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INDUCTION OF RAT HEPATIC CYTOCHROMES P-450 BY ENVIRONMENTAL NITROPOLYCYCLIC AROMATIC HYDROCARBONS

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Abstract—Nitrated polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants that result from various incomplete combustion processes. We have examined the activity of hepatic microsomal enzymes in rats pretreated with a series of environmentally occurring nitrated PAHs including: 1- and 4-nitropyrene, 1,3-, 1,6- and 1,8-dinitropyrene, 6-nitrochrysene, 7-nitrobenz[*a*]anthracene, 3-nitrofluoranthene, and 1-, 3-, and 6-nitrobenzo[*a*]pyrene. None of the compounds increased the cytochrome P-450 content more than 2-fold. 1,8-Dinitropyrene, 6-nitrochrysene, and 1- and 3-nitrobenzo[*a*]pyrene significantly increased arylhydrocarbon hydroxylase activity 2- to 8-fold higher than solvent-treated controls. The induction of 7-ethoxycoumarin *O*-deethylase activity paralleled that found with arylhydrocarbon hydroxylase. The maximum induction of aminopyrine *N*-demethylase was only 1.5-fold, and none of the nitrated PAHs caused significant increases in epoxide hydrase or NADPH–cytochrome *c* reductase. 1-Nitropyrene reductase activity was induced by each of the compounds with the exception of 6-nitrobenzo[*a*]pyrene. The greatest increase was caused by 1-nitrobenzo[*a*]pyrene followed by 1,3-dinitropyrene, 3-nitrobenzo[*a*]pyrene and 6-nitrochrysene. These data suggest that nitrated PAHs may potentiate the effects of subsequent exposures to various chemical carcinogens.

Hepatic microsomal monooxygenases, including cytochromes P-450, are the principal enzymes responsible for the metabolic detoxification of foreign compounds, although some chemicals are converted into mutagenic and carcinogenic derivatives [1]. These enzymes can be induced by a variety of drugs, insecticides, and other xenobiotics, in particular by polycyclic aromatic hydrocarbons (PAHs)‡ [2]. Recently, nitrated derivatives of PAHs have been detected as contaminants in airborne particulates, coal fly ash, and diesel emission [2, 3]. Since nitrated PAHs are structurally related to PAHs, they may also induce hepatic microsomal enzymes. In this study, we have examined several hepatic microsomal monooxygenase and reductase activities in rats pretreated with a series of environmentally occurring nitrated PAHs including: 1- and 4-nitropyrene, 1,3- 1,6- and 1,8-dinitropyrene, 6-nitrochrysene, 7-nitrobenz[*a*]anthracene, 3-nitrofluoranthene, and 1-, 3-, and 6-nitrobenzo[*a*]pyrene (Fig. 1). The activities assayed were aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin *O*-deethylase, epoxide hydrolase, NADPH–cytochrome *c* reductase and 1-nitropyrene reductase. In addition to establishing the effects of the individual nitrated PAHs, these results have been compared to those obtained with their parent PAHs in order to determine the effect of nitro substitution upon enzyme induction.

MATERIALS AND METHODS

Materials. Benzo[*a*]pyrene, pyrene, chrysene, benz[*a*]anthracene, 1-nitropyrene, 1-aminopyrene, 3-nitrofluoranthene, aminopyrine, 7-ethoxycoumarin and 7-hydroxycoumarin were purchased from the Aldrich Chemical Co., Milwaukee, WI. 1,3-, 1,6- and 1,8-Dinitropyrene were obtained from Midwest Research Institute, Kansas City, MO. 1-, 3-, and 6-Nitrobenzo[*a*]pyrene were synthesized as previously described [4]. 6-Nitrochrysene was prepared by the nitration of chrysene using the method of Newman and Cathcart [5]. 7-Nitrobenz[*a*]anthracene was synthesized according to the procedure of Fieser and Hershberg [6], while 4-nitropyrene was prepared as described by Bavin [7]. 1-Nitropyrene was purified as previously described [8]. All of the nitrated PAHs were examined by HPLC and were >99% pure. 1-Nitropyrene and the dinitropyrenes were also examined by gas chromatography to ensure that there was no isomeric contamination. Each of the PAHs was purified by chromatography on silica gel by eluting with hexane. Pyrene 4,5-oxide and pyrene *trans*-4,5-dihydrodiol were prepared as described by Harvey *et al.* [9, 10]. Horse heart cytochrome *c*, $NADP^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase (type XII) were purchased from the Sigma Co., St. Louis, MO. All other solvents were HPLC grade.

