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Sources of the atmospheric contaminants, 2-nitrobenzanthrone and 3-nitrobenzanthrone

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Abstract

2-Nitrobenzanthrone (2NB) was synthesized by reaction of N_2O_5 in CCl₄ at room temperature and used to determine that the hydroxyl-radical- and nitrate-radical-initiated reactions of gas-phase benzanthrone (Bz) produced 2NB rather than 3-nitrobenzanthrone (3NB), the powerful bacterial mutagen first identified by investigators using bioassay-directed fractionation of diesel emission particle extracts. Analysis of a diesel particle standard reference material (SRM 1975) showed the major NB in the sample to be 3NB. In contrast with the diesel SRM, an archived ambient sample with high levels of 2-nitrofluoranthene (2NF) and 2-nitropyrene, which demonstrated that the air mass sampled had undergone significant atmospheric reaction, contained mainly 2NB and much less 3NB. The 2NF/2NB ratio in the ambient sample was estimated to be ~ 35 , consistent with the expected lower gas-phase Bz concentration relative to the gas-phase fluoranthene concentration in ambient air. This work reiterates the need for isomer-specific identification of nitrated polycyclic aromatic compounds (nitro-PAC) when assessing the relative contributions of atmospheric formation reactions versus direct emissions to the nitro-PAC present in ambient air samples. © 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Nitrobenzanthrone; Nitro-polycyclic aromatic compound; Combustion product; Atmospheric formation of nitro-PAC

1. Introduction

Nitrated polycyclic aromatic compounds (nitro-PAC), including nitrated polycyclic aromatic ketones, have been found to be mutagenic (Tokiwa and Ohnishi, 1986; Enya et al., 1997; Kawanishi et al., 1998; Phousongphouang et al., 2000) as well as carcinogenic (Tokiwa and Ohnishi, 1986). 3-Nitrobenzanthrone (3NB), a powerful bacterial mutagen, was first identified by investigators using bioassay-directed fractionation and chemical analysis of diesel emission particle extracts (Enya et al., 1997). Our laboratory further identified the

genotoxic effects of 3NB in two human B-lymphoblastoid cell lines, MCL-5 and h1A1v2 (Phousongphouang et al., 2000). The results indicated that 3NB is an effective human cell mutagen, significantly inducing mutations at the tk and hprt loci in both cell lines, and inducing micronuclei in the h1A1v2 cell line (Phousongphouang et al., 2000). Investigators have reported the formation of DNA adducts by 3NB both in vitro (Enva et al., 1998a; Bieler et al., 1999; Borlak et al., 2000; Kawanishi et al., 2000) and in vivo (Arlt et al., 2001), and urinary metabolites of 3NB have been found in workers occupationally exposed to diesel exhaust (Seidel et al., 2002). There are currently no data available regarding the toxicity of the 2-nitrobenzanthrone (2NB) isomer, which has not previously been reported in ambient particles.

The identification in the early 1980s of 2-nitropyrene (2NP) (Nielsen et al., 1984; Pitts et al., 1985) and

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2-nitrofluoranthene (2NF) (Pitts et al., 1985; Nielsen and Ramdahl, 1986) in ambient particles at higher concentrations than the isomeric 1-nitropyrene (1NP) previously identified in diesel exhaust (Schuetzle et al., 1981; Paputa-Peck et al., 1983) demonstrated the need for isomer-specific identification of nitro-PAC, particularly when seeking to distinguish the relative contributions of atmospheric formation vs. direct emissions to ambient nitro-PAC levels (Atkinson et al., 1988; Atkinson and Arey, 1994; Arey, 1998; Arey and Atkinson, 2003). As noted, 3NB was first identified in diesel exhaust particles, but it was also suggested that atmospheric reactions of benzanthrone might contribute to its presence in ambient particles (Enya et al., 1997, 1998b).

