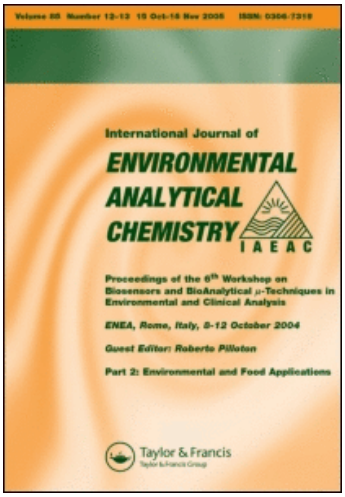

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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Detlev Helmig[a]; Janet Arey[a]
[a] Statewide Air Pollution Research Center, University of California, Riverside, CA, USA

To cite this Article Helmig, Detlev and Arey, Janet(1991) 'Analytical Chemistry of Airborne Nitrofluorenes', International Journal of Environmental Analytical Chemistry, 43: 4, 219 — 233
To link to this Article: DOI: 10.1080/03067319108027526
URL: http://dx.doi.org/10.1080/03067319108027526

Intern. J. Environ. Anal. Chem., Vol. 43, pp. 219–233
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ANALYTICAL CHEMISTRY OF AIRBORNE NITROFLUORENES

DETLEV HELMIG and JANET AREY

Statewide Air Pollution Research Center, University of California, Riverside, CA 92521, USA

(Received 14 June 1990; in final form 27 August 1990)

Analytical techniques for the analysis of 1-, 2-, 3- and 4-nitrofluorene were developed to enable these species to be determined in the gas and particle phases of the atmosphere. The nitrofluorene isomers were synthesized by electrophilic and gas-phase OH radical-initiated nitration of fluorene and identified by their ^{1}H-NMR and mass spectra. For quantitative analysis deuterated 2- and 4-nitrofluorene were also synthesized for use as internal standard compounds. The GC retention indices of all six compounds were determined on DB-5, DB-1701 and SB-Smectic columns.

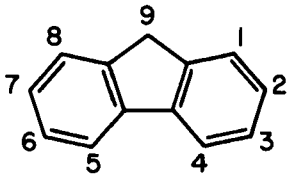
KEY WORDS: 1-, 2-, 3- and 4-nitrofluorene, NMR spectra, mass spectra, retention indices on DB-5, DB-1701 and SB-Smectic.

INTRODUCTION

Although the mutagenic and carcinogenic properties of organic extracts of ambient particulate matter are well established, the individual compounds responsible for much of the overall activity are still unknown. Recent research has shown that airborne nitro- and hydroxynitro-derivatives of polycyclic aromatic hydrocarbons (PAH) are responsible for a portion of the mutagenic activity.[1–3] These compounds can either be emitted during the combustion of organic materials or be formed in the atmosphere by transformations of the airborne PAHs. Due to the lower vapor pressures of these PAH-derivatives, they are partitioned more into the particle phase than the parent PAH.

Typical analyses of atmospheric nitro- and hydroxynitro-PAHs involve high volume sampling on inert filter (particle phase) and adsorbent (gas phase) materials, solvent extraction of the laden filters or adsorbents, fractionation of the extracts by liquid chromatography and identification and quantification by high performance liquid chromatography (HPLC) or gas chromatography (GC) using either nonspecific flame ionization detection (FID) or more specific detection techniques, e.g., mass spectrometric (MS) detection with different ionization techniques.[4,5] Thus, for correct and isomer-specific identifications, a knowledge of retention index data and mass spectra is essential.

Although, fluorene (I) is one of the major PAHs found in ambient air[6–11] reports of the identification of its nitroderivatives in ambient samples are limited.[12] The only mononitrofluorene isomer reported has been 2-nitrofluorene, which is the main product of the electrophilic nitration of fluorene[13] and the only commercially available nitrofluorene. This isomer, which has been identified in diesel exhaust



(I)

particulate extracts[14–19] is well known for its mutagenic activity and is often used as a standard mutagen compound in mutagenicity assays.[12]

In contrast to electrophilic nitration of fluorene, gas-phase reactions of fluorene in the atmosphere are expected to lead primarily to nitro-isomers other than 2-nitrofluorene. The most important atmospheric degradation reaction of fluorene is expected to be initiated by OH radical attack, as has been shown for a series of structurally-related compounds.[20–23] The preferred sites of the OH radical addition are expected to be the 2- and 4-positions, which if followed by addition of NO_2 at the *ortho* position would lead, after elimination of H_2O, to 1- and 3-nitrofluorene.[24] To our knowledge these isomers have not been identified in ambient air, perhaps due to a lack of analytical procedures capable of distinguishing all four isomeric nitrofluorenes. Therefore, we have investigated the analytical properties of these compounds. All four nitrofluorene isomers were synthesized by appropriate procedures and spectroscopically and chromatographically characterized. For quantitative analysis purposes deuterated 2- and 4-nitrofluorene were also synthesized to be used for internal standard calibration.

SYNTHESIS AND ISOLATION

Nitrofluorenes were synthesized for the spectroscopic studies according to a procedure by Radner[25] in which fluorene (Aldrich, 98%) in acetic acid anhydride is treated with concentrated HNO_3 at 0 °C. The synthesis mixture was extracted with dichloromethane and ice water and the organic layer was further separated by preparative HPLC on an Ultrasphere Si column (Beckman Semi-Prep 25 cm × 10 mm, using a Beckman Gradient Liquid Chromatograph Model 334 system with Beckman Model 164 UV Detector, $\lambda = 254$ nm). All four nitrofluorenes were separated using a solvent program starting with 75% hexane and 25% dichloromethane. After all nitrofluorenes had eluted the solvent was programmed to 100% dichloromethane to elute more polar substances from the column. The flow rate was 3 ml min^{-1}. A chromatogram on which the fractions collected are indicated is shown in Figure 1. GC/MS analysis (Hewlett-Packard 5890 GC with Hewlett-Packard 5970 mass selective detector (MSD), GC column eluting directly into ion source, GC operating conditions given below under Gas Chromatography) showed that fractions D, E, G and H contained nitrofluorenes. The nitrofluorene present in fraction H was the main reaction product and was identified as 2-nitrofluorene by comparing its GC and HPLC retention time with that of a 2-nitrofluorene standard solution (Aldrich, 98%). The nitrofluorenes in