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‡ Abbreviations: PAH, polycyclic aromatic hydrocarbon; and AHH, aryl hydrocarbon hydroxylase.

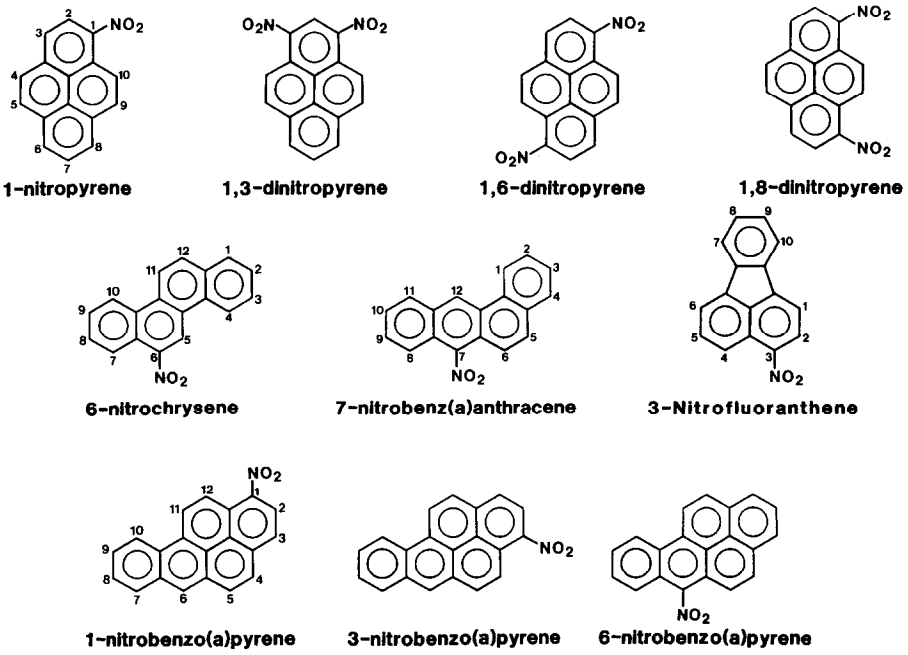

Fig. 1. Structures of the nitrated PAHs used in the induction of hepatic microsomal enzymes.

Animals. Young male Sprague–Dawley rats (CD23) (150 ± 20 g) were obtained from our breeding colony. For three consecutive days, groups of three rats were injected with the inducing agents dissolved in corn oil at a dose of 2.5, 5, or 25 mg per kg body weight. Control animals received corn oil daily. All animals received standard diet and water *ad lib.*

Preparation of microsomes. The rats were killed by cervical dislocation 1 day after the last injection, and their livers were perfused with ice-cold 1.15% KCl via the portal vein. Each liver was homogenized separately with ice-cold 50 mM Tris-acetate buffer, pH 7.4, containing 100 mM KCl and 1 mM EDTA. Microsomes were isolated by differential centrifugation, as previously described [11], and were suspended in 10 mM Tris–acetate buffer, pH 7.4, containing 1 mM EDTA and 20% (w/v) glycerol at a concentration of 10–15 mg protein/ml. Protein concentrations were measured by the method of Lowry *et al.* [12]. The microsomes were stored at −70° until use.

Enzyme assays. AHH activity was assayed fluorometrically by measuring the formation of 3-hydroxybenzo[*a*]pyrene as outlined by Nebert and Gelboin [13]. Cytochrome P-450 content was measured in dithionite-reduced microsomes by the method of Omura and Sato [14]. Aminopyrine demethylase activity was determined by measuring formaldehyde formation as described by Matsubara *et al.* [15]. 7-Ethoxycoumarin *O*-deethylase activity was assayed fluorometrically by measuring the enzymatic formation of 6-hydroxycoumarin by the method of Greenlee and Poland [16].

Epoxide hydrolase activity was determined by quantifying the catalytic hydration of pyrene 4,5-oxide using a procedure modified after Dansette *et al.* [17]. The reaction was initiated by adding 100 nmol pyrene 4,5-oxide in 40 μl methanol to 1 ml of 50 mM Tris–HCl buffer, pH 8.7, containing 50 μg microsomal protein. Following a 10-min incubation at 37°, the reaction was extracted with 2 ml of ethyl acetate. The pyrene *trans*-4,5-dihydrodiol was separated by reversed phase HPLC using a Du Pont ODS column (9.4 × 250 mm) and eluting isocratically with 95% methanol. The concentration of the dihydrodiol was calculated with a Hewlett–Packard 3390A integrator through comparison to a synthetic standard.

