questions as to the possible biological impact of such components. Although similar components have recently been tentatively identified in a diesel particulate extract (Paputo-Peck et al. 1983), very few nitroaza compounds have been tested for mutagenicity or carcinogenicity; therefore, no general guidelines as to structure/activity relationships are available. However, studies have shown that the addition of a single nitro-group to quinoline-1-oxide, producing 4-nitroquinoline-1-oxide, conferred substantial carcinogenicity to the previously inactive material as determined by assays using several rodent species (Clayson and Garner 1976). The detection of nitroaza compounds in airborne particulate matter and in mutagenic fractions from extracts of coal fly ashes underscores the importance of obtaining suitable structure/activity relationships for this class of potentially important biological hazards.

The identification of phenalen-1-one as a major constituent in the FBC fly ash Fraction 3 is important because this compound was previously identified as a product of fossil fuel combustion and was shown to be a potent mutagen (Leary et al. 1983). It cannot be responsible for the apparent direct-acting mutagenicity displayed by the HPLC fraction from which it was subfractionated (see Table 10, Fraction 10), because this compound was shown to be only a weak mutagen in the absence of metabolic activation. Further work will be required to determine the major direct-acting mutagens in this fraction of high mutagenic activity. Nevertheless, the presence of PAH-oxidation products, such as ketones, quinones, lactones, aldehydes and anhydrides, in both the FBC and PCC fly ashes is of interest in light of recent demonstrations that oxidation reactions can confer genotoxic activity on certain lower molecular weight PAH (Leary et al. 1983; Salamone et al. 1979; Pitts 1983).

Major findings from these studies may be summarized as follows:

1. Results from mutagenicity testing of the HPLC fractions of PCC fly ash extract show a bimodal distribution of mutagenic activity, with the less polar, mutagenically active components eluting between 15 and 30 min and the more polar compounds eluting between 40 and 50 min.

2. 1-Nitropyrene has been identified among the less polar, mutagenically active compounds but accounts for less than 1% of the total mutagenic activity of the PCC fly ash extract HPLC fractions. Other major constituents of these fractions include a series of alkylated nitrophenanthrenes and a series of alkylated nitrofluorenones. The contribution of these components to the biological activity of the extract is under evaluation.

3. The major components in the more polar, mutagenic HPLC fractions of PCC fly ash extract consist of a series of nitro derivatives of a single parent organic compound with the empirical formula $C_{13}H_{11}N$. Thus far, we have isolated and characterized two dinitro, two trinitro, and one tetranitro derivatives of this compound. These compounds may account for up to 25% of the total mutagenic activity. Attempts to determine the exact structure of the $C_{13}H_{11}N$ parent compound are in progress.

4. Results from mutagenicity testing of the HPLC fractions of the FBC fly ash extract show maximum mutagenic activity between 40 and 50 min, indicating a polar class of mutagens. Although data from nitroreductase-deficient bacteria indicate a strong contribution from nitro organics, the retention times virtually rule out any significant contributions from simple mononitro compounds.

5. The major components of the FBC fly ash HPLC fractions examined thus far appear to be oxy-PAH, including the known mutagen phenalene-1-one as well as compounds with formulas of $C_{19}H_{10}O$ and $C_{17}H_{10}O$.

CONCLUSIONS

In evaluating the results presented here, it should be kept in mind that the analytical data are preliminary. Compounds reported here constitute major components in the organic extracts as determined by UV detection, used in the HPLC separations. Future identification of higher molecular weight species or other minor components in these extracts may alter the present view of the chemical basis for the mutagenic activity observed. Furthermore, microbial mutagenicity assays are generally classified only as screening tests for carcinogens, and results in these assays may or may not correlate to possible activity in humans. The positive mutagenicity assay results presented here, however, give cause for concern and emphasize the need for further study.

Finally, caution should be used in interpreting the overall finding that FBC fly ash is lower in mutagenic activity and appears to have lower concentrations of nitro compounds in the mutagenic regions of the chromatograms than does PCC fly ash. Other studies comparing FBC and PCC fly ash have demonstrated that operational factors of combustors, such as temperature, air/fuel ratios, and combustion efficiency, can have a profound effect on fly ash mutagenicity (Hobbs 1983). Furthermore, as Kubitschek and Haugen (1980) have recently demonstrated, in addition to the problems of sampling, analyzing, and comparing effluents from coal technologies by use of S. typhimurium tester strains in mutagenicity assays, the intrinsic precision of these assays is often low, and the tests often fail to give additive results for mixtures of mutagens, thus making quantitative measurements and assessment of the biological hazards of coal combustion effluents very difficult. Nonetheless, our understanding of the relative operating characteristics of the two systems gives cause for optimism: FBC operates at lower temperatures, with combustion efficiencies generally comparable or superior to those of PCC. Both these trends are theoretically consistent with the apparent reduction in nitro PAH content in fly ash from the FBC unit. This initial round of biodirected analyses has demonstrated a possible chemical basis for the reduced mutagenicity of FBC fly ash extracts relative to their PCC counterparts.

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