When it was reported (Enva et al., 1998b) that the isomer distribution shifted from 3NB to 2NB when nitrating benzanthrone (Bz) using NO₂-O₃ mixtures in carbon tetrachloride rather than dichloromethane, it occurred to us that the behavior of Bz might be analogous to that of fluoranthene. We have previously shown that 2NF, rather than the electrophilic nitration product 3-nitrofluoranthene (3NF), is formed from reaction of fluoranthene with N₂O₅ in CCl₄ at 25°C (Zielinska et al., 1986). Ambient particles worldwide have been reported to contain 2NF (Ramdahl et al., 1986; Atkinson et al., 1988; Ciccioli et al., 1995, 1996) and its ubiquitous presence can be attributed to radicalinitiated reactions of gas-phase fluoranthene (Atkinson and Arey, 1994; Arey, 1998; Arey and Atkinson, 2003). Therefore, in this work we utilized nitration by N₂O₅ in CCl₄ at room temperature to produce 2NB and investigated the NB isomers formed from hydroxyl (OH)-radical-initiated and nitrate (NO₃)-radical-initiated reactions of gas-phase Bz. We also report here the results of analyses for NB isomers present in an archived ambient air sample whose high levels of 2NF indicated significant radical-initiated nitro-PAC formation in the air sampled, and contrast the nitro-PAC in this ambient air sample with those in a diesel exhaust particle sample.

2. Experimental

2.1. Chemicals

Benzanthrone (Bz; technical grade), carbon tetrachloride (CCl₄; 99 + %, anhydrous), and methanesulfonic acid (98%) were purchased from Aldrich Chemical Co. Acetonitrile (CH₃CN; OPTIMA), hexane (OPTIMA), methylene chloride (CH₂Cl₂; OPTIMA), and methanol (CH₃OH; OPTIMA) were purchased from Fisher Scientific. Dinitrogen pentoxide (N₂O₅) was synthesized and stored under vacuum at liquid nitrogen temperature as described previously (Atkinson et al., 1984). Dinitrogen tetroxide (N_2O_4) was prepared by inverting a cold cylinder of NO_2 with an attached glass pipette and allowing the liquid N_2O_4 to drip into a preweighed flask of CH₂Cl₂ (Gupta, 1995). Methyl nitrite (CH₃ONO) was prepared and stored as described previously (Atkinson et al., 1981).

2.2. Solution-phase syntheses of nitrobenzanthrones (NB)

Two nitro-PAC preparations were used to synthesize milligram quantities of 2NB and 3NB. The nitrations consisted of reaction of the parent compound with N_2O_5 in CCl₄ solution at room temperature (Zielinska et al., 1986) or with N_2O_4 in CH₂Cl₂ at room temperature (Radner, 1983). The N_2O_5 reaction may produce isomer profiles different from those formed in the N_2O_4 reaction, because in an aprotic solution the N_2O_5 undergoes dissociation to NO_3 and NO_2 , allowing nitration by way of a radical reaction mechanism (Zielinska et al., 1986; Gupta, 1995), whereas the N_2O_4 reaction in CH₂Cl₂ produces electrophilic nitration products (Radner, 1983; Ruehle et al., 1985).

3NB was synthesized by reacting \sim 550 mg of Bz with N_2O_4 in a 1:15 stoichiometric ratio in 150 ml CH₂Cl₂ at room temperature with a drop of methanesulfonic acid added to the mixture to catalyze the reaction (Radner, 1983). This reaction formed 3NB as the major product, along with minor amounts of another mono-NB (MNB) isomer and several di-NB (DNB) isomers. 3NB was separated from the other MNB and the DNB isomers by flash chromatography, and the fractions collected were analyzed by gas chromatography with mass spectrometry (GC-MS). The fraction containing the desired 3NB was then further purified by recrystallization and quantified by GC with flame ionization detection (GC-FID) and shown to be of >99% purity. 3NB was conclusively identified by HH-COSY and ¹H-NMR (Phousongphouang et al., 2000), which were in agreement with previously reported spectra (Enya et al., 1997; Suzuki et al., 1997).

2NB was synthesized by reacting ~200 mg Bz with N₂O₅ in a 1:4 stoichiometric ratio in 200 ml CCl₄ at room temperature (Zielinska et al., 1986). This reaction formed 2NB as the major MNB isomer, along with another minor MNB isomer and several DNB isomers. 2NB was separated and purified as described above for 3NB and quantified by GC–FID and shown to be of >99% purity. 2NB was also conclusively identified by HH-COSY and ¹H-NMR (CDCl₃): $\delta = 7.59$ (dt, J = 7.69, 0.97, 1H), 7.87 (dt, J = 7.71, 1.45, 1H), 7.97 (t, J = 7.82, 1H), 8.42–8.49 (m, 2H), 8.55 (dd, J = 7.92, 1.27, 1H), 8.93 (dd, J = 7.38, 1.03, 1H), 8.97 (ds, J = 2.12, 1H), 9.23 (ds, J = 2.13, 1H). This spectrum is in agreement with that previously reported by Suzuki et al. (1997).

