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Ambient Measurement of Nitrotriphenylenes and Possibility of Nitrotriphenylene Formation by Atmospheric Reaction

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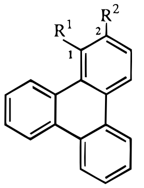
Environmental nitration of PAHs by nitrogen oxides on airborne particulates and identification and quantification of nitro-PAHs in them have been studied on fluoranthene and pyrene because of their large abundance. However, the direct mutagenic activity of these nitro-PAHs can account for only up to about 20% of the total direct mutagenic activity of airborne particulate samples, and the presence of unknown mutagenic nitro-PAHs was suggested. 1- and 2-Nitrotriphenylenes were first identified and quantified in ambient particulate samples collected in Tokyo. Their mutagenicity and formation by laboratory reactions of triphenylene deposited on a filter with NO_2/ NO_3/N_2O_5 were also investigated. The concentrations of 1- and 2-nitrotriphenylenes were much higher than that of 1-nitropyrene in the same samples. 2-Nitrotriphenylene showed over 2 orders of magnitude greater mutagenic activity than 1-nitrotriphenylene in both strains TA98 and YG1024 (18 and 480 revertants/nmol for 1-nitrotriphenylene and 3800 and 101 700 revertants/nmol for 2-nitrotriphenylene in TA98 and YG1024). The mutagenic 2-nitrotriphenylene was expected to contribute largely to the total mutagenic activity of the extracts from airborne particulates. In addition, more effective formation of nitrotriphenylenes by the nighttime reaction of triphenylene in the presence of O_3 in the dark can explain the observation of high nitrotriphenylene concentration in the nighttime samples.

Introduction

Some species of nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) which are ubiquitous in our atmospheric environment are known to exhibit high mutagenicity in in vitro test (*1*) and high carcinogenicity in animal experiments (*2*). Nitro-PAH has been attributed largely to direct emissions from combustion sources such as diesel engine smoke and soot from burning wood (*3, 4*). Nitro-PAH formation by the reaction of parent PAH with nitrogenous species has been discussed on gas-phase reaction (*5, 6*) and gas−solid-phase reaction (*7*−*11*).

CHART 1



1: R^1=R^2=H
2: R^2=NO_2; R^1=H
3: R^1=NO_2; R^2=H

Environmental nitration of PAHs by nitrogen oxides on airborne particulates has been studied particularly on fluoranthene and pyrene because of their large abundance in ambient particulate matter; nitrofluoranthenes and nitropyrenes have been identified and quantified in airborne particulate samples (*12*−*14*), and their mutagenic activities were determined using *Salmonella typhimurium* strains in the absence of S9 mix (*1, 14, 15*). However, the direct mutagenic activity of these nitro-PAHs can account for only up to about 20% of the total direct mutagenic activity of airborne particulate samples (*12*), and the presence of unknown mutagenic nitro-PAHs was suggested. Recently, nitro-PAH lactones were reported to contribute to the ambient mutagenicity (*16, 17*). 3-Nitrobenzanthrone was further shown to be a new class of powerful direct-acting mutagens of atmospheric origin, and it existed not only in diesel exhaust particles but also in airborne particulates, suggesting formation by the reaction of benzanthrone with NO_2 and O_3 (*18*).

In this study, we investigated the ambient amount of nitrotriphenylenes and their formation by the reaction of triphenylene (**1**) with NO_2 to elucidate the contribution of nitrotriphenylenes to be a direct active mutagen of airborne particulate samples (Chart 1). Though triphenylene is one of the 4-ring PAHs which are much abundant in airborne particulates, few studies have been carried out because of its low mutagenic activity. However, according to the reported high mutagenicity of 2-nitrotriphenylene (**2**) (*19*) and the enhanced nitration of triphenylene by acid catalysts (*20*), it is expected that nitrotriphenylene, especially 2-nitrotriphenylene, will contribute to the observed mutagenicity of airborne particulate samples and can be formed by atmospheric reaction. Thus we measured the amount of nitrotriphenylenes in airborne particulate samples collected both at daytime and at nighttime and investigated nitrotriphenylene formation in the nitration of triphenylene in the presence of O_3 or under light irradiation.

Experimental Section

Airborne Particulates Sampling. Airborne particulate matter was collected on a quartz fiber filter (Pallflex Product Co., 2500QATUP) using two high-volume air samplers with an impactor to remove particles larger than 10 μm in diameter (70 m^3/h) on the rooftop of a five-story building facing a traffic-heavy thoroughfare in central Tokyo (Minato-ku) during December 9−11, 1998. Daytime sample-collection was made for 9 h from 8 a.m. to 5 p.m., and the nighttime one was similarly done from 8 p.m. to 5 a.m. The samples for PAH analysis were extracted from collected airborne particulate matter with acetonitrile by sonication for 10 min, and the extract solution was concentrated to an adequate

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volume for the analysis. For nitro-PAH analysis, the sample was extracted with dichloromethane, and the extract solution was prefractionated by preparative HPLC using the solvent gradient (*n*-hexane for 5 min, linear gradient to 5% dichloromethane/*n*-hexane for 5 min, linear gradient to dichloromethane for 15 min, and hold for 5 min, and linear gradient to acetonitrile for 5 min, and finally hold for 10 min). Fractions eluted from 15 to 27 min were picked up and concentrated to an adequate volume for the analysis.