NADPH–cytochrome *c* reductase activity was measured according to the methods of Phillips and Langdon [18]. 1-Nitropyrene reductase activity was determined by assaying the formation of 1-aminopyrene in anaerobic incubations by a procedure modified after Saito *et al.* [19]. One milliliter of 50 mM potassium phosphate buffer, pH 7.4, containing 10 μmol glucose-6-phosphate, 0.5 μmol $NADP^+$, 25 nmol flavin mononucleotide, 1 unit of glucose-6-phosphate dehydrogenase and 0.2 mg of microsomal protein, was purged with a stream of argon through a rubber septum for 5 min at 0°. 1-Nitropyrene (8 nmol in 40 μl methanol) was then added to the mixture with a microsyringe, and the solution was incubated under argon at 37° for 20 min. The reaction was quenched by the addition of 1 ml of acetone, and then 3 ml of hexane was added. The tube was vortexed for 2 min and centrifuged at 1000 rpm for 5 min. The fluorescence in the hexane layer was determined using an excitation wavelength of 365 nm and an emission wavelength of 405 nm. In control experiments, the recovery of 1-aminopyrene was >99%. Similar results were obtained with HPLC analyses.

All the enzyme activities were linear with protein concentration up to 0.6 mg/ml and incubation time up to 20 min. Comparisons between groups were made by Student's *t*-test [20].

Instrumentation. Ultraviolet-visible spectra were recorded with a Varian Cary 219. Fluorescence was

Table 1. Cytochrome P-450 content and aryl hydrocarbon hydroxylase (AHH) activity in hepatic microsomes of rats pretreated with nitrated polycyclic aromatic hydrocarbons*

Compound	Dose (mg/kg)	Cytochrome P-450 (nmol/mg protein)	Ratio†	AHH (pmol/mg protein/min)	Ratio†
Control		0.70	1.0	35.3 ± 6.2	1.0
Pyrene	2.5	0.99	1.4	37.2 ± 10.8	1.1
1-Nitropyrene	2.5	1.12	1.6	39.6 ± 3.2	1.1
	25	0.74	1.1	44.9 ± 11.1	1.3
4-Nitropyrene	25	0.59	0.8	28.0 ± 5.9	0.8
1,3-Dinitropyrene	2.5	1.56	2.2	42.1 ± 6.2	1.2
1,6-Dinitropyrene	2.5	1.02	1.5	32.7 ± 5.1	0.9
1,8-Dinitropyrene	2.5	1.33	1.9	51.9 ± 1.1‡§	1.5
Chrysene	2.5	1.14	1.6	60.0 ± 5.0‡	1.7
	5.0	0.62	0.9	62.9 ± 7.1‡	1.8
6-Nitrochrysene	2.5	1.33	1.9	157.7 ± 49.9‡‖	4.5
	5.0	1.11	1.6	269.6 ± 10.1‡‖	7.6
7-Nitrobenz[*a*]anthracene	25	0.68	1.0	38.1 ± 3.5	1.1
3-Nitrofluoranthene	25	0.54	0.8	31.7 ± 2.2	0.9
Benzo[*a*]pyrene	2.5	1.07	1.5	44.1 ± 3.6	1.2
	5.0	0.84	1.2	115.3 ± 5.4‡	3.3
	25	1.13	1.6	346.2 ± 10.3‡	9.8
1-Nitrobenzo[*a*]pyrene	2.5	1.46	2.1	279.8 ± 68.4‡‖	7.9
	5.0	1.06	1.5	307.1 ± 1.5‡‖	8.7
3-Nitrobenzo[*a*]pyrene	2.5	1.17	1.7	184.6 ± 56.6‡‖	5.2
	5.0	1.33	1.9	334.8 ± 22.8‡‖	9.5
6-Nitrobenzo[*a*]pyrene	2.5	0.56	0.8	29.0 ± 2.1‖¶	0.8
	5.0	0.48	0.7	55.3 ± 4.0‡‖¶	1.6
3-Methylcholanthrene	5.0	1.38	2.0	279.6 ± 3.9‡	7.9

* Young male Sprague–Dawley rats were treated, i.p., for three consecutive days with the nitrated PAH at the dose indicated. One day after the last dose, the animals were killed, hepatic microsomes were prepared, and the cytochrome P-450 content and AHH activity were determined, as described in Materials and Methods. The AHH data represent the average ± SD of triplicate assays on each of three animals. Comparisons between groups were made by Student's *t*-test [20].