2.3. Gas-phase radical-initiated reactions of Bz

A 7000-l Teflon chamber was employed to study the NB isomers formed from the gas-phase OH-radical- or NO₃-radical-initiated reactions of Bz. The chamber is equipped with blacklamps for irradiation, a Teflon-coated fan for mixing, and sampling ports for reactant introduction and product collection. The reactions were performed at 298 ± 2 K and 740 Torr total pressure of purified air at ~5% relative humidity. Bz (~35 mg) was dissolved in 100 ml methanol and sprayed into the chamber by flowing nitrogen gas through a nebulizer with the chamber mixing fan on.

For the OH-radical-initiated reaction, 10 ppmv each of methyl nitrite and NO were added to the chamber and OH radicals were generated by photolysis of methyl nitrite in air (Atkinson et al., 1981):

 $CH_3ONO + hv \rightarrow CH_3O + NO$,

 $CH_{3}O + O_{2} \rightarrow HCHO + HO_{2},$

 $HO_2 + NO \rightarrow OH + NO_2$,

producing OH radical concentrations approximately 100-fold higher than those found in ambient atmospheres. After a 10-min irradiation period, the reaction was terminated by turning off the chamber lights, and the products were then collected as discussed below.

For the NO₃-radical-initiated reactions, initial reactant concentrations were 4 and 2 ppmv of N_2O_5 and NO₂, respectively. NO₃ radicals were generated by the thermal decomposition of N_2O_5 :

$$N_2O_5 \rightleftharpoons NO_3 + NO_2$$
.

After allowing 10 min for reaction, product collection was initiated.

For each radical-initiated reaction, approximately 90% of the chamber volume was collected onto two precleaned polyurethane foam (PUF) plugs placed in series in a 7 cm i.d. \times 24.5 cm length Pyrex tube connected directly to a high-powered vacuum motor. The front PUF from each reaction was Soxhlet extracted overnight in CH₂Cl₂. An aliquot of the extract was placed on a small silica gel column and eluted with CH₂Cl₂ before analysis by GC–MS.

2.4. Identification of nitro-PAC in an ambient particle extract

A large (over 12,000 m³ of air sampled) composite ambient air sample was prepared from 12-h daytime samples collected on high-volume filters at an industrial emissions-impacted site in Concord, CA, USA during January 1987 (Atkinson et al., 1988). The sample was collected on several co-located high-volume samplers equipped with Teflon-impregnated glass fiber filters (Pallflex T60A20) and 10 µm cut-off inlets. The filter sample was spiked with deuterated internal standards and Soxhlet extracted (Atkinson et al., 1988). An aliquot from the original whole, i.e., not fractionated, extract was stored in a freezer at -25° C. This archived extract was pre-cleaned by open-column silica chromatography with 100% CH₂Cl₂ followed by 100% methanol. The CH₂Cl₂ fraction was collected and concentrated by evaporation under reduced pressure for further fractionation by high-performance liquid chromatography (HPLC) using a Phase Sep (Norwalk, CT) normal-phase column ($25 \text{ cm} \times 1 \text{ cm}$). The HPLC system consisted of an HP Series 1050 gradient liquid chromatograph with a variable wavelength detector ($\lambda = 254 \text{ nm}$) and a Foxy 200 fraction collector. The solvent program (at a flow rate of 3 ml min^{-1}) was as follows: initially 100% hexane for 10 min. followed by a 5-min gradient to 95% hexane and 5% CH₂Cl₂. The solvent was programmed over the next 25 min to 100% CH₂Cl₂, where it was held for 10 min; then programmed to 100% CH₃CN over 10 min, held isocratic for 10 min; and then programmed back to the initial conditions. Beginning after 1 min, seven 9-min fractions of increasing polarity were collected.