Materials. Triphenylene was obtained from Tokyo Kasei Kogyo Co. Ltd. All solvents used were HPLC-grades from Kokusan Chemical Co. Ltd. Permical permeation tube, which was used to evolve NO_2, was purchased from Gastek Co. Ltd. Other chemicals were obtained from Aldrich Co.

Analytical Instrumentation. Fractionation of the samples were performed with a preparative high-performance liquid chromatograph (HPLC, Senshu, Model SSC-3100 and a Model SSC-3000A-II UV detector ($\lambda = 254$ nm) using a column of Nucleosil 100−1525 (10 mm i.d. × 250 mm)). Gas chromatographic/mass spectrometric (GC/MS) analysis was performed using a mass spectrometer (Hewlett-Packard, HP-5890A series II connected to JEOL JMS−HX110A); ionization voltage 70 eV; chamber temperature 280−290 °C; accelerating voltage 10 kV. Nitrotriphenylenes in the airborne particulate samples were identified by GC/MS with selected ion monitoring (SIM), and synthesized samples were characterized by operating in the scanning mode (45−500 u). The GC column used was a DB-5 column (5% phenylmethylsilicone, 0.25 mm i.d. × 30 m, J&W Scientific, Inc.). GC identification and quantification of nitrotriphenylenes and 1-nitropyrene was performed with a gas chromatograph equipped with a nitrogen−phosphorus detector (NPD, Hewlett-Packard, HP-5890A series II) using a HP-5 column (5% phenylmethylsilicone, 0.25 mm i.d. × 30 m, Hewlett-Packard). The temperature program for GC/MS and GC/NPD analysis was as follows: 70 °C for 1 min, to 300 °C with ramping rate of 7 °C/min, isothermal for 10 min. HPLC analysis of triphenylene, pyrene, and benzo[*e*]pyrene was performed with a Shimadzu LC-9A with a RF-550 spectrofluorometric detector using a Vydac 201TP54 (4.6 mm i.d. × 250 mm), and the mobile phase was acetonitrile/water (8/2, v/v) at 30 °C.

Synthesis of Nitrotriphenylenes. 1-Nitrotriphenylene (**3**) and 2-nitrotriphenylene (**2**) were synthesized according to the literature (*20*−*22*). To an acetic anhydride (Ac_2O) solution of triphenylene ((**1**), 662 mg, 2.5 mmol in Ac_2O of 2.5 mL), a solution of HNO_3 ($d = 1.38$, 0.18 mL) in Ac_2O (0.84 mL) added at 0 °C with continuous stirring for 30 min. After stirring for another 30 min, the mixture was poured onto ice/dichloromethane, and the organic layer was washed with water. When most of the solvent was evaporated, 1 g of silica gel was added, followed by complete evaporation of the solvent. The crude sample was placed on top of a column packed with silica gel and eluted with 10% dichloromethane/*n*-hexane. Purification was subsequently carried out by preparative HPLC using 10% dichloromethane/*n*-hexane. The purity of both isomers was above 99.9% on GC/NPD. 1-Nitrotriphenylene: yellow powder; EI-MS: m/z (%) = 273 (M^+, 22), 243 ($M^+ - NO$, 100), 226 ($M^+ - HNO_2$, 66), 215 ($M^+ - CNO_2$, 86); HR-EI-MS $C_{18}H_{11}-NO_2$ calcd 273.0790, found 273.0767. 2-Nitrotriphenylene (**2**): yellow powder; EI-MS: m/z (%) = 273 (M^+, 100), 243 ($M^+ - NO$, 4), 226 ($M^+ - HNO_2$, 86), 215 ($M^+ - CNO_2$, 33); HR-EI-MS $C_{18}H_{11}-NO_2$ calcd 273.0797, found 273.0797.

Mutagenicity Assay. The mutagenicity assay was performed following the method of Ames et al. (*23*) (*Salmonella typhimurium* strain, −S9 mix) with the slight modification of including a preincubation step (*24*). The bacterial strains used were *Salmonella typhimurium* His^- strains TA98 and YG1024 which is an *O*-acetyltransferase overproducing derivatives of TA98 (*25*). YG1024 exhibits the enhanced acetyltransferase activity 3−100 times as high as the original TA98. It was kindly provided by Dr. T. Nohmi, National Institute of Hygienic Sciences, Tokyo. The samples for the assay were concentrated by a rotary evaporator and dried under a stream of nitrogen. They were then dissolved in dimethyl sulfoxide and subjected to the mutagenicity test. Assays for synthesized nitrotriphenylenes were performed five times. For each mixture after the reaction of triphenylene with nitrogen dioxide, assay was carried out three times.

