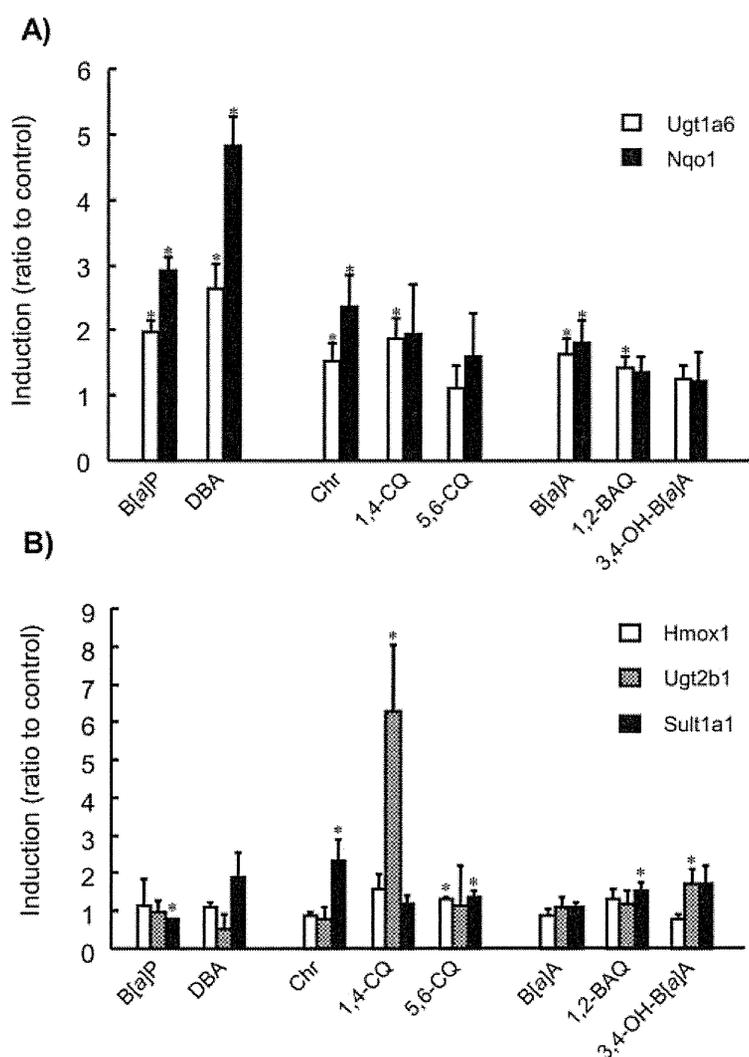


### Expression Changes of Genes Encoding Phase II Metabolizing Enzymes in PAH and PAH Derivative-exposed Cells

We examined changes of gene expression of Phase II metabolizing enzymes, *Ugt1a6*, *Nqo1*, *Hmox1*, *Ugt2b1*, and *Sult1a1*, which encode UDP-glucuronosyltransferase (Ugt) 1a6 and 2b1, NAD(P)H dehydrogenase quinone 1, heme oxygenase-1, and sulfotransferase 1a1, respectively. The expression of *Ugt1a6* and *Nqo1* were significantly induced by some of the PAHs or PAH derivatives (Figure 3A), which relatively strongly induced the genes of CYP1 family. On the other hand, the expression of *Hmox1*, *Ugt2b1*, and *Sult1a1* showed no or weak inductions by the compounds examined in this study (Figure 3B). Exceptionally, expression of *Ugt2b1* was up-regulated solely



**Figure 3:** mRNA expression of Phase II metabolizing enzymes by PAHs and PAH derivatives. (A) *Ugt1a6*, *Nqo1*; (B) *Hmox1*, *Ugt2b1*, *Sult1a1*. The cells were exposed to PAHs and PAH derivatives at concentration of 4  $\mu$ M for 24 h. Symbols and vertical bars respectively represent the mean and  $\pm$ S. D. ( $n = 3$ ). Asterisk (\*) shows significant difference ( $p < 0.05$ ).

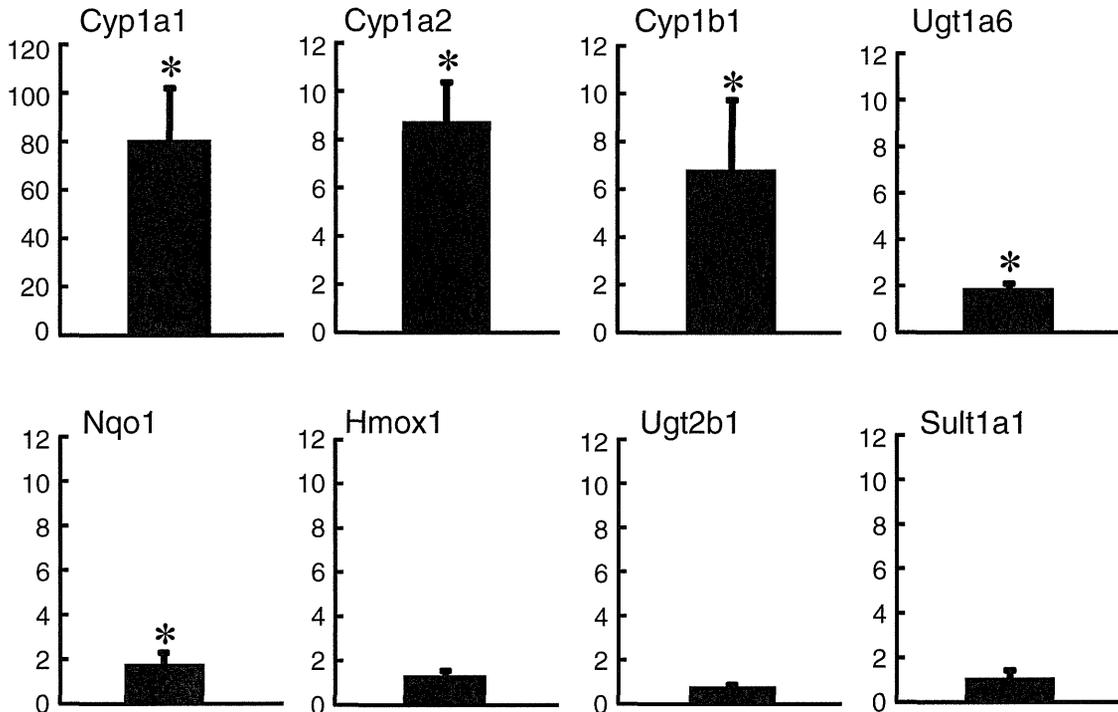
by exposure to 1,4-CQ, although Chr and 5,6-CQ did not up-regulate the expression of this gene.

