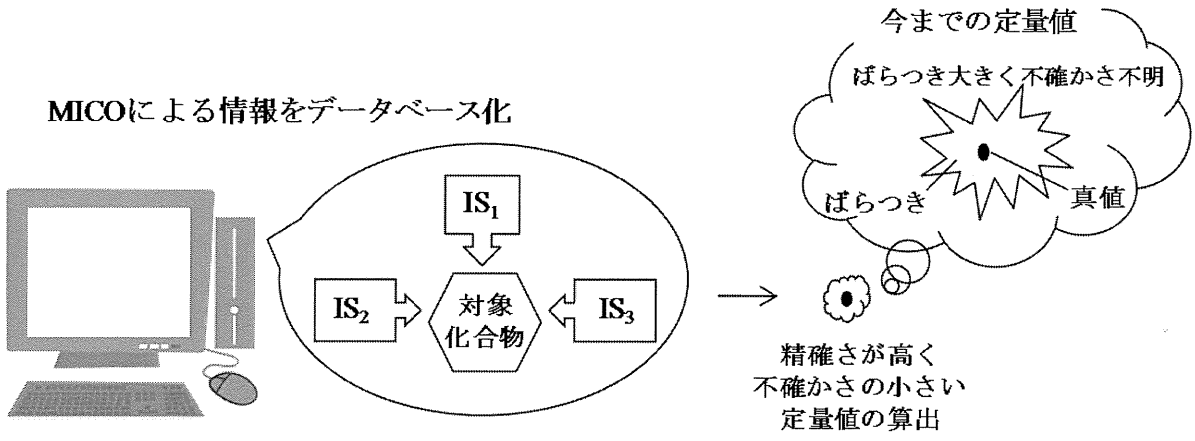

Fig. 5　MICO (Multi Internal standards Calibration Objective)の概念図

Table 1　PAHs 18 種混合標準液および試料調製における各濃度

No.	化合物	原液濃度	0.1%	0.2%	0.5%	1.0%	1.5%	2.0%
1	Naphthalene	100	0.1	0.2	0.5	1	1.5	2
2	1-Methylnaphthalene	100	0.1	0.2	0.5	1	1.5	2
3	2-Methylnaphthalene	100	0.1	0.2	0.5	1	1.5	2
4	Acenaphthylene	100	0.1	0.2	0.5	1	1.5	2
5	Acenaphthene	100	0.1	0.2	0.5	1	1.5	2
6	Fluorene	100	0.1	0.2	0.5	1	1.5	2
7	Phenanthrene	100	0.1	0.2	0.5	1	1.5	2
8	Anthracene	100	0.1	0.2	0.5	1	1.5	2
9	Fluoranthene	10	0.01	0.02	0.05	0.1	0.15	0.2
10	Pyrene	10	0.01	0.02	0.05	0.1	0.15	0.2
11	Benzo(a)anthracene	10	0.01	0.02	0.05	0.1	0.15	0.2
12	Chrysene	10	0.01	0.02	0.05	0.1	0.15	0.2
13	Benzo(b)fluoranthene	10	0.01	0.02	0.05	0.1	0.15	0.2
14	Benzo(k)fluoranthene	5	0.005	0.01	0.025	0.05	0.075	0.1
15	Benzo(a)pyrene	10	0.01	0.02	0.05	0.1	0.15	0.2
16	Indeno(1,2,3-cd)pyrene	10	0.01	0.02	0.05	0.1	0.15	0.2
17	Dibenzo(a,h)anthracene	10	0.01	0.02	0.05	0.1	0.15	0.2
18	Benzo(g,h,i)perylene	10	0.01	0.02	0.05	0.1	0.15	0.2

Table 2　PAHs 18 種および IS の定量イオンと保持時間

No.	化合物	定量イオン (*m/z*)	保持時間 (min)
1	Naphthalene	128	5.320
2	1-Methylnaphthalene	142	5.935
3	2-Methylnaphthalene	142	6.030
4	Acenaphthylene	152	6.920
5	Acenaphthene	153	7.135
6	Fluorene	166	7.930
7	Phenanthrene	178	10.230
8	Anthracene	178	10.395
9	Fluoranthene	202	13.780
10	Pyrene	202	14.345
11	Benzo(a)anthracene	228	17.425
12	Chrysene	228	17.530
13	Benzo(b)fluoranthene	252	20.655
14	Benzo(k)fluoranthene	252	20.750
15	Benzo(a)pyrene	252	21.700
16	Indeno(1,2,3-cd)pyrene	276	25.200
17	Dibenzo(a,h)anthracene	278	25.290
18	Benzo(g,h,i)perylene	276	26.025
IS-1	BTMSB	211	5.785
IS-2	DEP	149	7.665
IS-3	Bisphenol-A	213	14.580

Table 3　定量値の新規算出方法

	概要	検量線本数	計算式	得られる定量値
a	絶対検量線法	1	P	a
b	通常の内標準法（IS一つでの比）	1	P/AI or P/BI or P/CI	b1, b2, b3
c	IS三種それぞれにより比を算出し、 この3つの値を平均した後、検量線を作成	1	Ave (P/AI, P/BI, P/CI)	c
d	IS三種それぞれにより検量線を作成し、 得られた3つの定量値を平均	3	Ave (b1, b2, b3)	d
e	cに対し、IS三種それぞれで算出されるPAHsおよびIS それぞれの*m/z*における強度比の平均で補正	1	d×Ave[(PAHsTc/PAHsTs$_1$)/(ATc/ATs$_1$), (PTc/PTs$_1$)/(BTc/BTs$_1$), (PTc/PTs$_1$)/(CTc/CTs$_1$)]	e