Of the seven fractions collected, fraction 4 was concentrated for analysis of nitro-PAH, in particular the nitrofluoranthene (NF) and nitropyrene (NP) isomers. Fractions 5 and 6, which were predetermined to contain Bz and the NB isomers, were combined, concentrated, and further fractionated on a Vydac reverse-phase C18 column ($25 \text{ cm} \times 1 \text{ cm}$) with the same model HPLC with an HP 1040M Series II diode array detection system. The solvent program employed for further fractionation of the combined fractions 5 and 6 was 60% CH₃CN and 40% H₂O held isocratic for 20 min at a flow rate of 3 ml min^{-1} . Three fractions were collected as follows: (RP1) 1-15 min. (RP2) 15-17 min. and (RP3) 17-20 min. RP2, which was predetermined to contain Bz and NB, was concentrated to remove the CH₃CN and then solvent exchanged into CH₂Cl₂ for GC-MS analysis.

2.5. Identification of nitro-PAC in a diesel particle extract

Standard reference material (SRM) 1975, a diesel particulate matter extract, was obtained from the National Institute for Standards and Technology (NIST), Gaithersburg, MD, USA. The particle sample was collected from diesel-powered industrial forklifts and processed as described by NIST (2000). The contents of one ampoule (~ 1.2 ml) of extract was transferred to a 2 ml volumetric flask. The sample was spiked with an internal standard of 1-nitropyrene-d₉ (1NP-d₉) at an amount comparable to the concentration of 1-nitropyrene in the sample, based on measurements made at NIST (2000), and the volume was then brought

up to 2 ml with CH₂Cl₂. Of the 2 ml, $200 \,\mu$ l was processed for GC–MS analysis as described above, utilizing HPLC fractionation on the normal-phase column followed by further fractionation on the reverse-phase column.

2.6. Chemical analysis

GC-MS analyses were conducted of the products of the gas-phase radical-initiated reactions, solution-phase syntheses of NB, as well as the ambient and diesel particle sample extracts. The GC-MS analysis were performed on a 50 m DB-17 narrowbore column (0.25 mm i.d. and 0.25 µm film thickness) using an HP5890 Series II GC interfaced to an HP5971A MS detector in the cool on-column mode. Based on full-scan electron impact (EI) mass spectra, the following molecular ions and characteristic fragment ions were chosen for selected ion monitoring (SIM): Bz (m/z 230, 202) and NB (m/z: 275, 245, 229, 217, 201, 200, 189). The initial column temperature was 40°C, then temperature programmed to 300°C at 20°C min⁻¹, and held for 15 min. SIM analysis was also utilized to monitor the molecular ions and characteristic fragment ions for the analysis of NF and NP (m/z: 247, 217, 201, 200, 189), and 1NP-d₉ and 2NF-d₉ (*m*/*z*: 256, 226, 210, 208, 198). For this analysis the initial column temperature was 40°C, then temperature programmed to 200°C at 20° C min⁻¹, then to 300° C at 2° C min⁻¹, and held for 15 min.

GC–FID analyses were conducted to determine the purity of the NB in the cool on-column mode on a 24 m DB-1701 fused silica capillary column (0.32 mm i.d. and 1 μ m film thickness) using an HP5890 Series II GC with FID. The temperature program used was as follows: the initial column temperature was held at 35°C for 1 min, then temperature programmed to 150°C at 20°C min⁻¹, then to 280°C at 8°C min⁻¹, and held for 15 min.

A limited number of samples were analyzed by negative ion methane chemical ionization using an Agilent 5973N mass selective detector and a 30 m HP-5MS capillary column (0.25 mm i.d. and 0.25 μ m film thickness) for separation.

3. Results and discussion

3.1. Solution-phase synthesis of NB

It has been suggested that fluoranthene (I) may be used as a probe to distinguish between radical and electrophilic substitution pathways (Squadrito et al., 1987, 1990), with radical reaction pathways giving 2NF and the electrophilic positional selectivity being 3NF > 8NF > 7NF > 1NF > 2NF. The reaction of fluoranthene with N₂O₅ in CCl₄ at 25°C gives 2NF as the sole mononitro-isomer (Zielinska et al., 1986), while nitration of fluoranthene by N₂O₄ in CH₂Cl₂ gives 3NF > 8NF (Radner, 1983). The reaction of Bz (II) with N₂O₅ in CCl₄ solution at room temperature produced mainly 2NB, along with minor amounts of another



Fig. 1. Electron impact mass spectra of (A) 2-nitrobenzanthrone (from reaction of Bz with N_2O_5 in CCl₄ at 25°C) and (B) 3-nitrobenzanthrone (from reaction of Bz with N_2O_4 in CH₂Cl₂ at 25°C).