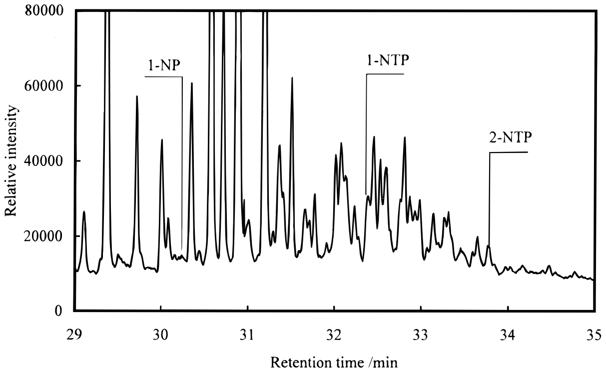

FIGURE 1. Gas chromatogram with NPD of the 12/9 nighttime samples. The identity of the peaks are as follows: 1-NP, 1-nitropyrene; 1-NTP, 1-nitrotriphenylene; and 2-NTP, 2-nitrotriphenylene.

Laboratory Nitration. One hundred micrograms of triphenylene dissolved in acetone/ether (1/1, v/v) was uniformly deposited on a 40 cm^2 piece of Teflon-coated glass fiber filter (Pallflex Product Co., TX40H120WW) and was air-dried. The apparatus for the exposure of triphenylene to NO_2 was the same as that previously described (*9*, *11*, *18*). The light source for photochemical reaction was a high-pressure mercury lamp (100 W) with cooling water and a Pyrex filter to cut off ultraviolet light shorter than 290 nm in wavelength. After the filter was installed in the reaction vessel, the dry air containing 10 ppm of NO_2 was supplied to the vessel at a flow rate of 0.7 L/min. The sample was then irradiated by the ultraviolet light. In the case of the dark reaction in the presence of O_3, an artificial air containing 10 ppm of NO_2 and 5 ppm of O_3 was employed. The reaction products were dissolved in acetonitrile with sonication for 10 min. Triphenylene and nitrotriphenylenes were analyzed by HPLC with spectrofluorometric detection and GC/NPD (or GC/MS), respectively. The reaction products were fractionated by preparative HPLC to identify the mutagens in them, and the effluents from the column were fractionated at intervals of 1 min.

Results and Discussion

Identification of Triphenylene and Nitrotriphenylenes in Airborne Particulates. Triphenylene was determined in acetonitrile extracts of the ambient daytime and nighttime samples collected at Minato-ku, Tokyo. Triphenylene and chrysene of molecular weight 228 were eluted at 14.7 and 15.7 min after injection, respectively. Triphenylene was successfully separated and quantitatively analyzed using a column of Vydac 201TP54 (4.6 mm i.d. × 250 mm).

1- and 2-Nitrotriphenylenes in the purified fractions from airborne particulate samples were analyzed by GC/NPD. A gas chromatogram with NPD of the 12/9 nighttime sample is shown in Figure 1. The identification of 1- and 2-nitrotriphenylenes was based on retention time matching with authentic 1- and 2-nitrotriphenylenes. To show that the identification of 1- and 2-nitrotriphenylenes in the ambient samples by our analytical system was little affected by the coexisting isomers such as nitrobenz[*a*]anthracenes and

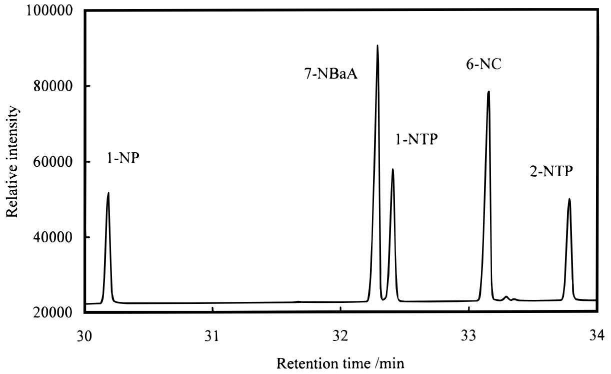

FIGURE 2. Gas chromatogram with NPD showing resolution of 1- and 2-nitrotriphenylenes (1- and 2-NTPs), 7-nitrobenz[*a*]anthracene (7-NBaA), and 6-nitrochrysene (6-NC). 1-Nitropyrene and the isomers in order of elution are as follows: 1-nitropyrene, 7-nitrobenz[*a*]-anthracene, 1-nitrotriphenylene, 6-nitrochrysene, and 2-nitrotriphenylene.