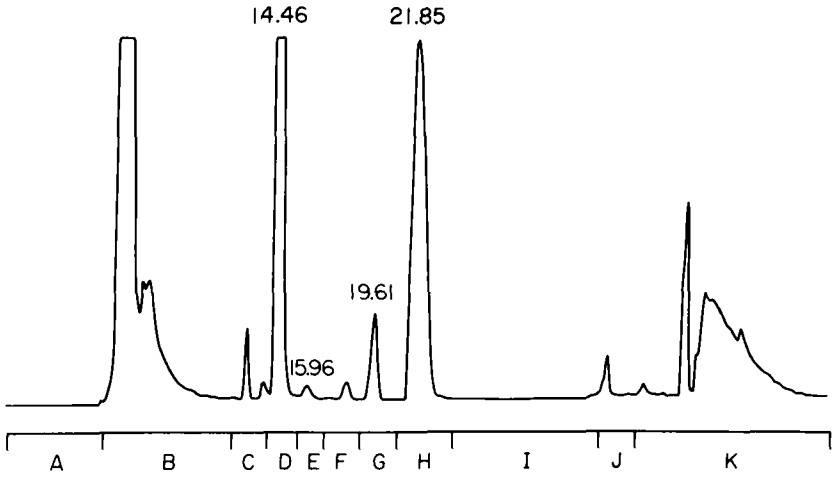


Figure 1 HPLC chromatogram of 100 μl (fluorene + HNO_3) synthesis mixture with the collected fractions indicated.

fractions D and G were identified by ^{1}H-NMR as described below. The yield of the nitrofluorene isomer at RT = 15.96 (Figure 1) was too low to produce sufficient material for NMR identification.

In a study of the gas-phase OH radical-initiated reaction of fluorene, samples were produced which contained all four nitrofluorene isomers.[26] Fluorene was introduced into a 6400 liter indoor all-Teflon chamber overnight by flowing dry nitrogen through a tube filled with crystalline fluorene. The fluorene concentration prior to the irradiation was about 100 ppb. OH radicals were generated by irradiation of methyl nitrite as described previously.[22] Approximately 2000 liters of the chamber volume were sampled onto polyurethane foam plugs (PUF), which were then Soxhlet extracted with dichloromethane. The extracts were filtered (0.45 μm Acrodisc CR, Gelman Sciences), concentrated and separated by HPLC (preparative Spherisorb S5W silica column, 25 cm × 10 mm, Regis, using a Spectra Physics model 8100 gradient liquid chromatograph with Spectra Physics model 8400 UV/VIS detector and ISCO fraction collector; solvent program starting with 100% hexane, then a linear gradient to 95% hexane and 5% dichloromethane from 10 to 15 min, then programmed to 100% dichloromethane by 40 min, held at 100% dichloromethane for 10 min, then programmed to 100% acetonitrile over 10 min, held isocratic for 10 min then programmed back to the initial conditions; solvent flow 3 ml min^{-1}). The fraction ranging from 28 to 37 min was collected, concentrated to 100 μl and analyzed by GC/MS. After quantification of the nitrofluorenes in the HPLC fractions, these chamber samples were utilized to isolate enough of the fourth nitrofluorene isomer to allow ^{1}H-NMR analysis.

The combined HPLC fractions of 13 chamber irradiation runs were further separated by reversed phase HPLC (Beckman Ultrasphere ODS 5 μ column, 10 mm × 25 cm, Beckman HPLC as described above; solvent program starting with 50% water and 50% methanol, from 10 to 40 min programmed to 100%

methanol, held at 100% methanol for 20 min and then programmed to the initial composition over 5 min; flow rate 3 ml min^{-1}). Under these conditions the desired isomer eluted last at RT = 40.1 min and was sufficiently separated from the other isomers.

Deuterated 2- and 4-nitrofluorene were obtained by treating deuterated fluorene (MSD Isotopes, 99.3%) with a solution of N_2O_4 in dichloromethane, adding a few drops of methanesulfonic acid as catalyst.[25] The synthesis mixture was separated by open column liquid chromatography on silica gel (LPS-2, 37–53 μm, Whatman). The extracts containing the mononitrofluorenes were combined and 2-nitrofluorene-d_9 (93%) was separated from 4-nitrofluorene-d_9 (7%) by fractional recrystallization. A GC/MS analysis of the isolated 2-nitrofluorene-d_9 and a MS probe analysis showed no foreign substances. The total reaction yield of 2- and 4-nitrofluorene-d_9 was 70%.

NMR SPECTRA

The identifications of the nitrofluorene isomers isolated from the fluorene + HNO_3 synthesis (HPLC fractions D and G in Figure 1) and the fourth isomer isolated from the chamber reactions were performed by taking the 300 MHz ^{1}H-NMR spectra (Nicolet 300 MHz pulsed Fourier transform spectrometer, spectra recorded in deuterated acetone as solvent and internal standard). The spectra of a fluorene and a 2-nitrofluorene standard solution were also taken to obtain additional information on the downfield shifting effects of the NO_2 group. The aromatic section of the spectra as recorded for 2-nitrofluorene, HPLC fractions D and G, and the isolated chamber product are shown in Figures 2 to 5. A summary of the spectroscopic data obtained is given in Table 1.