* P: PAHsのピーク面積値, A, B, C: IS三種, I: ISのピーク面積値
Tc: 検量線を作成した際のチューニング強度式から得られたPAHsおよびISの*m/z*における強度値の平均
Ts$_1$, Ts$_2$, Ts$_3$, Ts$_{4,}$ Ts$_5$: 試料測定日のチューニング強度式から得られたPAHsおよびISの*m/z*における強度値

Ⅲ．研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kameda, T., Akiyama, A., Yoshita, M., Tachikawa, C., Toriba, A., Tang, N., Hayakawa, K.,	Mutagenicities and endocrine-disrupting activities of 1-hydroxy-2-nitropyrene and 1-hydroxy-5-nitropyrene.	Journal of Health Science	57 (4)	372-377	2011
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Tang, N., Tokuda, T., Izzaki, A., Tamura, K., Ji, R., Zhang, X., Dong, L., Kameda, T., Toriba, A., Hayakawa, K.	Recent change in atmospheric polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (NPAHs) in Shenyang, China.	Environ. Forensics	12	342-348	2011
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Ⅳ. 研究成果の刊行物・別刷

Journal of Health Science, **57**(4) 372–377 (2011)

— **Rapid Communication** —

Mutagenicities and Endocrine-disrupting Activities of 1-Hydroxy-2-nitropyrene and 1-Hydroxy-5-nitropyrene

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The mutagenicities and endocrine-disrupting activities of two isomers of mononitrated 1-hydroxypyrene [1-hydroxy-*x*-nitropyrenes (1-OH-*x*-NPs); *x* = 2 and 5], which are not only photoreaction products of 1-nitropyrene (1-NP) but also constituent of ambient airborne particles, were evaluated for the first time using the Ames plate incorporation assay and the yeast two-hybrid assay, respectively. The mutagenicity of 1-OH-5-NP was weakly positive in the absence of rat liver S9, but was enhanced up to 3-fold with the metabolic activation by S9. On the contrary, 1-OH-2-NP did not exhibit significant mutagenicity in the presence or absence of S9. 1-OH-5-NP showed weak estrogenic activity, but 1-OH-2-NP did not show any estrogenic activity. The concentration of 1-OH-5-NP that gave 10% of activity of 1.0×10^{-6} M 17β-estradiol (E_2) was 5.4×10^{-7} M. 1-OH-5-NP exhibited stronger antiestrogenic and antiandrogenic activities than 1-OH-2-NP. 1-OH-5-NP at a concentration of 1.0×10^{-6} M inhibited 71 and 90% of β-galactosidase activity induced by 1.0×10^{-9} M of E_2 and 1.0×10^{-8} M of 5α-dihydrotestosterone (DHT), respectively. On the other hand, 1.0×10^{-6} M of 1-OH-2-NP inhibited 16 and 43% of β-galactosidase activity induced by 1.0×10^{-9} M of E_2 and 1.0×10^{-8} M of DHT, respectively. These findings point out the need for determining the environmental sources and distribution of 1-OH-2-NP and 1-OH-5-NP as well as the other hydroxynitropyrene isomers.

Key words——nitropyrene, nitropyrenol, polycyclic aromatic hydrocarbon, mutagen, endocrine disruptor

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INTRODUCTION

Numerous polycyclic aromatic hydrocarbons (PAHs) have been detected in organic extracts of airborne particles, and concerns are rising that they may affect human health through their mutagenic and carcinogenic effects. Nitrated polycyclic aromatic hydrocarbons (NPAHs) are also a class of mutagens/carcinogens found in the atmosphere, and some of them exhibit stronger mutagenicity/carcinogenicity than their parent PAHs.[1] 1-Nitropyrene (1-NP) is believed to be emitted into the atmosphere from combustion processes of fossil fuel such as diesel fuel[2] and is one of the most abundant NPAHs in the atmosphere.[3] 1-NP taken up by humans and animals is metabolized to hydroxynitropyrenes (OHNPs; Fig. 1), 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) in the presence of cytochrome P450 enzymes.[4,5] We recently found that these OHNP isomers were also produced from a photoreaction of 1-NP in the atmosphere as well as the other isomers, 1-hydroxy-2-nitropyrene (1-OH-2-NP) and 1-hydroxy-5-nitropyrene (1-OH-5-NP).[6] Several groups reported that 1-OH-3-NP, 1-OH-6-NP, and 1-OH-8-NP are weakly muta-

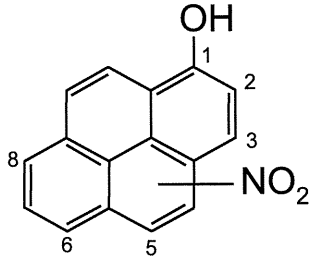

Fig. 1. Structure of OHNP

genic,[7–10] but the mutagenicities of 1-OH-2-NP and 1-OH-5-NP are unknown.

Recently several kinds of PAH derivatives have been found to act as endocrine disruptors which may cause the dysfunction of human and wildlife endocrine systems, abnormal development of reproductive systems, and immunodeficiencies. For example, several monohydroxylated derivatives of PAHs (OHPAHs) have significant estrogenic/antiestrogenic activities, as shown by a reporter gene assay[11] and by a yeast two-hybrid assay system based on the ligand-dependent interaction of the estrogen receptor (ER) and its co-activator.[12] Furthermore, mono- and dihydroxy metabolites of PAHs appear to act as antiandrogenic chemicals, as shown by a reporter gene assay based on Chinese hamster ovary (CHO) cells transiently cotransfected with a human androgen receptor (hAR) vector and an MMTV-LUC vector.[13] We have also found that 1-OH-3-NP, 1-OH-6-NP, and 1-OH-8-NP show significant estrogenic, antiestrogenic, and antiandrogenic activities in the yeast two-hybrid assay system.[14] These results imply that 1-OH-2-NP and 1-OH-5-NP, whose structures are similar to those of OHPAHs and other OHNP isomers, also exhibit endocrine-disrupting activities.