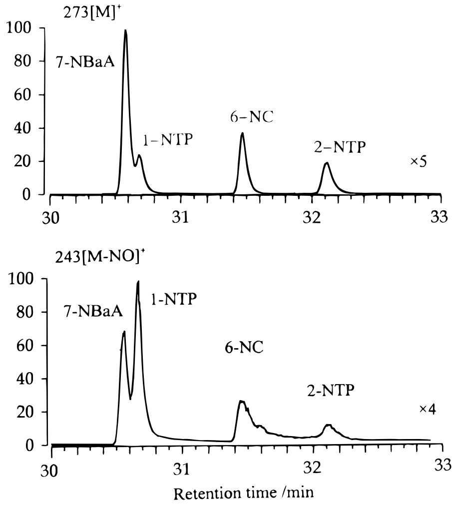

FIGURE 3. Mass chromatograms for molecular ions (*m/z* 273) and the $[M - NO]^+$ (*m/z* 243) fragment ions from the GC/MS-SIM analysis, showing resolution of 1- and 2-nitrotriphenylenes (1- and 2-NTPs), 7-nitrobenz[*a*]anthracene (7-NBaA), and 6-nitrochrysene (6-NC). The isomers in order of elution are as follows: 7-nitrobenz[*a*]anthracene, 1-nitrotriphenylene, 6-nitrochrysene, and 2-nitrotriphenylene.

nitrochrysenes, we then examined the resolution of 1- and 2-nitrotripenylenes from 7-nitrobenz[*a*]anthracene and 6-nitrochrysene by GC/NPD as shown in Figure 2. Because 7-nitrobenz[*a*]anthracene in diesel exhaust particulates and 6-nitrochrysene in airborne particulates have been detected (*26, 27*) and the preferred positions of nitration in benz[*a*]-anthracene and chrysene are predominantly the 7- and 6-position (*20, 28, 29*), respectively, there is quite a possibility that they will coexist in the samples. 7-Nitrobenz[*a*]anthracene and 6-nitrochrysene were eluted before the elution of 1-nitrotriphenylene and 2-nitrotriphenylene, respectively, indicating successful separation of 1- and 2-nitrotriphenylenes and the two isomers.

In addition, the purified fractions from the airborne particulate samples were analyzed by GC/MS-SIM. Mass chromatograms for molecular ions, *m/z* 273 ($[M^+]$), together with the characteristic fragment ions, *m/z* 243 ($[M - NO]^+$), also indicated resolution of 1- and 2-nitrotriphenylenes from the isomeric 7-nitrobenz[*a*]anthracene and 6-nitrochrysene on a 30-m DB-5 column (Figure 3). No identification of the six ambient daytime and nighttime samples was performed since the detect limits of GC-MS-SIM for 1- and 2-nitrotriphenylenes (10 and 10 ng, respectively) were 2 orders of magnitude larger than those of GC/NPD (0.1 and 0.1 ng, respectively). The composite sample made by mixing the six samples analyzed by GC/NPD was then used. As shown in Figure 4, 1- and 2-nitrotriphenylenes in the composite sample were confirmed on the basis of retention time and the molecular ion, *m/z* 273 ($[M^+]$), together with the characteristic fragment ions, *m/z* 243 ($[M - NO]^+$), of authentic 1- and 2-nitrotriphenylenes. 7-Nitrobenz[*a*]anthracene and 6-nitrochrysene were not detected in the composite sample.

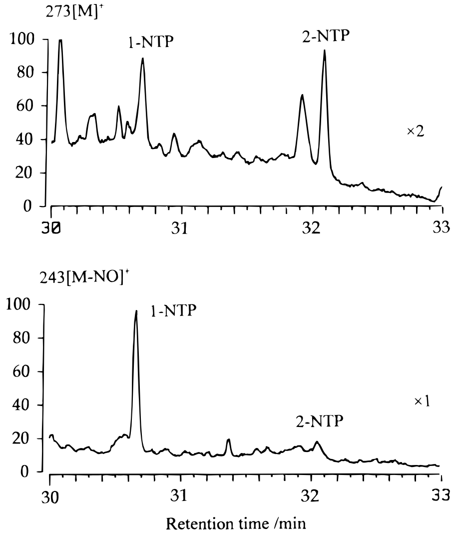

FIGURE 4. Mass chromatograms of ambient composite sample collected in Minato-ku, Tokyo during December 9−11, 1998.

Ambient Amount of Triphenylene and Nitrotriphenylenes. Figure 5(a) shows the ambient concentrations of triphenylene, pyrene, and benzo[*e*]pyrene in daytime and nighttime airborne particulate samples. Triphenylene and pyrene were present at higher levels in the daytime samples than in those of the nighttime. Triphenylene was found at concentrations ranging from 1.1 to 3.0 ng/m^3, and these were comparable levels to that of pyrene (1.0−2.8 ng/m^3). Benzo[*e*]pyrene also was determined as a reference polycyclic aromatic hydrocarbon (PAH) due to its high atmospheric stability. The concentrations of benzo[*e*]pyrene and pyrene obtained in this study coincided with those so far reported in Japan during the wintertime, and no reported value has been available for atmospheric concentration of triphenylene (*30*). On the contrary, the concentrations of both 1- and 2-nitrotriphenylenes were higher in the nighttime samples than in the daytime ones (Figure 5(b)). The difference between the daytime and nighttime samples is more pronounced with 1-nitrotriphenylene. The concentrations of 1- and 2-nitrotriphenylenes were much higher than that of 1-nitropyrene.