The peak assignments were made by comparing chemical shift data of structurally related nitroarene compounds[27–34] and by performing suitable decoupling experiments: Irradiation of the fluorene sample at 7.57 ppm reduced the triplet at 7.30 ppm to a doublet signal, showing that the proton at 7.30 ppm is adjacent to H-1. Irradiation of the sample from fraction G (Figure 4) at 7.85 ppm reduced the doublet at 8.21 ppm to a singlet signal and irradiation at 7.66 ppm caused the multiplet signal at 7.42 to 7.47 to be simplified which led to the assignments of H-1 and H-8 and the identification as 3-nitrofluorene. As even decoupling experiments did not allow assignment of the signals observed in the spectrum of fraction D (Figure 5), a 500 MHz spectrum (Varian VXR) of this sample was recorded which led to the identification of this sample as 4-nitrofluorene. With the higher resolution of this instrument the multiplet signal at 7.87 to 7.95 ppm split into three doublets at 7.89, 7.92 and 7.93 ppm. Because of its lower coupling constant (J = 6.41 Hz), the signal at 7.92 ppm was assigned to H-5. The remaining two protons (coupling constants 8.24 and 8.39 Hz) were distinguished by a nuclear Overhauser enhancement (NOE) experiment on H-9. The NOE difference spectrum showed enhancements of 5.4% for the doublet at 7.93 ppm and 4.6% for the split doublet at 7.68 ppm. The doublet at 7.93 ppm could thus be assigned to H-1

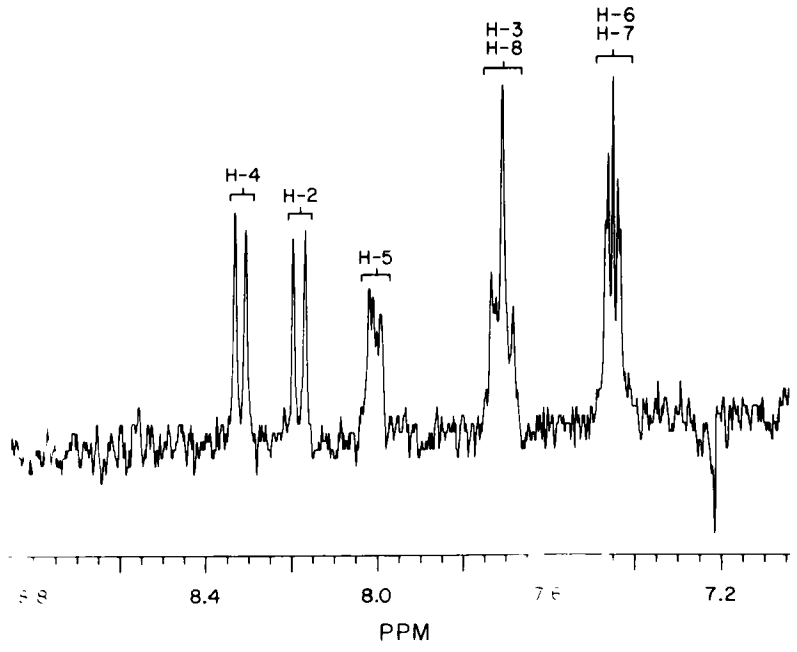


Figure 2 ^{1}H-NMR spectrum of 1-nitrofluorene (isolated from combined chamber samples).

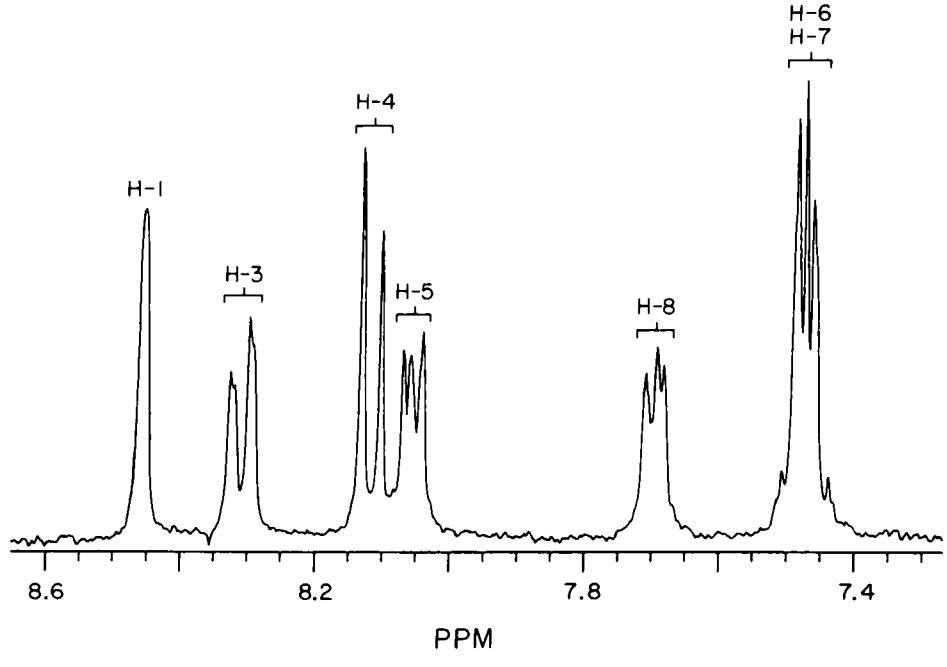


Figure 3 ^{1}H-NMR spectrum of 2-nitrofluorene (standard solution).