### Expression Changes of Genes Encoding CYP1 Family and Phase II Metabolizing Enzymes by the Extracts of Airborne Particles

The extracts of airborne particles strongly induced the expression of *Cyp1a1* (Figure 4), and mildly that of *Cyp1a2* and *Cyp1b1*. In addition, they slightly, but significantly, up-regulated the expression of *Ugt1a6* and *Nqo1*, but not that of *Hmox1*, *Ugt2b1*, and *Sult1a1*. There were no large differences between each sample of airborne particles.

### DISCUSSION

Liver metabolizes xenobiotic substances entering into the body, leading to the formation of numerous kinds of PAH derivatives. Not only PAHs but also their derivatives formed from this metabolism pathway induce CYP1 family and Phase II metabolizing enzymes. Since PAHs and PAH derivatives are



**Figure 4:** mRNA expression of CYP1 family and Phase II metabolizing enzymes by airborne particles. The cells were exposed to airborne particles for 24 h. Symbols and vertical bars respectively represent the mean and  $\pm$ S. D. ( $n = 3$ ). Asterisk (\*) shows significant difference ( $p < 0.05$ ).

distributed among various organs in the body, this metabolism is linked to toxicity in many cases. Therefore, toxicological analysis using liver cells is a useful method for toxicological evaluation of xenobiotic substances.

We showed here PAHs- and PAH derivatives-induced changes of expression levels of *Cyp1a1*, *Cyp1a2*, *Cyp1b1*, *Ugt1a6*, *Nqo1*, *Hmox1*, *Ugt2b1*, and *Sult1a1*. CYP1 family is well known as the Phase I enzyme, and play a pivotal role. Ugt family facilitates detoxification of the carcinogen of derivatives formed by CYP1 family (15).

In this study, in order to know the induction of genes encoding the metabolizing enzymes through AhR activation, we used two 5-rings PAHs, B[a]P and DBA, which tremendously induced the expression of *Cyp1a1*, *Cyp1a2*, and *Cyp1b1* (Figure 2A), 5'-flanking regions of which have several AhR binding sites, termed xenobiotic responsive element (XRE). B[a]P and DBA also up-regulated the levels of *Ugt1a6* and *Nqo1* (Figure 3A). *Ugt1a6* and *Nqo1* also have the XRE in their enhancer regions, and are regulated through binding of AhR/ARNT complex to this *cis*-element (15,16). These results were consistent with the previous reports (17). On the other hand, *Hmox1*, *Ugt2b1*, and *Sult1a1* were not induced by exposing to these two strong AhR agonists (Figure 3B), indicating that *Hmox1*, *Ugt2b1*, and *Sult1a1* are not regulated directly and indirectly by the AhR. It has been reported that expression of *Hmox1*, *Ugt2b1*, and *Sult1a1* is regulated by several transcriptional factors other than AhR, such as the activator protein 1 (AP-1) (18), androgen receptor (AR) (19), constitutive androstane receptor/constitutive active receptor (CAR) (20), glucocorticoid receptor (GR) (19), and nuclear factor erythroid 2-related factor 2 (Nrf2) (20). In addition, our results show that B[a]P significantly inhibited the expression of *Sult1a1*. This result is supported by the previous study showing that 3-methyochoranthrene, a strong AhR agonist, decreases the expression of *Sult1a1* (21), although its mechanism remains unclear.

In order to study the effects of derivatization on gene expression changes in CYP1 family and Phase II enzymes, 4-rings PAHs, Chr and B[a]A, were examined in this study. These two PAHs induced the expression of CYP1 family at a relatively low level, compared with 5-rings PAHs, suggesting that 4-rings PAHs have weaker potency for AhR activation than 5-rings PAHs. Quinone derivatization of B[a]A did not induce extreme changes of gene expression of CYP1 family elicited by B[a]A. We also found that 3,4-OH-B[a]A did not change the expression of CYP1 family, *Ugt1a6* and *Nqo1*, while it has a tendency to increase the expression of a part of Phase II genes that are not regulated by the AhR, suggesting a possibility that the increasing polarity of PAHs leads to the shift of inductive pattern of metabolizing enzymes.

Interestingly, 1,4-CQ more strongly induced the expression of these CYP1 family than the parent compound (Chr), while 5,6-CQ that is a structural isomer of 1,4-CQ inversely show lower induction of CYP1 family than Chr. This tendency was almost in parallel with AhR binding activities of the compounds

by DR-CALUX (10). Our data suggest that there are cases where metabolism of some PAHs rather enhance the induction of CYP1 family.

The expression levels of *Cyp1a2* and *Cyp1b1*, which are also known as the AhR target genes, were up-regulated by exposure to PAHs and PAH derivatives examined in the present study (Figure 2). Our data show that B[a]P and DBA increased the expression of *Cyp1b1* stronger than that of *Cyp1a2*, while B[a]A, Chr and 1,4-CQ mildly induced the same levels of expression of *Cyp1a2* and *Cyp1b1*. This discrepancy of induction pattern of two genes among PAHs may be due to the difference of transcriptional regulation between *Cyp1a2* and *Cyp1b1*. Although both genes are induced by the AhR agonist, XRE motif has not identified in the up-stream region of *Cyp1a2*, and AhR/ARNT heterodimer is not able to bind to this region. Sogawa et al. reported a novel function of AhR/ARNT as a coactivator (22). In addition, enhancer region of *Cyp1a2* has AP1 binding site (23). On the other hand, steroidogenic factor-1 (SF-1) motif, cAMP responsive element (CRE), and E-Box were found in 5' flanking region of *Cyp1b1* (24,25). Therefore, future study is needed to examine whether there is difference of activation of transcriptional factors involved in the regulation of genes of CYP1 family other than AhR between 4- and 5-rings PAHs.