Ambient concentrations depend on the level of emissions and meteorological conditions. However the result that triphenylene, pyrene, and benzo[*e*]pyrene were present at higher levels in the daytime than in the nighttime indicates that the concentration of these PAHs sampled in this work depended on the level of emissions strongly, because these PAHs are emitted from automobile engine (*31*) and traffic is usually heavy in the daytime. If 1- and 2-nitrotriphenylenes as well as PAH were directly emitted mainly from a diesel engine, they should be present at higher levels in the daytime samples than in the nighttime ones. The high concentration of 1- and 2-nitrotriphenylenes in the nighttime samples is probably due to atmospheric reaction of triphenylene to form 1- and 2-nitrotriphenylenes. The most reactive PAHs such as pyrene toward electrophilic nitration produced their electrophilic nitration products under ambient high NO_x

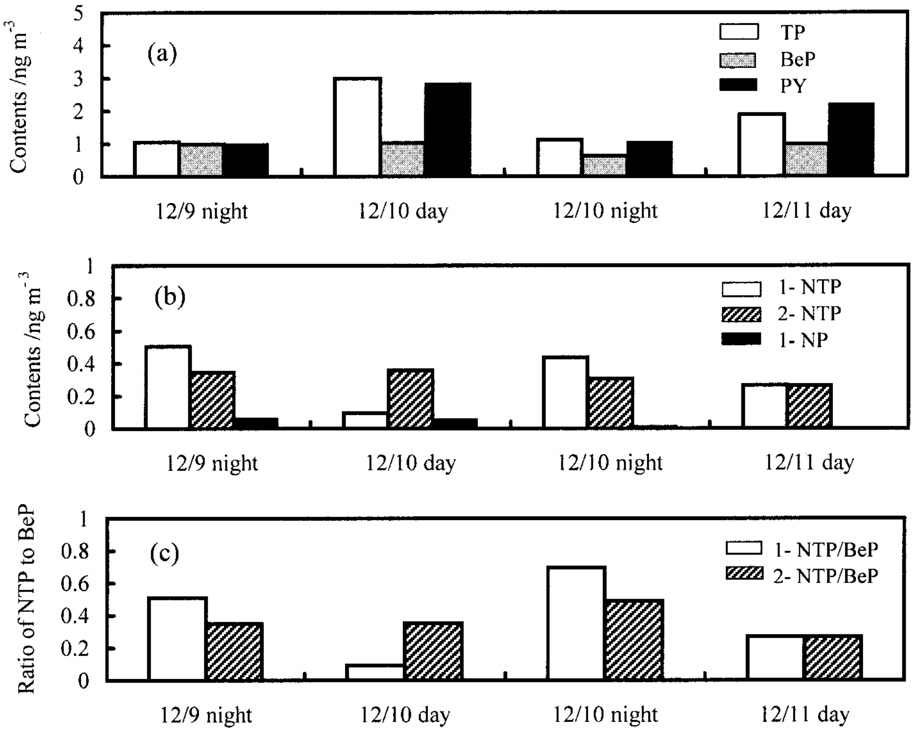

FIGURE 5. Ambient concentration of triphenylene (TP), benzo[*e*]pyrene (BeP), pyrene (PY), 1- and 2-nitrotriphenylenes (1- and 2-NTPs), and 1-nitropyrene (1-NP) in daytime and nighttime airborne particulate samples and concentration ratio of NTPs to BeP: (a) TP, BeP, and PY, (b) 1-NTP, 2-NTP, and 1-NP, and (c) 1-NTP/BeP and 2-NTP/BeP.

TABLE 1. Direct-Acting Mutagenic Activities (Revertants/nmol) of Nitrotriphenylenes

	***Salmonella typhimurium* strain (revertants/nmol ± SD) (−S9 mix)**	
	TA98	**YG1024**
1-Nitrotriphenylene (**3**)	18 ± 6	480 ± 55
2-Nitrotriphenylene (**2**)	3800 ± 250	101 700 ± 4160

conditions and using filters spiked with deuterated PAH (*32*). Since triphenylene is very unreactive toward electrophilic nitration, 1- and 2-nitrotriphenylenes will not be formed during collection (*33*). Figure 5(c) shows the concentration ratio of 1- and 2-nitrotriphenylenes to benzo[*e*]pyrene in the daytime and nighttime samples. The ratios of 1-nitrotriphenylene/benzo[*e*]pyrene and 2-nitrotriphenylene/ benzo[*e*]pyrene were much higher in the nighttime samples than in the daytime ones. This fact leads to the conclusion that 1- and 2-nitrotriphenylenes are at least partly formed by nitration of triphenylene in the atmosphere and that the formation of nitrotriphenylenes occurs easily during the nighttime.