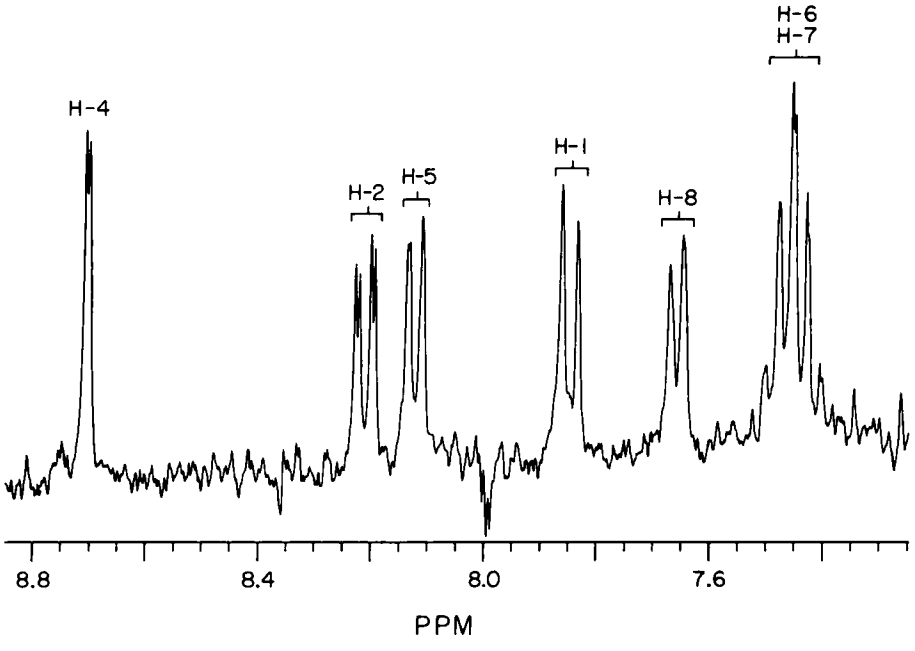


Figure 4 ^{1}H-NMR spectrum of 3-nitrofluorene (HPLC fraction G).

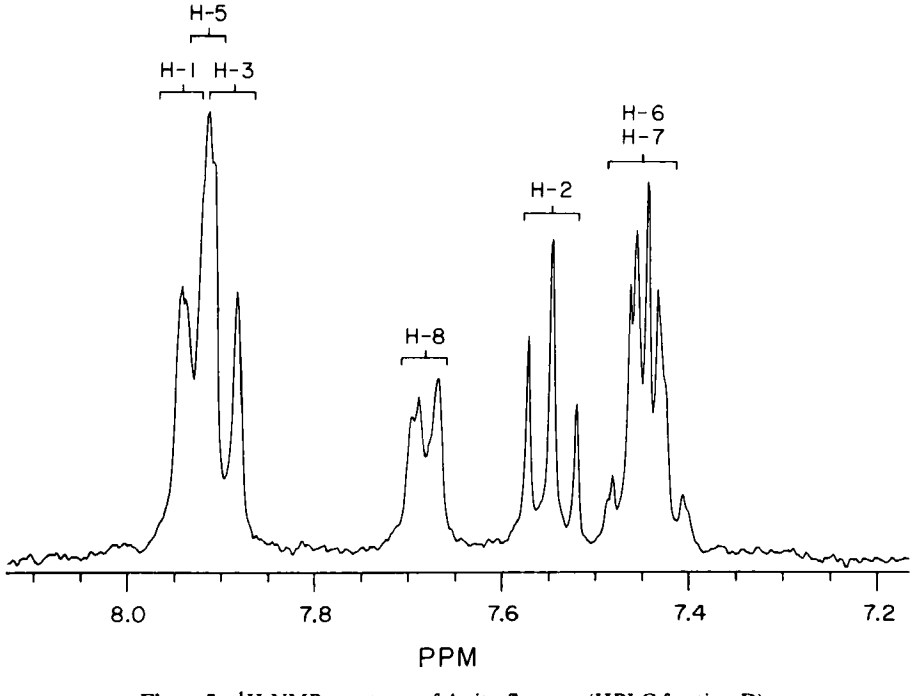


Figure 5 ^{1}H-NMR spectrum of 4-nitrofluorene (HPLC fraction D).

Table 1 ^{1}H-NMR data obtained from fluorene, 1-nitrofluorene (isolated from chamber samples), 2-nitrofluorene, 3-nitrofluorene (HPLC fraction G) and 4-nitrofluorene (HPLC fraction D)

Compound	*Chem. shift (ppm)*	*Number of protons*	*Coupling*[a]	*Assignment*	*Coupling constant (Hz)*
Fluorene	3.90	2	s	H-9	
	7.30	2	t	H-2, H-7	$J(1, 2) = 7.3$
	7.37	2	t	H-3, H-6	$J(3, 4) = 7.4$
	7.57	2	d	H-1, H-8	
	7.86	2	d	H-4, H-5	
1-Nitrofluorene	4.42	2	s	H-9	
	7.43–7.46	2	m	H-6, H-7	
	7.68–7.73	1	m	H-8	
	7.70	1	t	H-3	
	7.99–8.02	1	d	H-5	
	8.18	1	d	H-2[b]	$J(2, 3)^b = 8.0$
	8.32	1	d	H-4[b]	$J(3, 4)^b = 7.6$
2-Nitrofluorene	4.12	2	s	H-9	
	7.43–7.51	2	m	H-6, H-7	
	7.67–7.71	1	m	H-8	
	8.03–8.06	1	m	H-5	
	8.12	1	d	H-4	$J(3, 4) = 8.4$
	8.31	1	d	H-3	$J(1, 3) = 1.8$
	8.46	1	s	H-1	
3-Nitrofluorene	4.11	2	s	H-9	
	7.42–7.47	2	m	H-6, H-7	
	7.66	1	d	H-8	
	7.85	1	d	H-1	$J(1, 2) = 8.2$
	8.12	1	d	H-5	$J(5, 6) = 7.0$
	8.21	1	d	H-2	$J(2, 4) = 2.1$
	8.70	1	s	H-4	
4-Nitrofluorene	4.09	2	s	H-9	
	7.40–7.49	2	m	H-6, H-7	
	7.54	1	t	H-2	
	7.68	1	d	H-8	
	7.89[c]	1	d	H-3	$J(2, 3) = 8.24$
	7.92[c]	1	d	H-5	$J(5, 6) = 6.41$
	7.93[c]	1	d	H-1	$J(1, 2) = 8.39$

[a]s = singlet, d = doublet, t = triplet, m = multiplet.
[b]These assignments may be reversed.
[c]Data obtained from 500 MHz acquisition.

and the remaining signal at 7.89 ppm to H-3. This experiment also confirmed the assignment of H-8.