In this study, theirfore, we first examined the mutagenicities and endocrine-disrupting activities of 1-OH-2-NP and 1-OH-5-NP. For these analyses, we used the Ames plate incorporation assay and the yeast two-hybrid assay, respectively.

MATERIALS AND METHODS

Synthesis of OHNPs —— 1-OH-2-NP was synthesized by nitration of 1-hydroxypyrene (OHPy) by 4-nitro-4-methyl-2,3,5,6-tetrabromo-2,5-cyclohexadien-1-one in diethyl ether at room temperature for 2 hr according to the literatures.[6, 15, 16] 1-OH-5-NP was obtained by a photoreaction of 1-NP according to the previous report.[6] Each OHNP isomer was purified by preparative normal phase HPLC (SUPELCO, St. Louis, MO, U.S.A.; Supelcosil PLC-SI, 21.2 mm ID × 25 mm, eluted with CH_2Cl_2 containing 0.5 mM CH_3COOH at 10 ml/min). To identify the synthetic compounds, their EI-MS and ^1H NMR analyses were performed.

Chemicals —— 4-Nitro-4-methyl-2,3,5,6-tetrabromo-2,5-cyclohexadien-1-one was purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). 1-NP and OHPy were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). 17β-Estradiol (E_2) and 5α-dihydrotestosterone (DHT) were purchased from Wako Pure Chemicals (Osaka, Japan). 4-Hydroxytamoxifen (4-OHT) and hydroxyflutamide (OHFl) were obtained from Sigma-Aldrich and Toronto Research Chemical Inc. (North York, Canada), respectively. Test compounds were dissolved in ethanol and stored at −20°C until use. All other chemicals were of the highest quality available from commercial sources.

Mutagenicity Assay —— Mutagenic activities of OHNPs were assayed with *Salmonella typhimurium* strains TA98 and TA100 according to the method developed by Maron and Ames[17] including a slight modification of preincubation[18] in the presence or absence of S9 mix.

Yeast Two-hybrid Assay —— Estrogenic, antiestrogenic, androgenic, and antiandrogenic activities of OHNPs were evaluated with the yeast two-hybrid assay following Nishikawa's method with some modifications.[12, 19, 20] Briefly, yeast cells (*Saccharomyces cerevisiae* Y190) expressing human estrogen receptor (hERα) and hAR or two-hybrid system control yeast cells (*Saccharomyces cerevisiae* Y190 transfected with the pGBK7-53 and pGADT7-T) were grown overnight at 30°C with shaking in synthetic defined medium free from tryptophan and leucine, and treated with each test compound at 30°C for 4 hr. After the incubation, the treated cells were collected and enzymatically digested with 1 mg/ml Zymolyase 20T at 37°C for 30 min. 2-Nitrophenyl-β-D-galactoside was added to the lysate to a final concentration of 4 mg/ml. After incubation at 30°C for 45 min, the reaction was terminated by the addition of 1 M Na_2CO_3. The yeast debris was removed by centrifugation and the absorbance of supernatant was measured at 415 nm. Estrogenic activity was evaluated by the 10% relative effective concentration (REC_{10}), which is defined as the concentration of the test compounds showing 10% of the highest β-galactosidase activity of E_2. Antiestrogenic and antiandrogenic activities were evaluated by IC_{20}, which is the concentration of the test compounds that inhibit 20% of β-galactosidase activity induced by 1.0×10^{-9} M E_2 and 1.0×10^{-8} M DHT, respectively.

RESULTS AND DISCUSSION

Table 1 shows the mutagenicities of OHNPs ob-

Table 1. Specific Mutagenicities of OHNPs and 1-NP, and Relative Mutagenicities of OHNPs to 1-NP Evaluated by Ames Assay

Compound	TA98		TA100	
	−S9	+S9	−S9	+S9
Specific mutagenicity [a]				
1-OH-2-NP	28	46	118	164
1-OH-5-NP	73	255	209	589
1-NP	1605	165	539	236
DMSO	22	29	122	127
Relative mutagenicity to 1-NP [b]				
1-OH-2-NP [a]	0.02	0.3	0.2	0.7
1-OH-5-NP [a]	0.05	1.5	0.4	2.5
1-OH-3-NP	0.3[c]	0.6[c]	1.4[d]	0.8[d]
1-OH-6-NP	0.06[c]	1.5[c]	2.5[d]	2.4[d]
1-OH-8-NP	0.09[c]	0.3[c]	—[e]	—[e]

DMSO: used as a negative control. *a*) This study. Specific mutagenicity, expressed as revertants/40 nmol-test compound, was calculated by least squares linear regression from linear portion of dose-response curve. *b*) Calculated based on the specific mutagenicities. *c*) Obtained from reference 9. *d*) Obtained from reference 10. *e*) Not available in reference 10.