Mutagenic Activity of Nitrotriphenylenes. Table 1 lists the mutagenic activity of 1- and 2-nitrotriphenylenes in two *Salmonella typhimurium* strains TA98 and YG1024 in the absence of S9 mix. The strain YG1024 that exhibits the enhanced *O*-acetyltransferase activity is extremely sensitive to nitroarenes and aromatic amines (*25*). We determined the activity of 1- and 2-nitrotriphenylenes in strain YG1024 for the first time. The activities of 1- and 2-nitrotriphenylenes in strain TA98 determined in this study were 18 and 3800 revertants/nmol, respectively, and these values are lower than those reported by Greibrokk et al. (*19*). The mutagenicity of 2-nitrotriphenylene was over 2 orders of magnitude greater than that of 1-nitrotriphenylene in both strains TA98 and YG1024. 1-Nitropyrene has always been analyzed when the direct mutagenic activity of airborne particulate samples was determined because it is a well-known nitroarene in airborne particulates. The greater mutagenic activity and higher concentration of 2-nitrotriphenylene in the airborne particulate samples than those of 1-nitropyrene clearly indicate that 2-nitrotriphenylene is an important contributor to the total mutagenic activity of the sample of airborne particulate matter.

Formation of Nitrotriphenylenes by Laboratory Nitration of Triphenylene. To understand the atmospheric formation of nitrotriphenylene, a portion of 100 μg of triphenylene deposited on a filter was exposed to 10 ppm NO_2 in the dark with O_3 or with light irradiation for 1−30 h. Figure 6 shows the amounts of nitrotriphenylenes in the reaction products and the remaining triphenylene plotted against reaction time. The reaction of triphenylene with NO_2 in the dark without O_3 did not occur. When triphenylene was exposed to NO_2-O_3 in the dark (Figure 6(a)), 1- and 2-nitrotriphenylenes were immediately formed, and their amount increased with the decrease in the amount of remaining triphenylene. After the reaction for 8 h, nine kinds of dinitrotriphenylenes were also formed probably due to sequential nitration of nitrotriphenylenes. At the reaction time of 19 h, the maximum yields of 1- and 2-nitrotriphenylenes (9% and 15%, respectively) were attained, and 78% of triphenylene was degraded. The amounts of dinitrotriphenylenes increased gradually until 24 h and then slowly decreased. The maximum total yield reached 14%. On the other hand, in the photoreaction (Figure 6(b)), the amount of 2-nitrotriphenylene was small with a yield of up to 2% and seemed to be constant after the reaction for 8 h, although the degradation profile of triphenylene was almost the same in both the dark reaction and the photoreaction. 1-Nitrotriphenylene and dinitrotriphenylenes were not formed in any significant amounts under light irradiation. It is clearly shown that the nitration of triphenylene occurs more actively in the presence O_3 in the dark than in the absence of O_3 with light irradiation.

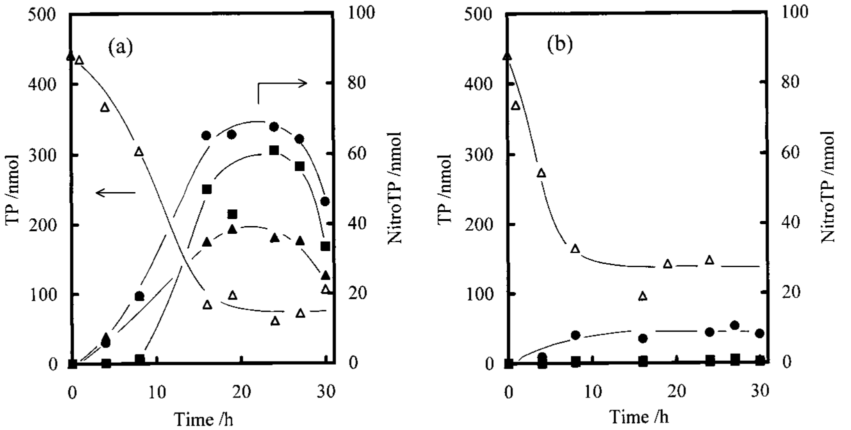

FIGURE 6. Formation of nitrotriphenylene (nitro TP) by the exposure of 100 μg of triphenylene (TP) to 10 ppm NO_2: (a) dark reaction (with O_3) and (b) photoreaction (▲) 1-nitrotriphenylene; (●) 2-nitrotriphenylene; (■) dinitrotriphenylenes; and (△) triphenylene. Dinitrotriphenylenes were estimated by using authentic 1-nitrotriphenylene on the assumption that molar sensitivity of all dinitrotriphenylenes for GC/NPD is consistent with that of monotriphenylenes.

The nitration of PAH in the presence of O_3 in the dark occurs mostly in the nighttime because the NO_3 radical and N_2O_5 are formed at night in polluted ambient air from the reaction sequence

$$NO_2 + O_3 \rightarrow NO_3 + O_2 \quad (1)$$

$$NO_3 + NO_2 \rightleftarrows N_2O_5 \quad (2)$$

Under our experimental conditions, N_2O_5 and the NO_3 radical are most probably the reactive species because N_2O_5, NO_3 radical, and NO_2 are in equilibrium (*34*). In the reaction of gas-phase PAH such as fluoranthene and naphthalene, an addition−elimination sequence involving the NO_3 radical species has been proposed (*5*). We reported that 3-nitrobenzanthrone is a new class of powerful direct-acting mutagens of atmospheric origin and would be formed by the reaction of benzanthrone in NO_2−O_3 in the atmospheric environment (*18*). From the result of the variation in isomer distribution on the conditions such as polarity of the organic solvent and the temperature employed, the reaction of benzanthrone in NO_2−O_3 would take place via the competition between the homolytic process involving the NO_3 radical and the heterolytic process involving NO_2^+ ion (*35*). We think that the nitrotriphenylene formation by the dark reaction with O_3 is also different from the reaction involving the NO_3 radical which is proposed in the reaction of gas-phase PAH.