Uniquely, the expression of *Ugt2b1* was increased remarkably only by 1,4-CQ, although this mechanism remains unclear. As shown in Figure 2, 1,4-CQ strongly induced the expression of *Cyp1a1*, suggesting that this compound activates the AhR. In addition, PAHQ produces ROS (4), resulting in activation of the transcriptional factors related to the oxidative stress, such as Nrf2, which regulates the expression of *Ugt2b1*(20). However, other than *Ugt2b1*, Nrf2 has been reported to regulate the expression of *Hmox1*(26), which was not significantly induced by 1,4-CQ. Therefore, regulation of *Ugt2b1* by 1,4-CQ may be involved in unidentified other transcriptional factor(s).

Finally, we analyzed the induction potency of genes encoding CYP1 family and Phase II metabolizing enzymes by the extracts from airborne particles collected in Beijing, China. The airborne samples strongly induced up-regulation of *Cyp1a1*, and mild up-regulation of *Cyp1a2* and *Cyp1b1* in the liver cells (Figure 4). In addition, the extracts significantly up-regulated the expression of *Ugt1a6* and *Nqo1*, but not *Hmox1*, *Ugt2b1*, and *Sult1a1*. Since these pattern of gene expression changes are similar to those by B[a]P and DBA, it seems that the effects of the extracts at the transcriptional levels of CYP1 family and Phase II genes are mainly attributed to the AhR activation. In addition, the induction potency of *Cyp1b1* by the extracts from the airborne particles was almost same level with that of *Cyp1a2*. This result was similar to those by 4-rings PAHs and their derivatives. However, the extracts did not up-regulate the expression of Phase II enzymes that were regulated by 4-rings PAHs and their derivatives, and gene expression changes of metabolizing enzymes

between exposure to single compound and crude extracts were slightly different. The airborne particles used in this study were collected from China, because this sample contains very much higher concentration of various PAHs than that from other locations, such as England, Greece, and Japan (27–29). Therefore, the similarity between gene expression patterns of airborne particles in China and some PAHs obtained in this report may be due to the relatively high concentration of PAHs.

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# Atmospheric Formation of Hydroxynitrofluoranthene from Photochemical Reactions of 2-Nitrofluoranthene

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The formation of hydroxynitrofluoranthene (OHNF) via photochemical reactions of 2-nitrofluoranthene (2-NF) was demonstrated using a UV irradiation system. The photoreaction of 2-NF in acetonitrile/water (3/1, v/v) gave a product that was hydroxynitro-substituted. Moreover, we detected an OHNF isomer, which was found in the 2-NF photoreaction products, in soluble organic fractions of ambient airborne particles collected in Kanazawa, Japan. The atmospheric concentration of the OHNF isomer was estimated to be less than  $2 \text{ fmol m}^{-3}$ , which was comparable to that of 1-hydroxy-3-nitropyrene, but lower than that of 1-hydroxy-6-nitropyrene by a factor of 10. The results suggest that atmospheric OHNF is partly formed *via* secondary formation processes, i.e., photochemical reactions of 2-NF appear to have a significant effect on the occurrence of OHNF in the atmosphere.

**Key Words:** 2-nitrofluoranthene, airborne particles, atmospheric secondary formation, hydroxynitrofluoranthene, photochemical reaction

## INTRODUCTION

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are a class of mutagens/carcinogens found in the atmosphere, and some of them exhibit stronger

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mutagenicity/carcinogenicity than their parent polycyclic aromatic hydrocarbons (PAHs) (1). Some types of NPAH are formed via gas-phase reactions of semi-volatile PAHs. For example, 2-nitropyrene (2-NP) is formed from the gas-phase reaction of pyrene with OH radicals in the presence of NO<sub>2</sub>, and 2-nitrofluoranthene (2-NF), one of the most abundant NPAHs in the atmosphere, is formed via two pathways, i.e., OH or NO<sub>3</sub> radical-initiated reactions in the gas phase (2). On the contrary, 1-nitropyrene (1-NP) is a representative NPAH formed through combustion of fossil fuels such as diesel fuel. Nitropyrenes and nitrofluoranthenes taken up by humans and animals are transformed to various metabolites such as hydroxynitropyrenes (OHNPs) and hydroxynitrofluoranthenes (OHNFs) in the presence of cytochrome P450 enzymes (3, 4). Several isomers of OHNP, such as 1-hydroxy-3-nitropyrene (1-OH-3-NP), 1-hydroxy-6-nitropyrene (1-OH-6-NP), and 1-hydroxy-8-nitropyrene (1-OH-8-NP) have also been observed in airborne particles (5, 6) and diesel exhaust particles (DEP) (7–9). Several studies have found that most OHNP and OHNF isomers have lower mutagenic activity than the parent NPAHs (9–11). Recently, however, we have found that several OHNPs act as endocrine disruptors, i.e., they act as estrogenic, anti-estrogenic, and anti-androgenic compounds (12, 13), which may cause dysfunction of human and wildlife endocrine systems, abnormal development of reproductive systems, and immunodeficiencies. In view of the influence of hydroxynitro-aromatics on human health, we need to learn more about their environmental concentrations, sources and behavior.