Since the nitrofluorenes in HPLC fractions D, G and H were identified as 4-nitrofluorene, 3-nitrofluorene and 2-nitrofluorene, respectively, the fourth nitrofluorene found in HPLC fraction E and isolated from the chamber irradiation runs had to be 1-nitrofluorene (Figure 2). The peak assignments of its NMR spectrum were performed by one NOE and two irradiation experiments. Irradiation of the overlapping multiplet and triplet signals at 7.68 to 7.73 ppm reduced both doublets at 8.18 and 8.32 ppm to singlets and simplified the multiplet at 7.43 to 7.46 ppm. Irradiation of the split doublet signal at 7.99 to 8.02 ppm only simplified the multiplet at 7.43 to 7.46 ppm showing that the former signal can be assigned to H-5 and the overlapping signal at 7.68 to 7.73 ppm to H-8. Distinguishing between the two doublets at 8.18 and 8.32 ppm was attempted by an NOE experiment on H-5 using the 500 MHz instrument. The difference spectrum showed only a very small but not unequivocal enhancement for the doublet at 8.32 ppm. This signal was assigned to H-4 and the remaining doublet at 8.18 ppm to H-2. This assignment is consistent with the expected chemical shifts calculated on the basis of the observed data for the other nitrofluorene isomers, i.e., on the downfield shifts in comparison to fluorene which result from the NO_2 substitutions. However, an unequivocal assignment was not achieved and the reversed assignment cannot be excluded.

MASS SPECTRA

The 70 eV electron impact (EI) mass spectra of the nitrofluorenes were recorded from the GC/MS analysis of a sample containing all four isomers and are shown in Figures 6 to 9. The intensities of the most abundant fragments are summarized in Table 2. The data were evaluated from the average of six scans around the peak maxima. It is apparent that for the isomers with the NO_2 group at the 1 or 4 position the loss of HNO_2 (-47) is the dominant fragmentation, whereas for the nitrofluorenes with the nitro group on position 2 or 3 the NO_2 loss dominates, giving a base peak at $m/z = 165$ (-46). The loss of OH as seen from the abundances of the $m/z = 194$ peak, found for all four isomers with an intensity range of 18 to 55%, is strikingly high. Other related nitroarenes generally show lower abundances for this fragmentation process,[27,35,36] with the exception of nitro-PAHs having an NO_2 group in a bay position.[35] Consistent with bay region NO_2 groups giving an OH loss, the abundance of the $m/z = 194$ peak is highest for 4-nitrofluorene (followed by 1-nitrofluorene) which indicates that the adjacent aromatic bay H atom (H-5) in 4-nitrofluorene as well as the nonaromatic H-9 atom in the *peri* position in 1-nitrofluorene are favoring this elimination. Additionally, recent work[37] has shown that OH loss can be one of the most abundant fragmentations in methylnitronaphthalenes having an NO_2 group in an *ortho* or *peri* position to the methyl group and thus allowing the abstraction of a nonaromatic H atom.

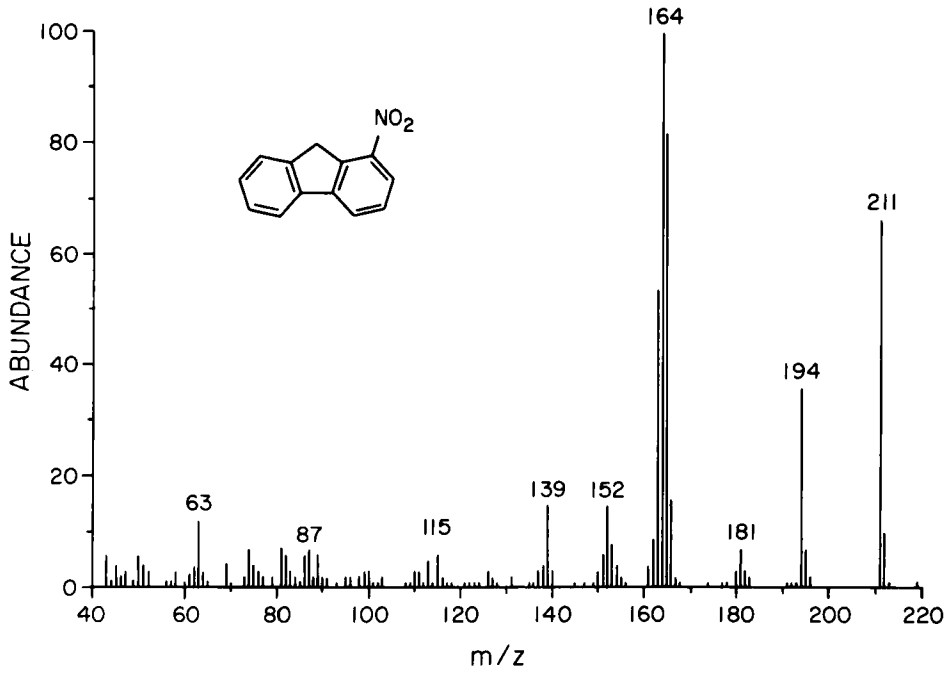


Figure 6 Mass spectrum of 1-nitrofluorene.