Under photolysis conditions also, the NO_3 radical and N_2O_5 are formed since O_3 is formed from the following reactions

$$NO_2 + h\nu \rightarrow NO + O \quad (3)$$

$$O + O_2 + M \rightarrow O_3 + M \quad (4)$$

Furthermore, it is also possible that HONO will be generated from such a high NO_2 concentration and could photolyze, producing OH radicals. The formation of nitro-PAH from the OH radical-initiated reactions of gas-phase PAH has been well documented (*5*). The photoreaction of triphenylene in our experimental system showed high positional selectivity at the 2-position, though the amount of formed 2-nitrotriphenylene was small. These results indicate that a different nitration mechanism is operative under dark conditions with NO_2 and O_3 and under photolysis conditions in our systems since the usual nitration of triphenylene with HNO_3 in acetic anhydride gave a small excess of a nitro substituted product at the 1-position which is more susceptible to attack by an electrophile such as NO_2^+ ion (*20, 21*).

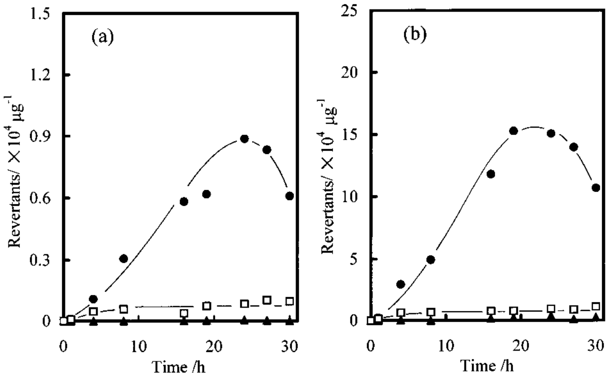

FIGURE 7. Mutagenic activities of the reaction products by the exposure of 100 μg of triphenylene to 10 ppm NO_2 as a function of reaction time. The mutagenicity tests were performed on strains TA98 (a) and YG1024 (b) in the absence S9 mix: (●) dark reaction (with O_3); (▲) dark reaction (without O_3); and (□) photoreaction.

Mutagenic Activity of Nitration Products of Triphenylene. Dinitrotriphenylenes as well as mononitrotriphenylenes were formed by the dark reaction of triphenylene with NO_2 in the presence of O_3 in our study. Consequently, the contribution of products such as dinitrotriphenylenes other than mutagenic 2-nitrotriphenylene to the mutagenic activities of the whole reaction products should be understood. In Figure 7, the change with time of mutagenic activities of the reaction products is shown for two *Salmonella typhimurium* strains TA98 and YG1024 in the absence of S9 mix. The change with time of mutagenic activities of the reaction products for strain TA98 (a) and YG1024 (b) was similar and reached the maximum at 24 h in agreement with the change with time of the total nitrotriphenylene amount as shown in Figure 6. The mutagenic activities for strain YG1024 were about 15 times higher than those for strain TA98. This also agrees that main mutagenic products were nitrotriphenylenes since YG1024 is more sensitive to nitroarenes than TA98. While the mutagenic activities of the products in the dark reaction under NO_2 in the absence of O_3 was a little low, those in the presence of O_3 increased linearly until the reaction time of 24 h and slowly decreased with an increase in exposure time. The mutagenic activities of the photoreaction products were lower than those of the dark reaction products in the presence of O_3 for both strains and reached a constant value after increasing linearly at 1−8 h.

To separate some direct-acting mutagens formed by the dark reaction in the presence of O_3 and photoreaction of triphenylene with NO_2 for 24 h, samples extracted from the