Photoreactions of 1-NP have been studied both in solvents (14–23) and on solid substrates (15, 19, 24–26). The products of these reactions include OHNPs such as 1-hydroxy-2-nitropyrene (1-OH-2-NP) (14), 1-OH-3-NP, 1-hydroxy-5-nitropyrene (1-OH-5-NP), 1-OH-6-NP, 1-OH-8-NP (6), 2-hydroxy-1-nitropyrene (2-OH-1-NP) (18), and 9-hydroxy-1-nitropyrene (9-OH-1-NP) (17). However, nothing is known about the photoreaction products of 2-NF, which is the most abundant NPAH in the atmosphere. Although several OHNP isomers have been observed in ambient samples (5, 6), there is not yet any evidence that OHNFs, which may be formed from the photoreaction of 2-NF, exist in the atmosphere. Therefore, in this study, we examined the formation of OHNF from photochemical reactions of 2-NF by laboratory experiments in order to clarify whether OHNF occurs in the atmosphere. Furthermore, we report that a particle-associated OHNF that was found in the laboratory photoreaction products was also detected in the atmosphere.

## EXPERIMENTAL SECTION

### Experimental Setup for Photoreaction of 2-NF

Photoreaction of 2-NF was performed in a Pyrex sleeve (1 cm in thickness) which surrounded an annular Pyrex vessel (6.6 cm ID × 60 cm length) as

previously reported (6). The external sleeve has a port for sampling the photoreaction products and the precursor 2-NF. The radiation equipment has 6 black-light lamps (20 W, Toshiba, FL20S-BLB) and a cooling device for isothermal reaction conditions at  $299 \pm 2$  K. The  $1 \times 10^{-6}$  mol L<sup>-1</sup> of 2-NF in acetonitrile/water (3/1, v/v) in the external sleeve was irradiated by the black light lamps placed around the reaction vessel under the presence of air. The total incident photon flux reaching to the surface of the sleeve measured with a Hatchard-Parker actinometer using potassium ferrioxalate photoreduction was  $5.2 \times 10^{16}$  photons cm<sup>-2</sup> s<sup>-1</sup>. The maximum intensity of the photon flux was obtained at a wavelength of 350 nm. A product collected after 2 h of the reaction was analyzed by high-performance liquid chromatograph equipped with a chemiluminescence detector (HPLC/CL) or liquid chromatographic-tandem mass spectrometer (LC/MS/MS) after acid-base and preparative HPLC fractionations.

#### *Airborne Particle Collection*

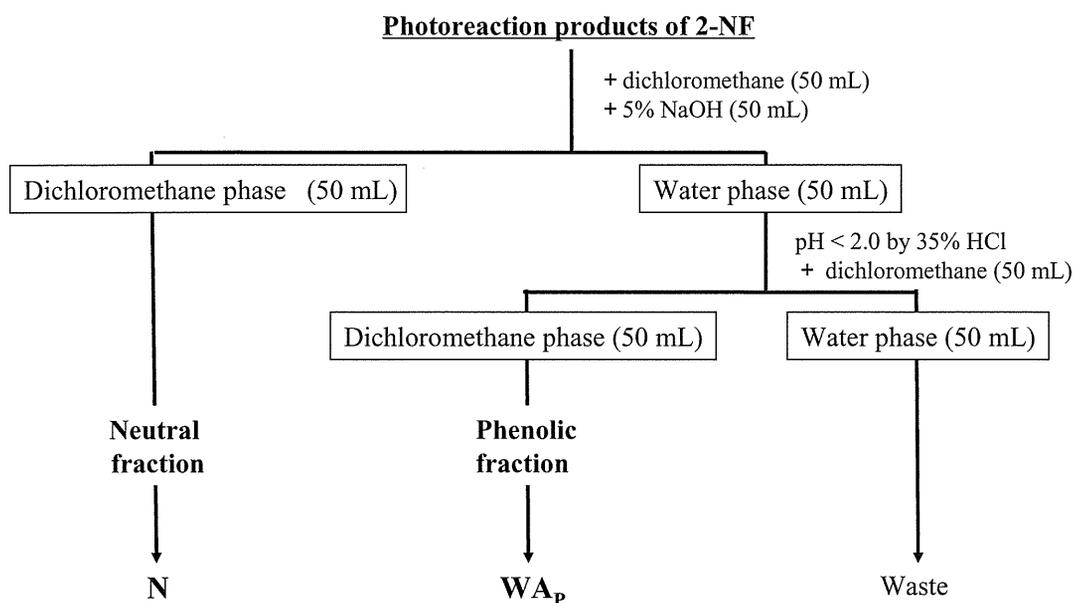
Airborne particles were collected at the rooftop level of a 7-story building approximately 30 m above ground level at Kanazawa University, Kanazawa, Ishikawa, Japan. This sampling site is located in a typical suburban area. The sampling campaign was performed using a high-volume air sampler (Kimoto Electric, Model 120) on quartz fiber filters (QFF; Advantec MFS, QR100), at a flow rate of 1500 L min<sup>-1</sup>, on November 12, 2007 for 6 h. The airborne particle samples were stored at 253 K until subjected to analysis.

#### *Extraction of Soluble Organic Fractions (SOF) from Airborne Particulate Samples*

The filter samples were cut into fine pieces before extraction. SOF from the filter samples were extracted twice with 100 mL of dichloromethane under sonication for 20 min. The extract solution was filtered with a cellulose acetate filter to remove solid residue, followed by concentrating to ca. 5 mL by a rotary evaporator. The sample solution after acid-base and preparative HPLC fractionations was subjected to the quantification by HPLC/CL.