† The ratio is the activity of the experimental animal divided by that of the control.

‡ Significantly different from solvent-treated control ($P < 0.05$).

§ Significantly different from 1-nitropyrene and 1,6-dinitropyrene ($P < 0.05$).

‖ Significantly different from parent PAH ($P < 0.05$).

¶ Significantly different from 1- and 3-benzo[*a*]pyrene ($P < 0.05$).

measured with an Aminco–Bowman spectrophotofluorometer. Reversed phase HPLC was conducted with a Waters Associates system consisting of two 6000A pumps, a 660 solvent programmer, and a 440 absorbance monitor adjusted to 254 nm.

RESULTS

The effects of the nitrated PAHs and their parent PAHs on the cytochrome P-450 content and AHH activity of hepatic microsomes are summarized in Table 1. Due to the low solubility of the nitrated PAHs in corn oil, only two low doses of 2.5 and 5.0 mg/kg were used with 6-nitrochrysene and the nitrobenzo[*a*]pyrenes, and 2.5 mg/kg was used with the dinitropyrenes. For comparison, an additional group was treated with 3-methylcholanthrene.

None of the nitrated PAHs increased the cytochrome P-450 content more than 2-fold (Table 1). In contrast, there were marked differences in the induction of AHH activity (Table 1). When administered at a dose of 2.5 mg/kg, 1,8-dinitropyrene, 6-nitrochrysene, and 1- and 3-nitrobenzo[*a*]pyrene significantly increased AHH activity compared to solvent-treated controls, whereas 6-nitro-benzo-[*a*]pyrene caused a significant increase only at 5 mg/kg. Furthermore, 6-nitrochrysene and 1- and 3-nitrobenzo[*a*]pyrene were significantly more active than their parent PAHs, chrysene and benzo[*a*]pyrene. However, 6-nitrobenzo[*a*]pyrene was significantly less active than benzo[*a*]pyrene as well as the other two nitrobenzo[*a*]pyrenes. When tested at doses up to 25 mg/kg, 1- and 4-nitropyrene, 7-nitrobenz-[*a*]anthracene, and 3-nitrofluoranthene did not cause induction of AHH activity.

The effects of the nitrated PAHs administered at 2.5 mg/kg on 7-ethoxycoumarin *O*-deethylase, aminopyrine *N*-demethylase, and epoxide hydrolase activity are shown in Table 2. The induction of 7-ethoxycoumarin *O*-deethylase activity by these compounds paralleled that of AHH activity. Thus, 1,8-dinitropyrene, 6-nitrochrysene, and 1- and 3-nitrobenzo[*a*]pyrene caused significant increases compared to solvent-treated controls, with the latter two compounds showing the greatest induction. 6-Nitrochrysene and 1- and 3-nitrobenzo[*a*]pyrene also induced 7-ethoxycoumarin *O*-deethylase to a greater extent than their parent PAHs. The maximum induction of aminopyrine *N*-demethylase compared to control animals was caused by 1,8-dinitropyrene, but this was only 1.5-fold (Table 2). None of the nitrated PAHs increased epoxide hydrolase activity, although benzo[*a*]pyrene did result in a statistically significant reduction in this activity (Table 2).

With the exception of 6-nitrobenzo[*a*]pyrene, each

Table 2. 7-Ethoxycoumarin *O*-deethylase, aminopyrine *N*-demethylase, and epoxide hydrolase activity in hepatic microsomes of rats pretreated with nitrated polycyclic aromatic hydrocarbons*