MNB isomer and DNB isomers. The reaction of Bz with N_2O_4 in CH_2Cl_2 at room temperature formed 3NB as the major MNB isomer, together with minor amounts of other MNB and DNB isomers. Thus, Bz and fluoranthene display the same radical (giving 2-position substitution) vs. electrophilic (giving 3-position substitution) isomer distributions.

The EI mass spectra of 2NF and 3NF (Pitts et al., 1985) are distinguished by the 3-NO₂-substituted isomer showing a larger $[M-NO]^+$ fragment ion than the 2-NO₂-substituted isomer. As shown in Fig. 1 the EI mass spectra of 2NB and 3NB have analogous patterns of fragmentation, with the more abundant loss of NO occurring for the 3NB isomer where the NO₂ group is in a *peri* position.

3.2. Gas-phase radical-initiated reactions of Bz

Scheme 1 shows mechanisms for the radical nitration of fluoranthene and Bz producing 2NF and 2NB, respectively. The OH-radical-initiated reaction of fluoranthene (X=OH in Scheme 1) has been shown to produce 2NF (and lesser amounts of 7NF and 8NF) with an estimated 2NF yield of ~3% (Atkinson et al., 1990). The NO₃-radical-initiated reaction of fluoranthene (X=NO₃ in Scheme 1) has been shown to produce solely 2NF with an estimated yield of ~24% (Atkinson et al., 1990).

3.2.1. NO₃-radical-initiated reaction of Bz

GC–MS SIM analysis of the extract of the PUF plug used to sample the products from the gas-phase reaction of Bz with the NO₃ radical in the presence of NO₂ showed 2NB and a very small 3NB peak (Fig. 2A). Since 3NB is the electrophilic nitration product of Bz, artifactual formation of 3NB during sampling or from Bz adsorbed on the chamber wall reacting with N₂O₅ cannot be ruled out as the source of the minor amount of 3NB observed.

It has previously been shown that reactions of 1- and 2-methylnaphthalene (Gupta, 1995) and fluoranthene (Zielinska et al., 1986) with N_2O_5 in CCl₄ give nitroisomer profiles similar to those observed from the gasphase NO₃-radical-initiated reaction of the parent aromatic compound. Thus, the identification of 2NB as the dominant product from the gas-phase reaction of Bz with the NO₃ radical is consistent with the similarity of mono-nitrated products formed from certain PAC from solution-phase nitrations by N_2O_5 in aprotic solvents at room temperature with their gas-phase reactions with NO₃ radicals in the presence of NO₂.

3.2.2. OH-radical-initiated reaction of Bz

GC–MS SIM analysis of the extract of the PUF plug used to sample the products from the gas-phase reaction of Bz with the OH radical in the presence of NO_x showed only 2NB (Fig. 2B). Thus, as suggested in Scheme 1, both Bz and fluoranthene produce predominantly the 2-substituted mononitro-isomers from both OH-radical- and NO₃-radical-initiated reactions. This behavior may be contrasted with that of pyrene, where the OH-radical-initiated reaction produces 2NP with an estimated yield of ~0.5% (presumably by addition of the OH radical to the most electron-rich 1-position of pyrene followed by *ortho* addition of NO₂ and loss of water to form 2NP), but the NO₃ radical reaction produces only a trace of 4NP (Atkinson et al., 1990).

To maximize the gas-phase Bz in the Teflon chamber, it was introduced by spraying Bz into the chamber, thereby coating the chamber walls. Because the Bz adsorbed onto the walls was in equilibrium with the gas phase, determining the amount of Bz reacted would be difficult and therefore we did not attempt to determine the yields of 2NB from the radical reactions. It should be



Scheme 1.