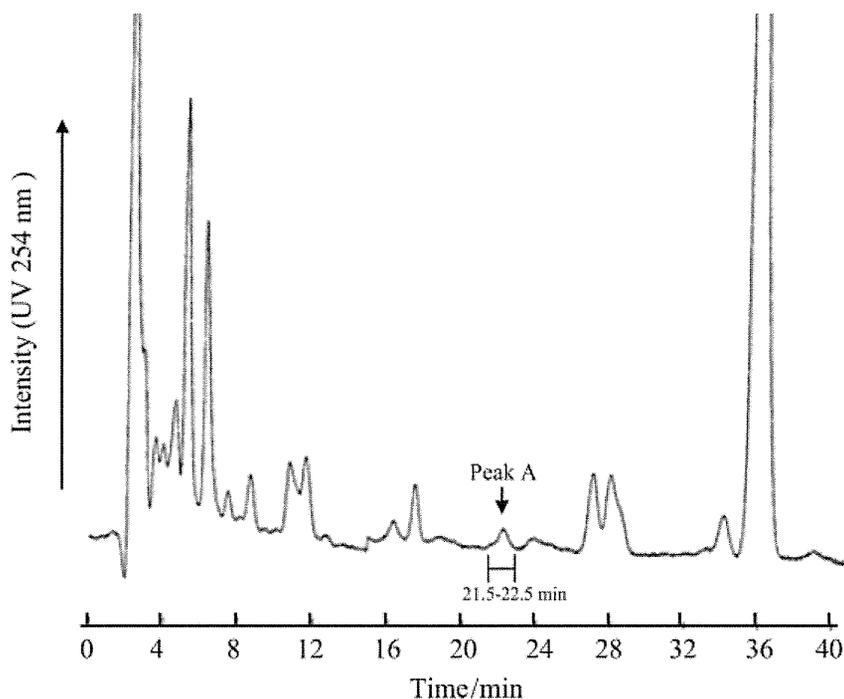
#### *Sample Fractionation*

In order to analyze the OHNF in 2-NF photoreaction products and the SOF from airborne particles, sample fractionation was performed by the following procedure. The crude SOF extracted from airborne particles or obtained from the photoreaction of 2-NF were separated by acid-base partitioning according to the previous reports (6, 28) in order to remove basic and neutral fractions containing interfering compounds such as parent 2-NF (Figure 1). The obtained weak-acidic fraction (WAP) with phenolic compounds such as OHNFs was applied to further fractionation by preparative HPLC (Hitachi, L-6200)



**Figure 1:** Fractionation of the photoreaction products of 2-NF.

equipped with a UV detector (Hitachi, L-4200,  $\lambda = 254$  nm). Fractions corresponding to the OHNF isomer eluted isocratically with acetonitrile/water (1/1, v/v) at a flow rate of  $10 \text{ mL min}^{-1}$  on a Inertsil ODS-P column (GL Science, 10 mm i.d.  $\times$  250 mm) were collected separately as shown in Figure 2. The photoreaction was repeatedly carried out with ca. 10 mg (40  $\mu\text{mol}$ ) of 2-NF in total amount.



**Figure 2:** Chromatogram at 254 nm for HPLC fractionation of 2-NF photoreaction products using a UV detection system. A fraction from 21.5–22.5 min was collected (Peak A).

### Analytical Instrumentation

LC/MS/MS analysis was performed as previously described (6) using the Agilent 1100 series LC system (Agilent Technologies) with an API 4000 Q-Trap tandem mass spectrometer (Applied Biosystems) equipped with an electrospray ionization (ESI) interface and operated in a negative ion mode. Chromatographic separation of 2-NF photoreaction products was performed on a Zorbax Extend-C18 column (150 mm  $\times$  2.1 mm i.d., Agilent Technologies). The column temperature was kept at 303 K. A gradient elution using 0.01%  $\text{NH}_4\text{OH}$  in water (eluent A) and 0.01%  $\text{NH}_4\text{OH}$  in methanol (eluent B) was carried out (B, 25–75% linear gradient for 40 min) at a flow rate of 0.2 mL  $\text{min}^{-1}$ . Sample volumes of 5  $\mu\text{L}$  were typically used for each analysis. The mass spectrometer was operated under multiple reaction monitoring (MRM) mode, and the monitored precursor (Q1) and product (Q3) ions were  $m/z$  262 and 232, respectively. The structures of 2-NF photoreaction products were elucidated using the enhanced product ion (EPI) scan mode in which the product ions are trapped in Q3 (in trap mode) before mass analysis. The EPI scan rate was 1,000  $\text{amu s}^{-1}$ , and the scan range was 100–400  $\text{amu}$ .

An HPLC system with column-switching and chemiluminescence detection previously described (6, 29) was employed for quantification of the 2-NF photoreaction product and the ambient sample. Briefly, the system consists of four HPLC pumps, a 6-port switching valve, a clean up column (GL Sciences, Inertsil ODS-P, 3.0 mm ID  $\times$  250 mm), separation columns (GL Sciences, Inertsil ODS-EP, 3.0 mm ID  $\times$  250 mm or Inertsil ODS-3, 3.0 mm ID  $\times$  250 mm  $\times$  2), a reducer column (Jasco, NPPak-RS, 4.6 mm ID  $\times$  10 mm), a trapping column (GL Sciences, Inertsil ODS-3, 4.0 mm ID  $\times$  30 mm), and a chemiluminescence detector (Soma Optics, S-3400). The chemiluminescence reagent solution was an acetonitrile solution containing 0.03  $\text{mmol L}^{-1}$  bis(2,4,6-trichlorophenyl)oxalate and 15  $\text{mmol L}^{-1}$   $\text{H}_2\text{O}_2$ . Mobile phases were methanol/water (3:1, v/v) for the clean up and reduction of OHNF, and acetonitrile/imidazole-perchloric acid buffer (45:55, v/v) for the separation. The reduction of OHNF into the corresponding amino compounds, which are strongly fluorescent, was performed at 373 K in the reducer column.

### Chemicals

2-NF was obtained from Chiron AS. All solvents and other chemicals used were HPLC or analytical grades from Wako Pure Chemical Ind., Ltd.