Compound	Enzyme activity (nmol/mg/min) 7-Ethoxycoumarin *O*-deethylase	Ratio†	Aminopyrine *N*-demethylase	Ratio†	Epoxide hydrolase	Ratio†
Control	1.44 ± 0.29	1.0	3.11 ± 0.88	1.0	19.80 ± 1.40	1.0
Pyrene	1.61 ± 0.78	1.1	3.52 ± 0.55	1.1	21.55 ± 1.85	1.1
1-Nitropyrene	1.99 ± 0.25	1.4	3.98 ± 0.60	1.3	18.77 ± 2.70	0.9
1,3-Dinitropyrene	1.65 ± 0.14	1.1	4.37 ± 0.29	1.4	15.20 ± 3.41	0.8
1,6-Dinitropyrene	2.03 ± 0.23	1.4	3.91 ± 0.39	1.3	16.23 ± 2.80	0.8
1,8-Dinitropyrene	2.03 ± 0.20‡	1.4	4.60 ± 0.26‡	1.5	18.15 ± 0.55	0.9
Chrysene	2.08 ± 0.17‡	1.4	3.76 ± 0.50	1.2	17.27 ± 1.89	0.8
6-Nitrochrysene	4.49 ± 1.14‡§	3.1	3.86 ± 0.31	1.3	24.50 ± 3.40	1.2
Benzo[*a*]pyrene	1.73 ± 0.16	1.2	4.28 ± 0.19	1.4	12.00 ± 1.63‡	0.6
1-Nitrobenzo[*a*]pyrene	10.26 ± 1.91‡§	7.1	3.97 ± 0.30	1.3	26.30 ± 7.30§	1.1
3-Nitrobenzo[*a*]pyrene	9.36 ± 0.83‡§	6.5	3.70 ± 0.31	1.2	20.87 ± 5.21§	1.1
6-Nitrobenzo[*a*]pyrene	1.57 ± 0.04‖	1.1	3.45 ± 0.29	1.1	17.03 ± 4.09	0.9

* Young male Sprague–Dawley rats were treated with 2.5 mg/kg of the nitrated PAHs, and hepatic microsomes were prepared as described in Table 1. The data represent the average ± SD of triplicate assays on each of three animals. Statistical analyses were performed as described in Table 1.

† The ratio is the activity of the experimental animal divided by that of the control.

‡ Significantly different from solvent-treated control ($P < 0.05$).

§ Significantly different from parent PAH ($P < 0.05$).

‖ Significantly different from 1- and 3-nitrobenzo[*a*]pyrene ($P < 0.05$).

Table 3. 1-Nitropyrene reductase and NADPH–cytochrome *c* reductase activity in hepatic microsomes of rats pretreated with nitrated polycyclic aromatic hydrocarbons*

Compound	1-Nitropyrene reductase (pmol/mg/min)	Ratio†	Cytochrome *c* reductase (nmol/mg/min)	Ratio†
Control	38.7 ± 12.4	1.0	213.5 ± 12.7	1.0
Pyrene	77.6 ± 35.8‡	2.0	197.8 ± 31.7	0.9
1-Nitropyrene	67.6 ± 6.1‡	1.7	233.2 ± 24.2	1.1
1,3-Dinitropyrene	148.0 ± 27.6‡§	3.8	226.5 ± 28.7	1.1
1,6-Dinitropyrene	97.6 ± 10.2‡§‖	2.5	222.2 ± 3.0	1.0
1,8-Dinitropyrene	112.7 ± 22.7‡§	2.9	243.2 ± 31.1	1.1
Chrysene	73.5 ± 6.1‡	1.9	224.7 ± 23.6	1.1
6-Nitrochrysene	122.1 ± 15.9‡¶	3.2	231.8 ± 33.6	1.1
Benzo[*a*]pyrene	112.5 ± 25.8‡	2.9	227.9 ± 5.7	1.1
1-Nitrobenzo[*a*]pyrene	163.9 ± 22.6‡	4.2	296.0 ± 21.2‡	1.4
3-Nitrobenzo[*a*]pyrene	134.4 ± 32.5‡	3.5	250.7 ± 44.8	1.2
6-Nitrobenzo[*a*]pyrene	55.5 ± 36.5**	1.4	234.0 ± 20.1	1.1

* Young male Sprague–Dawley rats were treated with 2.5 mg/kg of the nitrated PAHs, and hepatic microsomes were prepared as described in Table 1. The data represent the average ± SD of triplicate assays on each of three animals. Statistical analyses were performed as described in Table 1.

† The ratio is the activity of the experimental animal divided by that of the control.

‡ Significantly different from solvent-treated control ($P < 0.05$).

§ Significantly different from 1-nitropyrene ($P < 0.05$).

‖ Significantly different from 1,3-dinitropyrene ($P < 0.05$).

¶ Significantly different from parent PAH ($P < 0.05$).

** Significantly different from 1- and 3-nitrobenzo[*a*]pyrene ($P < 0.05$).

of the compounds, administered at 2.5 mg/kg induced a significant increase in 1-nitropyrene reductase activity compared to solvent-treated controls (Table 3). The greatest induction was observed with 1-nitrobenzo[*a*]pyrene, followed by 1,3-dinitropyrene, 3-nitrobenzo[*a*]pyrene and 6-nitrochrysene. Each of the dinitropyrenes was more active at inducing 1-nitropyrene reductase activity than 1-nitropyrene. 6-Nitrochrysene was significantly more active than chrysene, whereas the other nitro PAHs were not significantly more active than their parent PAHs. The maximum induction of NADPH–cytochrome *c* reductase was observed with 1-nitrobenzo[*a*]pyrene. None of the other compounds caused a significant increase in the activity of this enzyme (Table 3).