Table 2. REC_{10} and IC_{20} Values for OHNPs and Reference Chemicals in Yeast Two-hybrid Assay

Compound	REC_{10}[c]	IC_{20}[e]	
	Estrogenic activity	Antiestrogenic activity	Antiandrogenic activity
1-OH-2-NP [a]	—[d]	1.3×10^{-6}	3.7×10^{-7}
1-OH-5-NP [a]	5.4×10^{-7}	2.5×10^{-7}	3.2×10^{-8}
1-OH-3-NP [b]	6.0×10^{-7}	1.1×10^{-6}	2.3×10^{-7}
1-OH-6-NP [b]	6.0×10^{-8}	1.0×10^{-6}	3.1×10^{-7}
1-OH-8-NP [b]	9.0×10^{-7}	7.0×10^{-7}	5.1×10^{-8}
E_2 [a]	6.0×10^{-11}		
4-OHT [a]		5.3×10^{-6}	
OHFl [a]			5.3×10^{-6}

a) This study. *b*) Taken from reference 14. *c*) Concentration of the test compounds showing 10% of the highest β-galactosidase activity of E_2. *d*) Significant induction of β-galactosidase activity was not observed at concentrations between 1.0×10^{-8} and 1.0×10^{-6} M. *e*) Concentration of the test compounds that inhibit 20% of β-galactosidase activity induced by 10^{-9} M E_2 or 10^{-8} M DHT.

tained in this study. 1-OH-2-NP was not mutagenically active in either TA98 or TA100 with or without metabolic activation by S9. The mutagenicity of 1-OH-5-NP in the TA98 and TA100 strains was weakly positive in the absence of S9, *i.e.*, the numbers of revertants were 2–3-fold greater than the number of spontaneous revertants in the negative control [dimethyl sulfoxide (DMSO)]. On the other hand, the mutagenic activities of 1-OH-5-NP in both the strains were enhanced approximately 3-fold by the addition of S9. The mutagenicities of 1-OH-5-NP relative to the mutagenicity of 1-NP are given in Table 1 together with those of the other OHNP isomers. With S9 activation, they were 1.5 and 2.5 in TA98 and TA100, respectively, *i.e.*, the mutagenicity of 1-OH-5-NP was higher than that of 1-NP, as was the case of 1-OH-6-NP in the presence of S9. These results are consistent with previous findings that some OHNP isomers need metabolic activation to exhibit mutagenicity.[8, 9, 21]

Figure 2 shows the estrogenic activities of E_2 and the two isomers of OHNP. β-Galactosidase activity increased with increasing E_2 concentration, reaching a plateau at 1.0×10^{-6} M. A significant induction of β-galactosidase activity was also observed for 1-OH-5-NP ($REC_{10} = 5.4 \times 10^{-7}$ M), but no activity was observed for 1-OH-2-NP at concentrations between 1.0×10^{-8} and 1.0×10^{-6} M. The REC_{10} and IC_{20} values for the OHNP isomers obtained in this study and for the reference chemicals

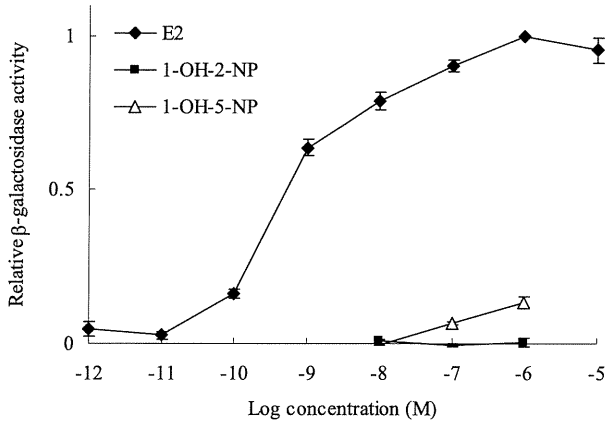

Fig. 2. Dose Response Curves of Estrogenic Activity of E_2 and 1-OH-x-NPs (x = 2 and 5) in a Yeast Two-hybrid Assay System

Each data point is the mean ± S.D. (n = 3).

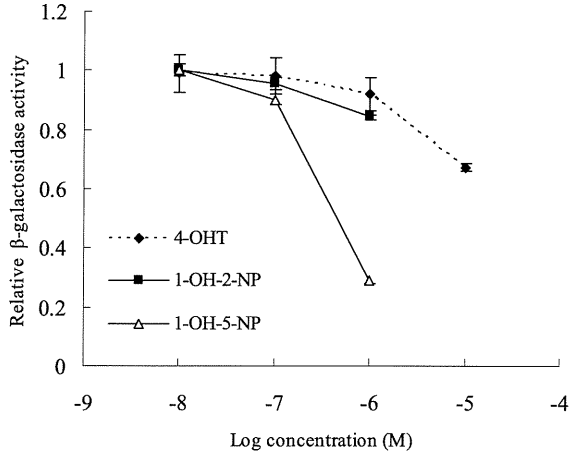

Fig. 3. Antiestrogenic Activity of 4-OHT and 1-OH-x-NPs (x = 2 and 5) against the Estrogenic Activity of E_2 in a Yeast Two-hybrid Assay System

Antiestrogenic activities of 4-OHT and 1-OH-x-NPs were expressed as β-galactosidase activity relative to the level induced by 1.0×10^{-9} M E_2. Each data point is the mean ± S.D. (n = 3).