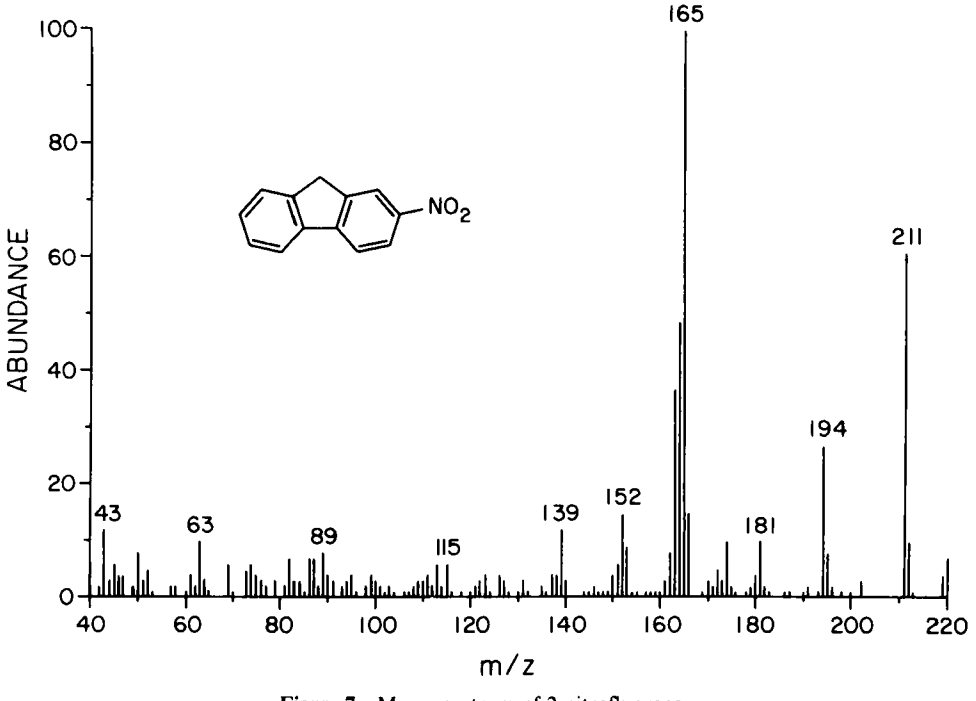


Figure 7 Mass spectrum of 2-nitrofluorene.

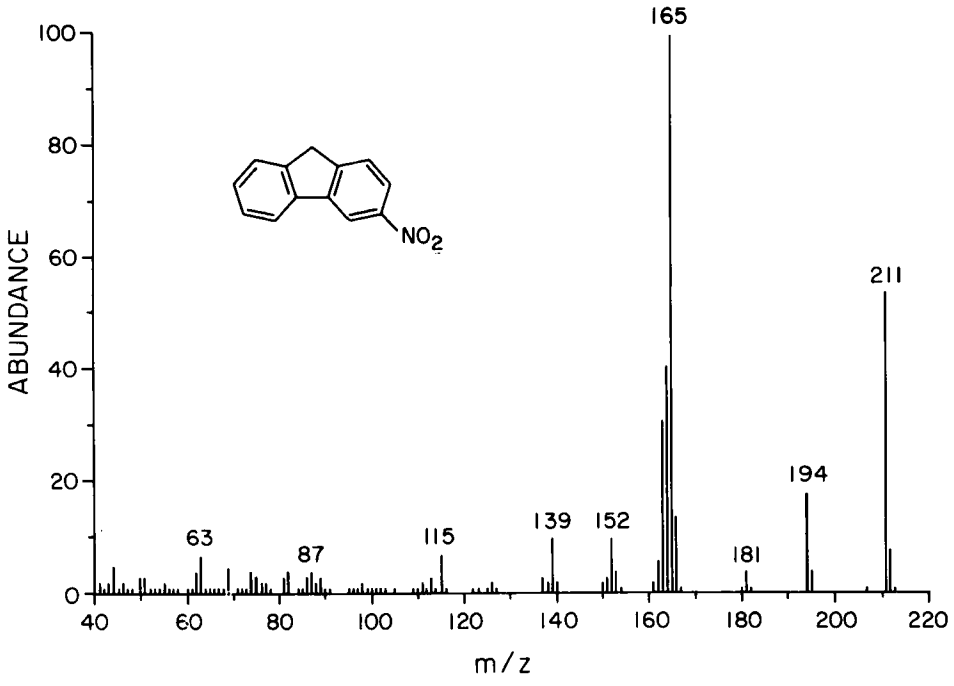


Figure 8 Mass spectrum of 3-nitrofluorene.

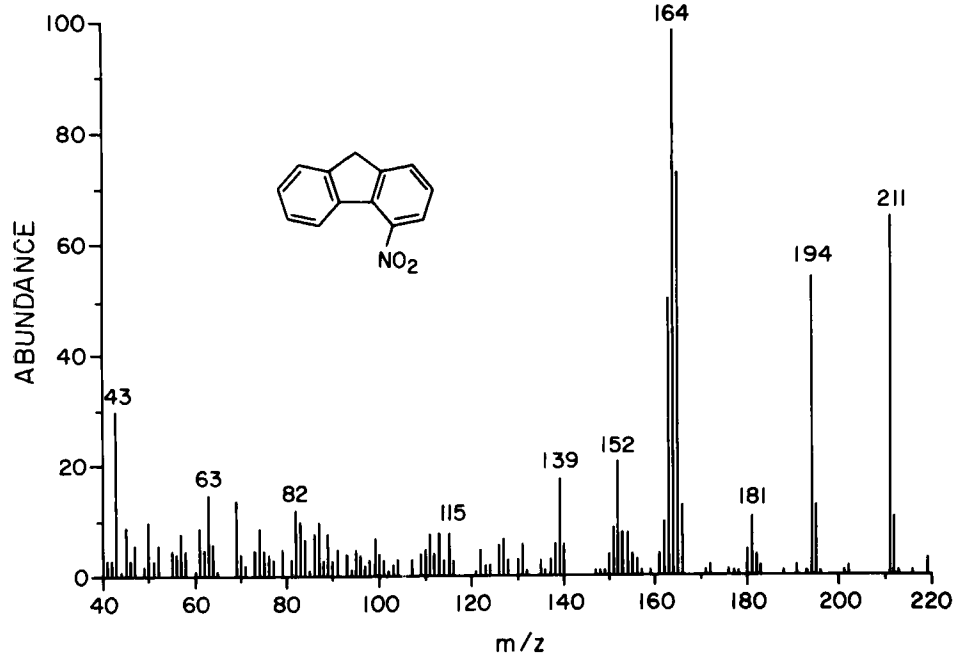


Figure 9 Mass spectrum of 4-nitrofluorene.