## RESULTS AND DISCUSSION

### Photoreaction of 2-NF

Figure 2 shows a profile of the preparative HPLC with UV absorption for the products from photoreactions of 2-NF in acetonitrile/water (3/1, v/v). Many

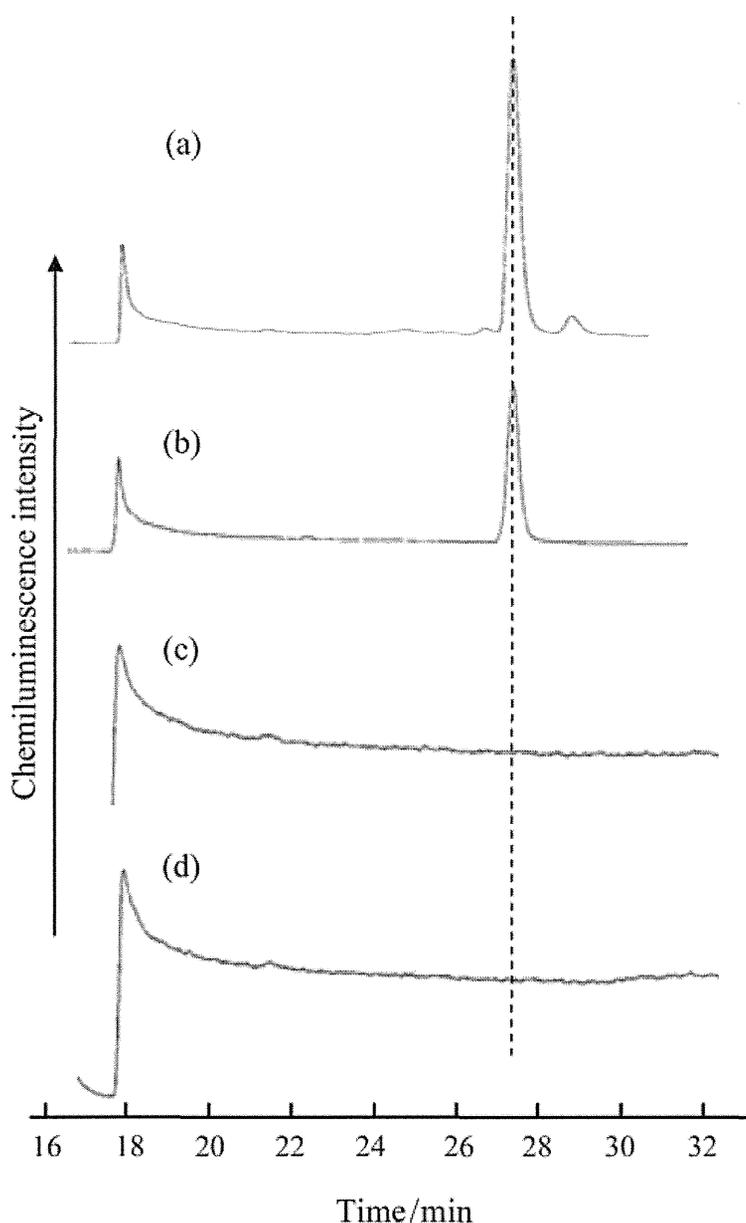
chromatographic peaks were observed in the chromatogram as 2-NF photoreaction products after acid-base fractionation. The HPLC fraction collected every 5 min was then analyzed by HPLC/CL which can selectively detect nitro aromatics with high sensitivity (29). A fraction obtained from 20–25 min, which contained peak A in Figure 2, yielded one major peak around 28 min in the HPLC/CL chromatogram (Figure 3a). The compound eluted around 28 min in the HPLC/CL chromatogram was also observed in the HPLC/CL analysis of a fraction obtained from 21.5–22.5 min by the HPLC fractionation (Figure 3b), which corresponds to the peak A in Figure 2.

When a 2-NF photoreaction sample was analyzed by HPLC/CL without a reducer column, the peak of the photoreaction product was completely eliminated (Figure 3c). This indicates that the photoreaction product is a nitro aromatic compound that can be reduced into a fluorescent amino aromatic compound by the reducer column. After washing the photoreaction product with 5% NaOH/water, the peak of the product also disappeared from the chromatogram (Figure 3d). This result confirms that the peak of the photoreaction product originates from an OHNF that has nitro and phenolic hydroxyl groups in its structure.

This compound gave a characteristic MS/MS spectrum with a molecular-related ion  $m/z$  262 and fragment ions  $m/z$  232 and  $m/z$  216 in EPI mode of LC/MS/MS analysis (Figure 4). The similarity between the fragmentation patterns of the 2-NF photoreaction product and OHNPs (6) indicates that the product is an isomer of OHNF. The structure of the OHNF isomer obtained by the 2-NF photoreaction could not be determined by analysis of its  $^1\text{H-NMR}$  spectrum due to a low yield. A lack of material also made it impossible to prepare a standard solution of the OHNF from the purified 2-NF photoreaction product.