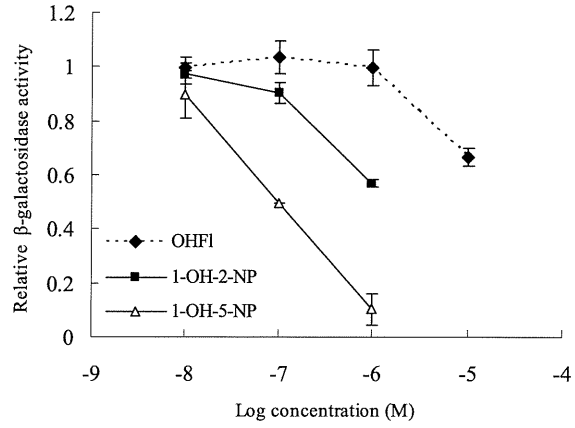

Fig. 4. Antiandrogenic Activity of OHFl and 1-OH-x-NPs (x = 2 and 5) against the Androgenic Activity of DHT in a Yeast Two-hybrid Assay System

Antiandrogenic activities of OHFl and 1-OH-x-NPs were expressed as β-galactosidase activity relative to the level induced by 1.0×10^{-8} M DHT. Each data point is the mean ± S.D. (n = 3).

are summarized in Table 2. The estrogenic activity of 1-OH-5-NP was lower than that of 1-OH-6-NP, but higher than the estrogenic activities of the other OHNP isomers previously reported[14] or bisphenol A (REC_{10} = 3×10^{-6} M),[12] a known estrogenic compound. Figure 3 shows the antiestrogenic activities of the tested OHNPs in the concentration range from 1.0×10^{-8} to 1.0×10^{-6} M. To obtain these data, we used an E_2 concentration of 1.0×10^{-9} M, which induced about 50% of the maximum β-galactosidase activity. At a concentration of 1.0×10^{-6} M, each of the OHNP isomers decreased the induction of β-galactosidase activity by E_2. 1-OH-2-NP and 1-OH-5-NP showed 4 and 21 times higher antiestrogenic activity, respectively, than 4-OHT, a typical ER antagonist (Table 2). Figure 4 shows the results of the antiandrogenic activities for the tested OHNPs. In the presence of OHNPs at concentrations between 1.0×10^{-8} and 1.0×10^{-6} M, the activity of 1.0×10^{-8} M DHT, which induced about 50% of the highest β-galactosidase activity of DHT, was inhibited concentration-dependently. The highest inhibitory effect among the five OHNP isomers was observed with 1-OH-5-NP as was the case with antiestrogenic activity. The antiandrogenic activities of 1-OH-2-NP and 1-OH-5-NP were 14 and 166 times higher than the antiandrogenic activity of OHFl (Table 2). At concentrations less than 1.0×10^{-6} M, neither OHNP isomer was cytotoxic to the control yeast cells, which supports the idea that the decreases of β-galactosidase induction observed in this study were due to antiestrogenic/antiandrogenic effects rather than cytotoxic effects. Neither 1-OH-2-NP nor 1-OH-5-NP showed androgenic activity at concentrations between 1.0×10^{-8} and 1.0×10^{-6} M. OHPAHs having four aromatic rings, such as hydroxybenz[a]anthracenes, hydroxychrysenes, and hydroxybenzo[c]phenanthrenes, were shown to have strong endocrine-disrupting activities.[20] In addition, it was reported that the four rings and a phenolic hydroxyl group needed to be in a rectangular plane in order for OHPAHs to bind to the site of the receptor.[20] The OHNP isomers have the same planar structure, which could account for their endocrine-disrupting activities.

Because of the significant biological effects of OHNPs, further studies of their environmental sources, sinks, and distributions are needed to better assess their risks.

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Short communication

Hydrogen peroxide–sodium hydrosulfite chemiluminescence system combined with high-performance liquid chromatography for determination of 1-hydroxypyrene in airborne particulates

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ABSTRACT

In this research, a highly sensitive chemiluminescence method based on a sodium hydrosulfite ($NaHSO_3$)–hydrogen peroxide (H_2O_2) reaction for the determination of 1-hydroxypyrene (1-OHP) was developed. The response of this system was linear in the range from 0.5 to 50 pmol ($R^2 = 0.9983$). The limit of detection for 1-OHP was 100 fmol ($S/N = 3$). 1-OHP in airborne particulates was well separated from interfering compounds using an ODS column with 75% methanol as the mobile phase in isocratic mode. The proposed method was successfully applied to determine the 1-OHP in airborne particulates collected in Kanazawa, Japan. The average concentration of 1-OHP in the atmosphere was 2.0 pg/m^3 (9.2 fmol/m^3).

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants [1,2] that are generated from incomplete combustion of organic matters such as fuel oil and coal [3,4]. In the atmosphere, PAHs react with the co-pollutants, such as ozone and nitrogen oxides (NO_x) to produce substituted PAHs [5,6]. One type of substituted PAHs is hydroxylated PAHs (OH-PAHs). They may be generated from photochemical reactions of PAHs or combustion of coal [7–9]. OH-PAHs have endocrine disrupting activities such as estrogenic activities or antiestrogenic activities [10,11]. Atmospheric concentrations of OH-PAHs were reported to be in the pg/m^3 range, which are 1–3 orders of magnitude lower than that of PAHs [8,9]. Therefore, it is necessary to establish a highly sensitive and reliable method for determination of OH-PAHs in atmosphere [12].

Gas chromatography/mass spectrometry (GC–MS) is one of the most commonly used methods in detecting OH-PAHs in the atmosphere [7–9,13,14]. A disadvantage of GC–MS is that it requires a derivatization step before analysis, which is time-consuming, and increases the risk of contamination or loss of OH-PAHs [15]. Another method for determining OH-PAHs is high-performance liquid chromatography (HPLC) with electrochemical detection [16], but the sensitivity was too low to detect OH-PAHs in the atmosphere. Kishikawa et al. determined six kinds of OH-PAHs by HPLC with a fluorescence detector using the internal standard method [15]. However, interference peaks were found in the chromatogram, which may affect the accuracy of the method.