Table 2 Relative abundances of major ions observed in the mass spectra of the four nitrofluorene isomers

	Relative abundance (%)							
Fragment lost	—	*OH*	*NO*	NO_2	HNO_2	H_2NO_2	$HCNO_2$	$H_2C_2NO_2$
m/z	*211*	*194*	*181*	*165*	*164*	*163*	*152*	*139*
1-Nitrofluorene	66	36	7	82	100	54	15	15
2-Nitrofluorene	60	27	10	100	49	37	15	13
3-Nitrofluorene	54	18	4	100	41	31	10	10
4-Nitrofluorene	66	55	11	76	100	51	21	18

GAS CHROMATOGRAPHY

Initial attempts to use a 50 m HP-5 column (crosslinked 5% phenylmethylsilicone, 0.2 mm i.d., 0.33 μm film thickness, Hewlett-Packard) to separate the isomeric nitrofluorenes resulted in poor resolution of 1- and 4-nitrofluorene and of 3- and 2-nitrofluorene. Better resolution was achieved on a column with a DB-1701 liquid phase (14% cyanopropylphenyl [equivalent to OV-1701], 30 m × 0.26 mm, 0.25 μm film thickness, J & W Scientific, carrier gas He, 50 °C on column injection, then programmed to 280 °C at 6 °C min^{-1}), which then was used routinely. A typical chromatogram showing the ion trace for the molecular ion at m/z = 211 for a standard mixture containing all four isomers is shown in Figure 10. Although 1- and 4-nitrofluorene still are closely eluting, the resolution is sufficient for quantitative analysis of all four isomers. The observed peak tailing is due to the polarity of the nitrofluorenes since nonpolar substances showed a distinctly sharper peak shape under the same analysis conditions. Corresponding to their higher dipole moments, the strongest tailing is observed for 2- and 3-nitrofluorene.

For the determination of linear retention indices (RI) a solution containing approximately equal amounts of all four isomers and the deuterated compounds was spiked with a series of bracketing substances. Since two different retention index systems have been used for the analysis of nitro-PAHs, a total of four internal standards was added to allow calculation of the indices in both systems. The solution was spiked with phenanthrene (RI = 300) and chrysene (RI = 400) for determining the indices in the most commonly applied system using parent PAHs as bracketing standards.[38-44] Additionally, two nitrated bracketing substances (1-nitronaphthalene [RI = 200] and 9-nitrophenanthrene [RI = 300]), were added to calculate the retention indices in the system recently described by Robbat *et al.*[45,46] This index system is of special advantage when more selective and sensitive GC detection techniques [e.g. electron capture detectors (ECD), nitrogen phosphorous detectors (NPD), negative ion chemical ionization mass spectrometry (NICIMS), chemiluminescent detectors (CD)] with enhanced sensitivity for nitrogen containing compounds are applied, since these detectors respond poorly to the parent non-nitrated PAHs.

A total of three columns was tested: a 60 m × 0.24 mm DB-5 column (5% phenylsilicone, film thickness 0.25 μm, J & W Scientific), a second DB-1701 column having the same parameters as the one described above and a 25 m × 0.20 mm SB-

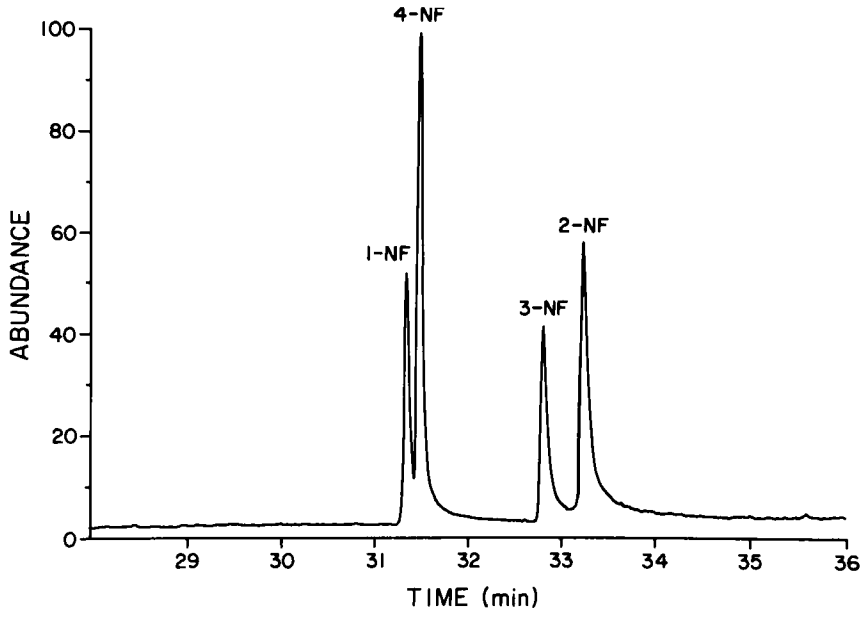


Figure 10 Portion of the GC/MS separation (ion trace at m/z = 211) of a standard solution containing all four nitrofluorenes (NF) on a 30 m DB-1701 column. The elution sequence is 1-nitrofluorene (RT = 31.33 min), 4-nitrofluorene (RT = 31.48 min), 3-nitrofluorene (RT = 32.80) and 2-nitrofluorene (RT = 33.23).