## **OHNF in Ambient Airborne Particles**

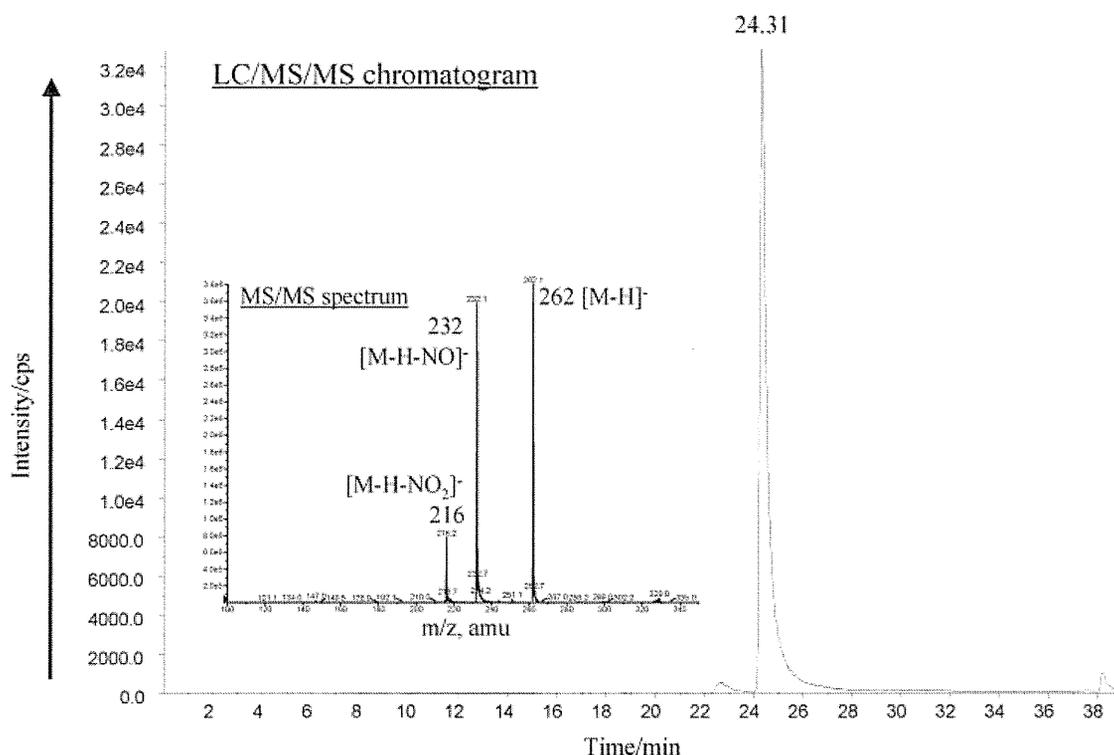
HPLC/CL chromatograms of the 2-NF photoreaction product and extracted SOF from airborne particles are shown in Figures 5a and 5b, respectively. A compound, whose retention time was consistent with that of the 2-NF photoreaction product, was detected by HPLC/CL. The concentration of airborne particle-bound OHNF was estimated to be  $1.7 \text{ fmol m}^{-3}$ , assuming that the response factor of OHNF for the HPLC/CL analysis was the same as that of 1-OH-6-NP. The concentration of OHNF associated with airborne particles was comparable to that of 1-OH-3-NP, but lower than that of 1-OH-6-NP by a factor of 10 (6, 29). Little is known about the concentrations of OHNF in airborne particles. The ambient airborne concentration of hydroxynitro-PAH isomers (MW 263, mainly OHNPs and OHNFs) was reported to be about  $0.01 \text{ ng m}^{-3}$  ( $40 \text{ fmol m}^{-3}$ ) by Nishioka et al. (30). In their study, OHNPs appear to have significantly contributed to the total concentration of hydroxynitro-PAH isomers.



**Figure 3:** Typical chromatograms from the HPLC-chemiluminescence detection system for 2-NF photoreaction products after preparative HPLC fractionation: (a) fraction from 20–25 min; (b) fraction from 21.5–22.5 min; (c) fraction from 21.5–22.5 min without the reduction process; (d) fraction from 21.5–22.5 min with washing process with 5% NaOH solution. OHNF are reduced into their corresponding amino compounds in the HPLC system, and then are detected by the chemiluminescence detector. In the case without the reduction or with the washing process, the peaks of the compounds were eliminated from the chromatograms (see text for details).

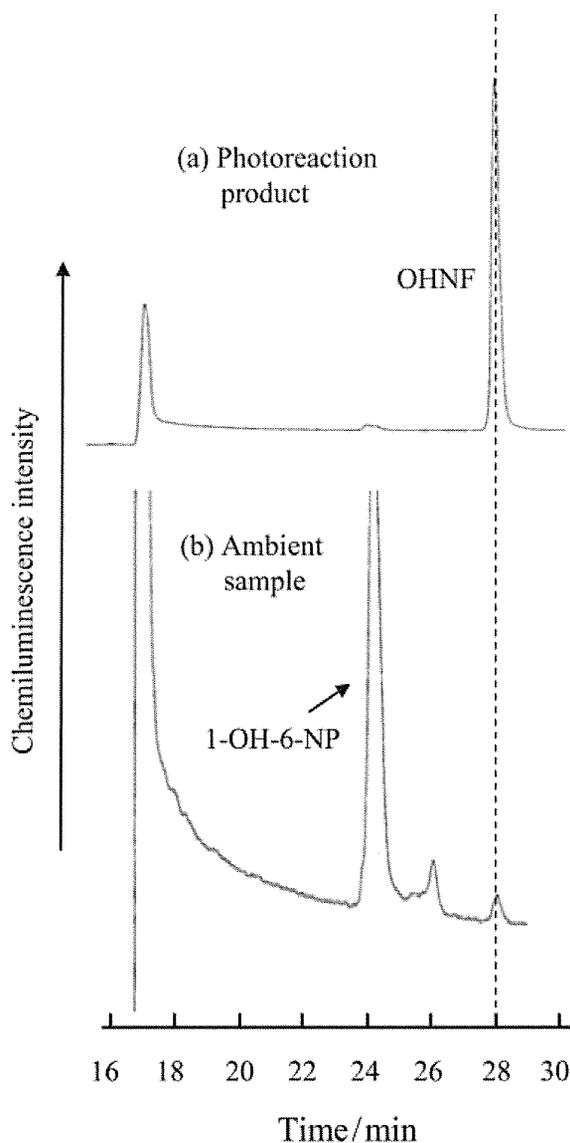
Gibson et al. did not quantitatively analyze airborne particle-bound OHNF although they quantified OHNPs (5).

It has been proposed that the first step of the 1-NP photoreaction is the formation of a nitrite intermediate via both intramolecular nitro-nitrite rearrangement and C-N bond dissociation-recombination mechanisms, and that



**Figure 4:** LC/MS/MS chromatogram ( $m/z$  262 $\rightarrow$ 232) and MS/MS spectrum for a fraction of a 2-NF photoreaction product obtained by the preparative HPLC fractionation from 21.5–22.5 min.

the second step is generation of nitrogen oxide (NO) and pyrenyloxy radicals (14). Pyrenyloxy radicals can react with the solvent to form the corresponding hydroxy compound. They identified 1-hydroxypyrene as a major product of the 1-NP photoreaction in methanol. The NO radicals are expected to recombine with the pyrenyloxy radicals at various carbon positions followed by the formation of OHNPs. We previously identified five kinds of OHNP isomer as 1-NP photoreaction products and also found them in airborne particles (6). This “nitro-nitrite rearrangement” mechanism was also proposed for a photoreaction of 9-nitroanthracene by Chapman et al. (31). However, it has been unclear if the same or similar mechanism is also responsible for the 2-NF photoreaction. In 2-NF, as well as in 2-NP, a nitro group lies in the same plane as the aromatic rings. According to previous studies, such a coplanar structure interferes with the nitro-nitrite rearrangement, resulting in photostability of 2-NF and 2-NP (14, 23). However, the observation of OHNF in the 2-NF photoreaction products indicates that the nitro-nitrite rearrangement cannot be ruled out as a 2-NF photoreaction mechanism although the yield of OHNF from the 2-NF photoreaction (less than 2%) was significantly lower than that of OHNPs from the 1-NP photoreaction ( $\sim$ 20%) (6). Thus, the low concentration of OHNF in the airborne particulate sample may also be caused by the difficulty with the nitro-nitrite rearrangement in 2-NF molecules in spite of the abundance of atmospheric 2-NF.