Fig. 2. GC–MS SIM traces of the m/z = 275 molecular ion of nitrobenzanthrones: (A) formed from gas-phase reaction of Bz with the NO₃ radical in the presence of NO_x and (B) formed from the gas-phase reaction of Bz with the OH radical in the presence of NO_x.

noted that for naphthalene, alkylnaphthalenes and fluoranthene, the mono-nitrated product yields from the NO₃-radical-initiated reactions are at least an order of magnitude higher than the yields from the OH-radical-initiated reactions (Atkinson et al., 1988; Atkinson and Arey, 1994; Arey, 1998; Arey and Atkinson, 2003).

These atmospheric simulation chamber results indicate that atmospheric reaction of gas-phase Bz with either OH radicals or NO_3 radicals will lead to the formation of 2NB.

3.3. Identification of nitro-PAC in ambient and diesel particle extracts

3.3.1. Nitrofluoranthenes and nitropyrenes

The nitro-PAC identified in combustion sources such as diesel exhaust are generally the isomers produced by electrophilic nitration of the parent PAC (Schuetzle et al., 1981; Paputa-Peck et al., 1983; Schuetzle, 1983). This is consistent with the NF and NP isomers observed in the diesel SRM 1975 (see Fig. 3B). 1NP, the electrophilic nitration product of pyrene, is the dominant isomer of molecular weight 247, with 3NF and a trace of 8NF also being observed. Although fluoranthene and pyrene are often present in combustion samples in similar amounts, fluoranthene is less reactive toward electrophilic nitration (Nielsen, 1984) and the dominance of 1NP in many diesel samples has been reported (Schuetzle et al., 1981; Paputa-Peck et al., 1983; Truex et al., 1998).

The ambient sample analyzed was an archived extract that had previously been analyzed for NF and NP isomers (Atkinson et al., 1988). Prior to extraction in



Fig. 3. GC–MS SIM traces of the m/z = 247 molecular ion of nitrofluoranthenes (NF) and nitropyrenes (NP) separated on a 50 m DB-17 capillary column: (A) an authentic standard; (B) a diesel particle extract (NIST SRM 1975); and (C) an ambient particle extract from filter samples collected during daytime in Concord, CA.

1987 the filters were spiked with 2NF-d₉ and 1NP-d₉. Based on these original internal standards, the NF and NP isomers were again quantified following HPLC separation and using GC–MS with selected ion monitoring (see Fig. 3C). The agreement with the original analysis was excellent (original, reanalysis; units of pg/m³ of air sampled): 2NF (503, 520); 1NP (19, 13); 2NP (56, 69), suggesting that the compounds are stable during storage (or possibly that any degradation of the deuterated and non-deuterated nitro-PAC was identical).

The Concord, CA ambient sample was a composite of daytime filter samples collected on three days in January 1987, and the presence of high levels of both 2NF and 2NP in the sample suggest that these nitro-PAC were formed by daytime OH-radical-initiated reactions of gas-phase fluoranthene and pyrene. It should be noted that ambient samples with high levels of 2NF and high ratios of 2NF/2NP have been reported and the presence of 2NF in these samples attributed to nighttime NO₃radical-initiated formation (Arey et al., 1988a; Atkinson et al., 1988; Zielinska et al., 1989a). The dominance of 2NF over the combustion emission 1NP has been reported throughout the world (Ramdahl et al., 1986; Arey et al., 1988a; Atkinson et al., 1988; Zielinska et al., 1989a; Ciccioli et al., 1995, 1996; Dimashki et al., 2000; Feilberg et al., 2001), indicating the important role of

atmospheric reactions in determining the nitro-PAC present in ambient air.

3.3.2. Nitrobenzanthrones

The GC-MS SIM analyses of the NB in the diesel SRM and the ambient sample are shown in Fig. 4. Based on the retention times and confirmed by the relative abundances of the fragment ions monitored, the diesel sample contains mainly 3NB (Fig. 4B) and the ambient air sample has 2NB (Fig. 4C) as the dominant NB. Enva et al. reported concentrations of 3NB in diesel exhaust particles ranging between 0.6 and $6.6 \,\mu g \, g^{-1}$ of particle, depending on the engine load (Enya et al., 1997). The presence of 3NB in diesel particles is consistent with this isomer being formed from reaction of Bz with N2O4 in CH₂Cl₂ (Phousongphouang et al., 2000; Feilberg et al., 2002) and with the presence of isomers that are electrophilic nitration products of the parent aromatic compounds being more abundant in combustion emissions (Schuetzle et al., 1981; Paputa-Peck et al., 1983; Schuetzle, 1983). It should be noted that there was a significantly larger and earlier-eluting peak (not shown in Fig. 4) in the analysis of the diesel SRM sample that was also apparently a nitro-PAC of molecular weight 275.