Smectic column (liquid crystalline polysiloxane, film thickness 0.15 μm, Lee Scientific). Helium was used as carrier gas in all cases. The measurements were performed on a HP 5880A GC using flame ionization detection. On-column injections with the same oven program as described above were used for both the DB-5 and DB-1701 columns. Injections on the SB-Smectic column were made on a split injector (300 °C) at 120 °C oven temperature and the oven was then programmed to 280 °C at a rate of 4 °C min^{-1}. The linear retention indices were calculated from the relationship given by Van Den Dool and Kratz.[47]

The RI values obtained are listed in Table 3. Limited literature data are available which may be compared with our results for the DB-5 column. Retention index data for 2- and 3-nitrofluorene were reported on SE-30, SE-52 and SE-54 liquid phases which have similar chemical structures and retention characteristics as the DB-5 column. There is generally good agreement between our RI value for 2-nitrofluorene of 350.98 on the DB-5 column and the literature values of RI = 346.54 on SE-54,[16] 348.56 on SE-30[48] and data ranging from 352.12 to 353.06 on SE-52.[40,41,43] A discrepancy between our retention index for 3-nitrofluorene (RI = 347.86) and a reported value of RI = 328.71[41,43] on SE-52 seemed too high to be explainable by differences in the selectivity of the stationary phase or in GC parameters. Furthermore, we observed the same elution pattern on the HP-5 column, which has a similar stationary phase, with 3-nitrofluorene eluting just before 2-nitrofluorene. This discrepancy between our RI value for 3-nitrofluorene

Table 3 Retention indices (RI) on DB-5, DB-1701 and SB-Smectic columns (mean values and standard deviations calculated from six replicates). PAH (bracketing standards phenanthrene and chrysene) and nitro-PAH (NPAH, bracketing standards 1-nitronaphthalene and 9-nitrophenanthrene) RI values

Nitrofluorene	*PAH retention index*	*NPAH retention index*
	DB-5	
4-Nitrofluorene-d_9	336.39 ± 0.05	269.56 ± 0.03
1-Nitrofluorene[a] / 4-Nitrofluorene[a]	337.35 ± 0.11	270.55 ± 0.08
3-Nitrofluorene	347.86 ± 0.02	281.35 ± 0.05
2-Nitrofluorene-d_9	350.07 ± 0.04	283.62 ± 0.04
2-Nitrofluorene	350.98 ± 0.04	284.56 ± 0.06
	DB-1701	
1-Nitrofluorene	344.44 ± 0.05	268.50 ± 0.10
4-Nitrofluorene-d_9	345.10 ± 0.03	269.19 ± 0.10
4-Nitrofluorene	345.83 ± 0.05	269.93 ± 0.07
3-Nitrofluorene	357.36 ± 0.03	281.97 ± 0.09
2-Nitrofluorene-d_9	360.42 ± 0.08	285.17 ± 0.01
2-Nitrofluorene	361.09 ± 0.03	285.85 ± 0.06
	SB-Smectic	
4-Nitrofluorene-d_9	331.97 ± 0.06	263.40 ± 0.06
4-Nitrofluorene	332.65 ± 0.04	264.27 ± 0.03
1-Nitrofluorene	341.82 ± 0.04	275.87 ± 0.02
3-Nitrofluorene	352.59 ± 0.03	289.49 ± 0.04
2-Nitrofluorene-d_9	370.72 ± 0.09	312.36[b]
2-Nitrofluorene	371.72 ± 0.09	313.65[b]

[a]Peaks not resolved.

[b]Values determined with additionally 6-nitrochrysene added as bracketing standard. Only one single run was performed at a program rate of 2.5 °C min $^{-1}$ as 6-nitrochrysene eluted 6 degrees above recommended maximum oven temperature.

and that reported in the literature cited has been resolved, since the literature value was based on an apparent compound misidentification.[49] The elution sequence on these columns is related to the polarity of the isomers, with the nonresolved 1- and 4-nitrofluorene, which have the lowest dipole moments, eluting first, and the 2- and 3-nitrofluorenes eluting later due to the more exposed position of the nitro group.

The DB-1701 columns showed the same elution sequence as the DB-5 column, but as illustrated in Figure 10, 1- and 4-nitrofluorene were resolved.

A different elution sequence was found on the SB-Smectic column, with 4-nitrofluorene eluting prior to 1-nitrofluorene. The spread of the RI values reflects that the four isomers were more widely spaced than on the other columns tested. The separation on this phase is reported to be strongly dependent on molecule geometry (especially on the length-to-breadth ratio) and is thus very suitable for the separation of isomeric compounds.[37,50,51] Consistent with the importance of molecular geometry, 4-nitrofluorene, the broadest nitrofluorene, elutes first and 2-nitrofluorene, the longest isomer, elutes last.

In summary, the described chromatographic techniques enable isomer-specific determination of all four nitrofluorenes in ambient samples. The liquid chromatographic elution sequence on the Beckman Ultrasphere Si HPLC column was determined to be: 4-nitrofluorene, 1-nitrofluorene, 3-nitrofluorene, 2-nitrofluorene whereas the elution sequence on the reversed phase Ultrasphere ODS column was: 4-nitrofluorene, 2-nitrofluorene, 3-nitrofluorene, 1-nitrofluorene. GC analysis of all four mononitrofluorene isomers can be achieved on either a DB-1701 or SB-Smectic stationary phase and quantification can conveniently be performed by adding deuterated 2- or 4-nitrofluorene, since the perdeuterated isomers are sufficiently separated from the non-deuterated compounds.

Acknowledgments

The financial support through the California Air Resources Board (Contract No. A732-154) and the U.S. Environmental Protection Agency through Assistance Agreement R814857-01 are gratefully acknowledged. We thank Dr. R. Lee, Professor D. L. Rabenstein and their co-workers (Department of Chemistry, University of California Riverside) for recording the NMR spectra and for helpful comments on the data interpretation. Dr. R. Atkinson, Statewide Air Pollution Research Center, University of California is thanked for valuable discussions and for performing the chamber experiments. The results described in this article have not been subjected to the agencies' review and therefore no official endorsement should be inferred.

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