In this study, we developed a highly sensitive and simple chemiluminescence (CL) method to determine 1-OHP in the airborne particulates for the first time. Airborne particulate sample might contain many interfering compounds. Therefore, an ODS separation column was connected to the system to separate 1-hydroxypyrene from interfering compounds. The method avoids the time-consuming derivatization step required by GC–MS method [14]. Moreover, the method was simple and convenient. The possible mechanism of this CL reaction was proposed. We used the method to determine 1-OHP in airborne particulates in Kanazawa city in Japan.

2. Experimental

2.1. Reagents and chemicals

Sodium hydrosulfite ($NaHSO_3$) and hydrogen peroxide (H_2O_2) were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan).

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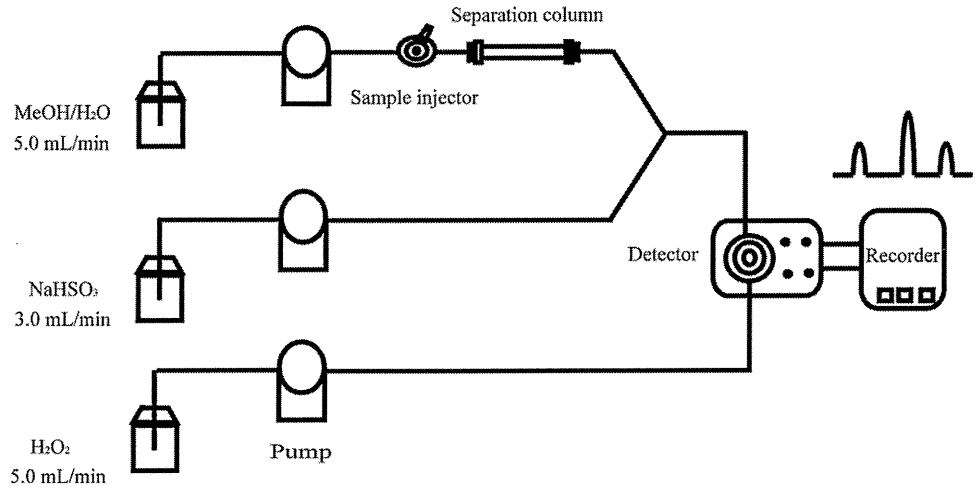

Fig. 1. Schematic diagram of the $NaHSO_3$–H_2O_2 CL system to detect 1-OHP.

$NaHSO_3$ solution was freshly prepared by dissolving appropriate amounts of $NaHSO_3$ powder in ultrapure water. H_2O_2 solution was freshly prepared by volumetric dilution of commercial 30% H_2O_2 solution. 1-OHP was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Solution of 1-OHP was prepared by dissolving powder with methanol. Standard solution of 1-OHP was diluted with 75% methanol. All the organic solvents (methanol, hexane, dichloromethane, diethyl ether, benzene, and ethanol) were HPLC grade and from Wako Pure Chemical Industries, Ltd. Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Sample collection

Airborne particulates were collected on the roof of a seven-storey building of Kanazawa University (Kakuma-machi) in Kanazawa, Japan. Sampling was conducted in August, 2010 using a high-volume air sampler (Model 120, Kimoto Electrics, Osaka, Japan) with a 2500 QAT-UP quartz fiber filter (8″ × 10″, Pallflex Products, Putnam, CT, USA) for 24 h at a flow rate of 1000 L/min (total volume 1434.9 m^3). The filters with airborne particulates were stored in a freezer at −20 °C until analysis.

2.3. Sample preparation

The whole sample filter was cut into small pieces and extracted ultrasonically with 100 mL benzene/ethanol mixture solvent (1:1, v/v) for 15 min twice. Both extracts were combined and filtered with a cellulose acetate filter (No. 5C, Advantec MFS, Dublin, CA, USA) to remove solid residue. Then the solution was evaporated to near dryness at 30 °C in a rotary evaporator under reduced pressure. The residues were reconstituted in 10 mL hexane (under ultrasonication for 2 min), and filtered with a 0.45 μm membrane filter.

The residues were loaded onto a Sep-Pak®Plus Silica cartridge (Waters, Milford, MA, USA) which was preconditioned with 10 mL hexane. The target compound was eluted by 10 mL hexane/ethyl acetate (7/1, v/v). The eluate was gently evaporated to near dryness under the gentle nitrogen gas stream, and then re-dissolved in 1.0 mL methanol/water mixture solvent (75/25, v/v). An aliquot of 100 μL of the sample solution was injected into the HPLC system.

2.4. HPLC-CL system

The HPLC system with a CL detector is shown in Fig. 1. This HPLC-CL system consists of three HPLC pumps (LC-10A, LC-10A, and LC-6000, Shimadzu, Tokyo, Japan), a sample injector with a 100 μL loop, a separation column (Cosmosil 5C18-AR-II; 10.0 mm i.d. 250 mm, Nacalai Tesque), and a column oven (Sugai, U-620). The temperature of the column oven was set at 30 °C. The CL detector is S-3400 (Soma Optics, Tokyo, Japan). An aliquot of 100 μL of the standard mixture or the airborne particulate sample was injected into the HPLC system, and detected directly by the CL detector.