Fig. 4. GC–MS SIM traces of m/z=275 molecular ion of nitrobenzanthrones (NB) separated on a 50 m DB-17 capillary column: (A) an authentic standard of 2NB and 3NB; (B) a diesel particle extract (NIST SRM 1975); and (C) an ambient particle extract from filter samples collected during daytime in Concord, CA.

As noted, the ambient sample contained mainly the 2NB isomer. Confirmatory analysis on an HP-5MS column with detection by negative ion chemical ionization showed 2NB and 3NB to be very closely eluting. By using a slow temperature ramp, sufficient resolution was obtained to confirm the presence of 2NB as the major isomer and suggest that the minor isomer (which eluted just in front of 2NB on the HP-5MS column) was 3NB. Although we did not have internal standards for the NB, using an external calibration the calculated concentrations were 15 and 0.4 pg/m^3 for 2NB and 3NB, respectively. Thus, the ratio of 2NF/2NB in the ambient sample was ~35.

One of the major controlling factors in the amounts of 2NF vs. 2NB formed from atmospheric radical-initiated reactions will be the gas-phase concentration of fluoranthene and Bz. There have been numerous reports of Bz in environmental samples (Spitzer and Takeuchi, 1995), including ambient particles (Konig et al., 1983; Ligocki and Pankow, 1989; Allen et al., 1997; Oda et al., 1998a, b; Nielsen et al., 1999), diesel exhaust particles (Yu and Hites, 1981; Handa et al., 1984; Tong et al., 1984; Rogge et al., 1993), and gasoline engine exhaust particles (Handa et al., 1984; Spitzer and Kuwatsuka, 1989; Rogge et al., 1993). For ambient air samples collected in Portland, OR, USA, Ligoki and Pankow reported a gas-phase Bz concentration approximately 1% of the fluoranthene concentration, with $\sim 94\%$ of the fluoranthene and $\sim 4\%$ of the Bz being in the gas phase (Ligocki and Pankow, 1989).

3.4. Implications for assessing atmospheric NB formation

3NB has been tentatively identified in ambient particles collected in Salt Lake City, UT, USA (Zhu et al., 2001), and observed in ambient particles collected from a semi-rural area (Feilberg et al., 2002) and an area impacted by heavy traffic (Enya et al., 1997) at several $pg m^{-3}$ levels. Higher levels (up to ~80 pg m^{-3}) of 3NB have been observed in ambient air of various underground salt mining work-places that are subjected to diesel exhaust (Seidel et al., 2002). In order to assess the relative importance of direct emissions vs. atmospheric formation of NB, isomer-specific analyses are necessary. Mass spectral analyses without chromatography, for example, the time-of-flight secondary-ion mass spectrometry employed by Zhu et al. (2001), will not distinguish between 2NB and 3NB (or among nitro-isomers formed from different molecular weight 230 parent PACs).

The nitrations producing 2NF and 2NB are gas-phase radical-initiated reactions, or reactions in aprotic solutions that proceed by radical pathways (Zielinska et al., 1986; Squadrito et al., 1987, 1990). Under ambient conditions adsorbed-phase, heterogeneous nitrations will produce electrophilic nitration products. For example, reactions of fluoranthene and Bz adsorbed on surfaces with N_2O_5 produce 3NF (Sweetman et al., 1986; Zielinska et al., 1986) and 3NB (Enya et al., 1997), respectively. Additionally, 3NF and 3NB are expected from combustion sources, such as diesel exhaust (see Figs. 3 and 4). Thus, gas-phase radical-initiated nitro-PAC formation from fluoranthene and Bz can be readily identified by the unique isomers (2NF and 2NB) formed and, as noted, the 2NF/2NB concentration ratio observed in the ambient sample reported here is consistent with the expected lower gas-phase concentration of Bz relative to gas-phase fluoranthene.