**Figure 5:** Chromatograms of HPLC-chemiluminescence detection system for (a) a 2-NF photoreaction product and (b) soluble organic fraction of airborne particles.

Another possible pathway for the formation of OHNFs in ambient air is a reaction of hydroxyfluoranthene, which can be produced from photoreactions of nitrofluoranthenes or the parent fluoranthene (FL), with  $\text{NO}_2$ . The addition of an activating group such as a hydroxyl group to the PAH species would be expected to greatly increase the reactivity of the ring toward further reaction, including nitration. Thus, this reaction pathway could partly participate in the formation of OHNF in ambient air. Zielinska et al. (32) reported that OHNF could be produced from the reaction of FL with  $\text{N}_2\text{O}_5/\text{NO}_3/\text{NO}_2$  in a  $\text{CCl}_4$  liquid phase-system. This also raises the possibility that atmospheric OHNFs are formed partly by reactions of FL initiated by OH or  $\text{NO}_3$  radicals in the presence of  $\text{NO}_2$ . In order to understand the factors affecting the formation of atmospheric OHNF, detailed reaction experiments and further observation

of ambient OHNF, which includes a comparison of daytime- and nighttime-concentrations of OHNF, are required.

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# Characteristics of Atmospheric Polycyclic Aromatic Hydrocarbons and Nitropolycyclic Aromatic Hydrocarbons in Hanoi-Vietnam, as a Typical Motorbike City

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We investigated polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (NPAHs) in airborne particulates collected at two sites (I and T) in Hanoi city, a typical city where traffic jams were caused by many motorbikes. The mean total concentration of 10 PAHs having 4–6 rings at sites I and T ranged from 9.6–63.7 pmol m<sup>-3</sup> and from 24.8–41.9 pmol m<sup>-3</sup>, respectively. The predominant PAHs determined were 6-ring PAHs: benzo[ghi]perylene (BghiPe), indeno[1,2,3-cd]pyrene (IDP) and 5-ring PAHs: benzo[b]fluoranthene (BbF), benzo[a]pyrene (BaP). The NPAHs with the highest mean concentrations were 9-nitroanthracene (9-NA) (297 ± 134 fmol m<sup>-3</sup>) at site I and 1-nitropyrene (1-NP) (331 ± 103 fmol m<sup>-3</sup>) at site T. Both PAH and NPAH concentrations were higher in winter at site I but higher in summer at site T. Diagnostic markers indicated that motorbikes strongly contributed to atmospheric PAHs and NPAHs in Hanoi city. The contribution of PAHs having 6 rings and 7-NBaA in Hanoi

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city was larger and the [NPAH]/[PAH] concentration ratios were slightly larger than those in typical automobile cities in Japan and a coal-burning city in China. A possible reason for the higher ratio of 7-NBaA to the total concentration of NPAHs may be that almost all of the motorbikes are not fitted with catalytic converters.

*Key Words:* airborne particulates, motorbike, nitropolycyclic aromatic hydrocarbons, polycyclic aromatic hydrocarbons

## INTRODUCTION

Several polycyclic aromatic hydrocarbons (PAHs) such as benzo[*a*]pyrene are carcinogenic and several nitropolycyclic aromatic hydrocarbons (NPAHs), such as 1,8-dinitropyrene and 1-nitropyrene, are probably or possibly carcinogenic to humans (1). Both PAHs and NPAHs are mainly formed during the incomplete combustion of organic matters such as coal, oil and wood from both natural and anthropogenic sources (2,3). PAHs are partitioned in the atmosphere between the gas and particle phases depending on several factors such as vapor pressure, ambient temperature, and the concentration of particulates (4,5). PAHs having 2 and 3 rings exist mainly in the gas phase, whereas PAHs having 5 rings or more are predominately adsorbed onto particulate matters (6,7). The total atmospheric concentration of PAHs is usually higher in winter than in summer (2,8,9). NPAHs, a group of at least 200 different substances, could be formed through reactions of parent PAHs with gaseous nitrogen oxides, during not only the above combustions but also transportation in the atmosphere (10).

Numerous studies of atmospheric PAHs and NPAHs conducted in Europe, North America, and Northeast Asia have found that automobiles were the major contributor to both PAHs and NPAHs in the urban atmosphere (6,8,11,12). On the other hand, motorbikes (two-wheel motor vehicle) are the most popular vehicle in many Asian countries such as Taiwan, Malaysia, Thailand, Cambodia, Vietnam, Indonesia, and the Philippines owing to its convenience and cheaper expense (13–15). They are also popular in several European countries, such as Italy (16,17). In these countries, the number of motorbikes per 1,000 people ranges from 26 in the Philippines to 590 in Taiwan and the ratio of registered motorbikes to registered cars ranges from 1.3 in Malaysia to 56 in Vietnam (18). This suggests that motorbikes might be major contributors to the atmospheric PAHs and NPAHs burden in urban areas of Asian countries. However, little is known of the occurrences of PAHs and NPAHs from motorbike exhausts in Southeast Asia. Several studies have examined PAH emissions from diesel engines (19–21), gasoline engines (22,23), and two-stroke and four-stroke motorbike engines (17,23). Nevertheless, how atmospheric PAHs and NPAHs distribution in motorbike cities differ from those in automobile cities have not been yet studied. The purpose of this study was to assess the