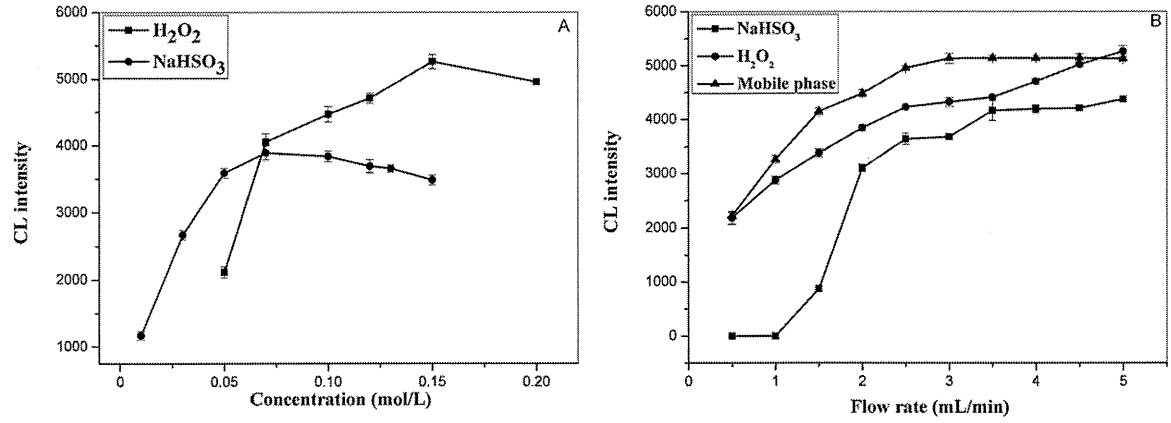

Fig. 2. Effects of concentrations and flow rates on CL intensity: (A) concentrations of $NaHSO_3$ and H_2O_2. Flow rates of $NaHSO_3$, H_2O_2 and mobile phase were 5.0, 3.0, and 5.0 mL/min, respectively; (B) flow rates of $NaHSO_3$, H_2O_2 and mobile phase. Concentrations of $NaHSO_3$ and H_2O_2 were 0.1 mol/L and 0.15 mol/L.

Table 1
Precision of the method for determining 1-OHP in airborne particulates and recovery of 1-OHP in airborne particulates.

	Intra-day ($n=3$)			Inter-day ($n=3$)		
Added (nmol/L)	0	50	100	0	50	100
Found (nmol/L)	7.0	53	110	7.0	53	110
Recovery (%)[a]	–	92%	103%	–	92%	103%
RSD (%)	9.5%	8.2%	2.4%	15.5%	11.5%	4.7%

[a] Expressed as [(found concentration – non-spiked concentration)/added concentration] × 100.

Mobile phase was methanol/water mixture (75/25, v/v) in isocratic mode at a flow rate of 5.0 mL/min.

3. Results and discussion

3.1. Optimization of HPLC-CL system

In order to obtain a high sensitivity for determination of 1-OHP, factors influencing CL intensity, such as concentration and flow rate of CL reagents were investigated independently.

We investigated the concentration of H_2O_2 in the range from 0.05 to 0.2 mol/L. CL intensity increased with H_2O_2 concentration up to 0.15 mol/L and then leveled off (Fig. 2A). In addition, too high concentration of H_2O_2 caused gas bubble to form in the tube, which would affect the stability of the CL system. Therefore, a H_2O_2 concentration of 0.15 mol/L was used for the following experiments.

The effect of $NaHSO_3$ concentration on CL intensity was studied in the range 0.01–0.15 mol/L. The CL intensity increased linearly with the increasing of $NaHSO_3$ concentration, but at concentrations of $NaHSO_3$ higher than 0.10 mol/L, the CL intensity began to decrease (Fig. 2A). Therefore, the concentration of $NaHSO_3$ solution was selected to be 0.1 mol/L for further study.

The flow rate of the CL reagent solution strongly affected sensitivity of the CL method. We investigated the flow rates of $NaHSO_3$, H_2O_2, and mobile phase from 0.5 to 5.0 mL/min. The optimized flow rates of $NaHSO_3$ and H_2O_2 were 5.0 mL/min and 3.0 mL/min (Fig. 2B). When the flow rate of the mobile phase was higher than 3.0 mL/min, the CL intensity remained constant (Fig. 2B). But the separation efficiency of the column was the highest at a flow rate of 5.0 mL/min. Therefore, the flow rate of the mobile phase was set at 5.0 mL/min.

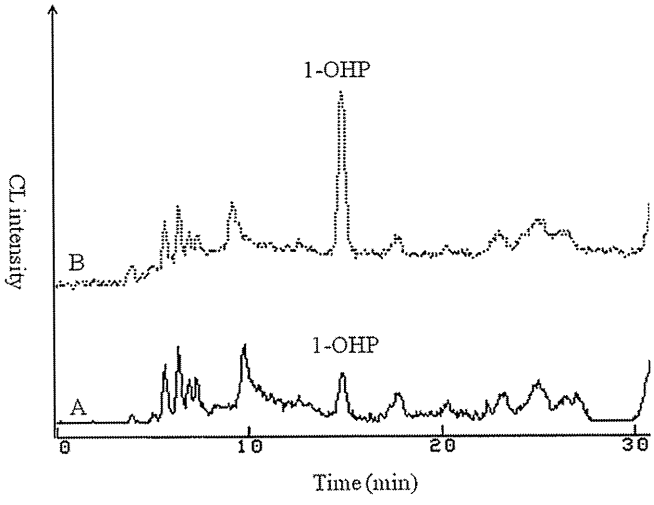

Fig. 3. Chromatograms of 1-OHP (A) in airborne particulates and (B) airborne particulates spiked with 1.0 pmol 1-OHP. Mobile phase was 75% methanol at 5.0 mL/min; concentrations of $NaHSO_3$ and H_2O_2 were 0.10 mol/L and 0.15 mol/L; their flow rates were 3.0 mL/min and 5.0 mL/min respectively.