It is less straightforward to assess the contribution of atmospheric reactions to the formation of the electrophilic nitration products, 3NF and 3NB. However, the dominance of 2NF over 3NF and 1NP (the electrophilic nitration product of pyrene) in ambient samples worldwide suggests that heterogeneous nitration reactions of fluoranthene and pyrene are less important than gasphase radical-initiated reactions.

Studies to assess artifactual formation of nitro-PAC during filter sampling, conducted by passing ambient air containing high levels of NO_x species, including HNO₃, over deuterated PAC added to particles on a filter, found little (Arey et al., 1988b) or no (Dimashki et al., 2000) formation of deuterated nitro-PAC. The amounts of deuterated nitro-PAC formed (no 3NF, small amounts of 1NP, more 3-nitroperylene) (Arey et al., 1988b) were consistent with the reported ease of electrophilic nitration (Nielsen, 1984) for the PAC involved. Consistent with these artifact studies and the high reactivity of anthracene toward electrophilic nitration (Nielsen, 1984), elevated levels of 9-nitroanthracene have been reported in ambient particles after long-range transport and the increases ascribed to heterogeneous formation reactions (Feilberg et al., 2001). As noted, fluoranthene has low reactivity toward electrophilic nitrations and Bz would be expected to be less reactive than fluoranthene (Brown and Okamoto, 1958; Zetzsch, 1982; Kwok and Atkinson, 1995). Thus, we agree with the conclusion of Feilberg and coworkers that the dominant sources of 3NB in ambient particles are combustion processes rather than atmospheric formation (Feilberg et al., 2002), but disagree with the proposal by Enya et al. (1997, 1998b) that facile nitration observed in the laboratory using levels of NO₂ (10 ppmv) and O₃ (5 ppmv) which are orders of magnitude above those relevant to ambient conditions can be extrapolated to suggest the occurrence of heterogeneous atmospheric formation of 3NB.

In an ambient sample in which atmospheric reactions produced high 2NF levels, 2NB has been identified. 2NB thus joins 2NF, 2NP, nitronaphthalenes and methylnitronaphthalenes (MNN) as nitro-PAC identified as being formed from atmospheric radical-initiated reactions (Atkinson et al., 1988; Atkinson and Arey, 1994; Arey, 1998; Arey and Atkinson, 2003). The MNN, in particular, results in specific isomer profiles that can be used to distinguish air masses in which gas-phase PAC have reacted with OH radicals or with NO3 radicals (Zielinska et al., 1989b; Gupta, 1995; Gupta et al., 1996; Arey, 1998). During daylight hours, OH radicals formed from the photolysis of O_3 (and other species) (Atkinson, 2000) will generally be available to participate in radicalinitiated formation of nitro-PAC. Nitrate radicals are formed from the reaction of O₃ with NO₂ and because NO₃ radicals rapidly photolyze they are present at significant levels only during evening and nighttime hours (Atkinson, 2000). Furthermore, because NO and NO₃ react rapidly and consequently are not present at significant concentrations simultaneously, NO₃ radicals will not be present close to sources of NO such as traffic (Atkinson, 2000). High levels of 2NF and MNN attributed to nighttime NO₃ radical chemistry have generally occurred downwind of polluted urban areas (Gupta, 1995; Gupta et al., 1996; Arey, 1998; Arey and Atkinson, 2003).

Ambient air data suggest that nitro-PAC formation from gas-phase radical-initiated reactions generally dominate over heterogeneous formation (Atkinson et al., 1988; Atkinson and Arey, 1994; Arey, 1998; Arey and Atkinson, 2003) and that heterogeneous reactions may be important only for PAC that are reactive toward electrophilic nitration (Feilberg et al., 2001). Due to the lower abundance of gas-phase Bz relative to fluoranthene in ambient air, it is expected that 2NB will not be formed in concentrations as high as 2NF and, indeed, in many ambient air samples 3NB from combustion sources may exceed 2NB formed from atmospheric reactions. However, it is important to recognize that gas-phase radical-initiated atmospheric reactions often produce nitro-PAC isomers distinct from those emitted in combustion sources and the health effects of these atmospherically formed nitro-PAC should be evaluated and included in risk assessments of the health impact of PAC emissions.

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