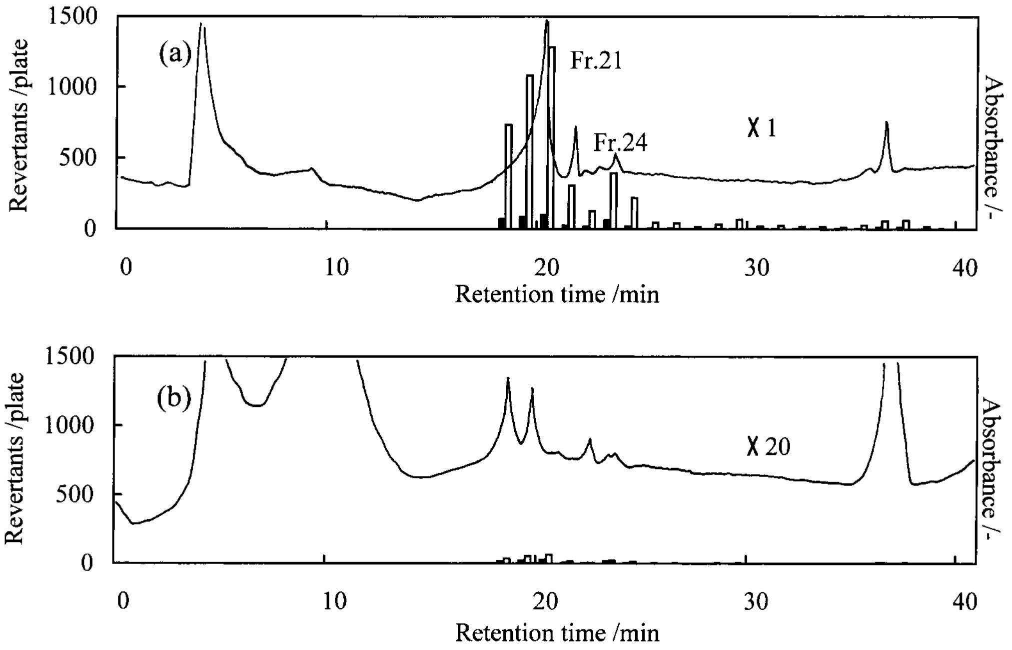

FIGURE 8. Preparative HPLC separation of the reaction products and mutagenic activities of each HPLC fraction tested with TA98 and YG 1024 in the absence of S9 mix: (a) dark reaction (with O_3) 24 h and (b) photoreaction 24 h (■) TA98 and (□) YG1024.

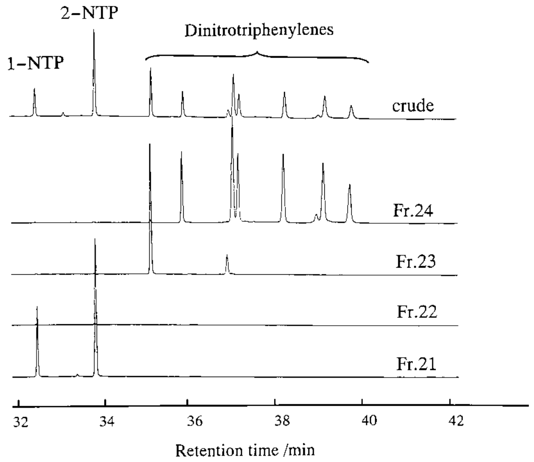

FIGURE 9. Gas chromatograms of the crude sample and HPLC fractions from 21 to 24 in the dark reaction product in the presence of O_3 for 24 h.

reaction products were fractionated by preparative HPLC. All of the effluents were assayed for mutagenic activities for *Salmonella typhimurium* strains TA98 and YG1024 in the absence of S9 mix. Fr.18−23 and Fr.25, which correspond to the range of 17−22 and 24 min in the retention time in chromatogram of the dark reaction products in the presence of O_3, showed high mutagenic activity as shown in Figure 8(a). Fr.21 was the most active fraction both in the dark reaction and the photoreaction.

Figure 9 shows the gas chromatograms of the crude sample and Fr.21−24 of the products by the reaction in NO_2−O_3 for 24 h. The 12 peaks were observed in the gas chromatograms of the crude sample. The compounds corresponding to two peaks in the most active Fr.21 were identified as 1- and 2-nitrotriphenylenes on the basis of retention time and the molecular ion together with the characteristic fragment ions of authentic 1- and 2-nitrotriphenylenes. The compounds corresponding to the nine peaks in relatively slightly active Fr.23 and highly active Fr.24 were identified as dinitrotriphenylenes by detecting the molecular ion, m/z 318 ($[M^+]$), together with the characteristic fragment ions, m/z 288 ($[M - NO]^+$), 276 ($[M - NO_2]^+$), and 275 ($[M - HNO_2]^+$).

These results indicate that 2-nitrotriphenylene can be largely attributed to the mutagenic activity of the dark reaction products in the presence of O_3 and dinitrotriphenylenes are not. Additionally also in the photoreaction, 2-nitrotriphenylene would be largely attributed to the mutagenic activity of the reaction products because Fr.21, which contains mononitrotriphenylenes, was the most active fraction.

The heterogeneous reactions studied herein indicate the possibility for atmospheric formation of mutagenic 2-nitrotriphenylene during the daytime and nighttime. The higher concentration of nitrotriphenylenes in the nighttime samples than in the daytime ones can be attributed to the effective formation of nitrotriphenylenes by the dark reaction of triphenylene with NO_2 and O_3. It is presumed impossible to extrapolate the laboratory nitration of triphenylene with ppm concentrations of reactants to the ambient conditions, since ambient NO_2 and O_3 concentrations in Tokyo Minato-ku were an average of 0.046 and 0.011 ppm, respectively, in December, 1996. However, the heterogeneous reactions studied herein should be regarded as an important process for atmospheric formation of mutagenic 2-nitrotriphenylene.

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