Methanol and acetonitrile are organic solvents that are often used as the mobile phase in HPLC analyses. The effect of methanol and acetonitrile on the CL intensity was compared. Methanol gave higher sensitivity for 1-OHP and better separation. Therefore, methanol was selected for further study.

3.2. Possible mechanism of CL system

In the $NaHSO_3$–H_2O_2 CL reaction, HSO_3^- was oxidized by H_2O_2 to produce sulfite radical ($^\bullet SO_3^-$) [17], which dimerized to dithionate ion ($S_2O_6^{2-}$) [18,19].

$$HSO_3^- + H_2O_2 \rightarrow {}^\bullet OH + SO_3^{\bullet -} + H_2O \quad (1)$$

The OH radical, a strong oxidant, rapidly reacted with HSO_3^- to give $^\bullet SO_3^-$ radical [20,21].

$$^\bullet OH + HSO_3^- \rightarrow SO_3^{\bullet -} + H_2O \quad (2)$$

$$k = 1.5 \times 10^9\ M^{-1}\ s^{-1}$$

$$^\bullet SO_3^- + {}^\bullet SO_3^- \rightarrow S_2O_6^{2-} \quad (3)$$

Excited sulfur dioxide (SO_2^*) was generated by the decomposition of $S_2O_6^{2-}$ [18,19]. The emission wavelength of SO_2^* is 260–480 nm [22,23], which overlaps the excitation wavelength of 1-OHP [15]. Therefore, when 1-OHP is present in the CL system, SO_2^* will transfer its energy to 1-OHP. This greatly increased the CL emission. CL intensity was highly correlated with 1-OHP concentration. Based on this mechanism, 1-OHP could be detected sensitively through the following reactions.

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2* \quad (4)$$

$$SO_2* \rightarrow SO_2 + h\nu \quad (5)$$

$$SO_2* + \text{1-OHP} \rightarrow SO_2 + \text{1-OHP}* \quad (6)$$

$$\text{1-OHP}* \rightarrow \text{1-OHP} + h\nu \quad (7)$$

3.3. Calibration curve, detection limit and reproducibility

Under the optimized conditions, standard 1-OHP was successfully determined by the $NaHSO_3$–H_2O_2 CL system without a separation column. The calibration curve was obtained with a good linear relationship between the concentrations of 1-OHP and peak heights. The linear range was from 0.005 to 10 pmol/injection (seven calibration points; $R^2 = 0.9994$). The slope and intercept of the regression equation of calibration curve (mean ± error) were $5.77 \times 10^{11} \pm 5.63 \times 10^9$ and 635 ± 220. The detection limit ($S/N = 3$) was 1.0 fmol/injection. This CL detection method for detecting 1-OHP showed higher sensitivity than the previous methods reported [15,24]. However, 1-OHP cannot be detected exactly only with the proposed CL method due to the interference compounds in airborne particulates. An ODS column was incorporated to separate 1-OHP and the interference compounds in airborne particulates. A good linear relationship was observed between the peak height and concentrations of 1-OHP in the range from 0.5 to 50 pmol/injection (ten calibration points) with 100 μL per injection

($R^2 = 0.9983$). The slope and intercept of the regression equation (mean ± error) were $2.92 \times 10^{11} \pm 8.94 \times 10^9$ and 674 ± 65.7. The detection limit ($S/N = 3$) for 1-OHP detected by the proposed method was 100 fmol/injection.

The precision of the determination of 1-OHP and the recovery of 1-OHP were investigated by spiking standard 1-OHP solutions into the airborne particulates. The results are shown in Table 1. The relative standard deviation (RSD) of intra-day and inter-day ranged from 2.4% to 11.5%. The recoveries for the spiked airborne particulates of intra-day and inter-day were in the range 93–103%.

3.4. Determination of 1-OHP in airborne particulates

Pyrene is one of major PAHs in the atmosphere and exists in both gas and particulate phase. The vapor pressure of 1-OHP is much lower than that of pyrene because of the hydroxy group; 1-OHP may mainly exist in particulate phase. Therefore, the proposed method was applied to determine 1-OHP in airborne particulates collected in Kanazawa, Japan in August 2010. The chromatogram of real airborne particulates showed the peak at the retention time of 1-OHP and the peak height increased by the addition of 1-OHP standard solution (Fig. 3(A) and (B)). From the chromatogram, the average concentration ($n = 3$) of 1-OHP in airborne particulates in Kanazawa city was 2.0 ± 0.2 pg/m^3 (9.2 ± 0.9 fmol/m^3). Although only a few reports have examined the concentrations of 1-OHP in airborne particulates, our results were in the range of previous values. For example, the concentration of 1-OHP in Nagasaki, Japan was 5.97–63.25 pg/m^3 [15].

4. Conclusions

In this research, a highly sensitive HPLC-CL method based on $NaHSO_3$–H_2O_2 coupled with an ODS separation column to detect 1-OHP in airborne particulates was proposed. This is the first time that the $NaHSO_3$–H_2O_2 CL system reaction was combined with an HPLC system to detect air pollutants. The proposed method was successfully applied to detect trace levels of atmospheric 1-OHP in Kanazawa city in summer.

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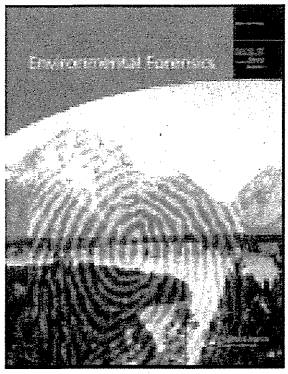

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Recent Changes in Atmospheric Polycyclic Aromatic Hydrocarbons (PAHs) and Nitropolycyclic Aromatic Hydrocarbons (NPAHs) in Shenyang, China

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