DISCUSSION

The results of this study demonstrate that nitrated PAHs can induce various hepatic microsomal enzymes, with the magnitude of induction depending on the particular compound being examined. In general, the nitrated PAHs induced AHH, 7-ethoxycoumarin *O*-deethylase, and 1-nitropyrene nitroreductase, but not aminopyrine *N*-demethylase, epoxide hydrolase and NADPH–cytochrome *c* reductase. 1- and 3-Nitrobenzo[*a*]pyrene caused the greatest increase followed by 6-nitrochrysene and 1,8-dinitropyrene. The pattern of high induction of AHH activity and low induction of aminopyrine *N*-demethylase, NADPH–cytochrome *c* reductase and epoxide hydrolase is similar to what has been observed for 3-methylcholanthrene, which induces high levels of cytochrome P-450c [21]. A similar finding has been reported recently by Asokan *et al.* [22] who applied mixtures of dinitropyrenes topically to newborn rats and assayed cutaneous and hepatic microsomal activities. Cytochrome P-450c catalyzes the *N*-oxidation of certain aromatic amine carcinogens, and it is also the major P-450 isozyme responsible for the metabolic activation of carcinogenic PAHs [23]. Thus, our data suggest that nitrated PAHs can potentiate the effects of subsequent exposures to various chemical carcinogens.

PAHs, in particular benz[*a*]anthracene and benzo[*a*]pyrene, are potent hepatic microsomal enzyme inducers [1, 13]. Depending upon the site of substitution, nitration had a dramatic effect upon this activity. For example, when administered at 5 mg/kg, benzo[*a*]pyrene caused a 3.3-fold increase in AHH activity (Table 1); this increase was 9-fold after administration of its 1- and 3-nitro derivatives. In contrast, only a slight increase in AHH induction was observed with 6-nitrobenzo[*a*]pyrene. Similarly, while benz[*a*]anthracene causes substantial increases in AHH activity [13], 7-nitrobenz[*a*]anthracene was devoid of this capability even when administered at 25 mg/kg. Pyrene, chrysene and fluoranthene were weak inducers of AHH activity (Table 1; see also Refs. 13 and 24). Among the nitro-derivatives, 3-nitrofluoranthene did not cause a significant induction, while of the nitrated pyrenes, only 1,8-dinitropyrene showed a significant capability. By comparison, 6-nitrochrysene was found to be a potent inducer of AHH activity. It is perhaps significant that the nitro groups of 7-nitrobenz[*a*]anthracene and 6-nitrobenzo[*a*]pyrene are orientated perpendicular to the aromatic ring system, while the nitro moieties in 1- and 3-nitrobenzo[*a*]pyrene are coplanar to the aromatic ring [25]. This perpendicular orientation may inhibit the interaction of these compounds with the *Ah* receptor. Some other biochemical activities of nitroarenes, such as binding with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin receptor sites, may also be associated with the orientation of the substituted nitro-groups [26].

Nitro PAHs can undergo sequential reduction to nitroso, *N*-hydroxy amino, and amino derivatives, and this nitroreduction has been implicated in the activation of certain of these compounds to mutagens and carcinogens [3, 27]. In the case of 1-nitropyrene and 1,8-dinitropyrene, DNA adducts have been characterized both *in vitro* and in mutagenesis assays that are indicative of the reaction of their *N*-hydroxy amino derivatives with C8 of deoxyguanosine [8, 28–30]. Similar DNA adducts have been detected *in vivo* in rats administered 1-nitropyrene [31, 32] and 1,6-dinitropyrene (Z. Djurić, Y. Yamazoe and F. A. Beland, manuscript in preparation), and these may be involved in the tumorigenic response reported for these nitrated PAHs. In the present study we found that, in addition to inducing AHH and 7-ethoxycoumarin deethylase, certain of the nitrated PAHs caused significant increases in 1-nitropyrene reductase activity. Since nitroreduction appears to be important in the metabolic activation of nitrated pyrenes, this suggests that chronic exposure to nitrated PAHs may result in increased DNA adduct formation which, in turn, could result in increased tumor yields.

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REFERENCES

1. A. H. Conney, *Cancer Res.* **42**, 4875 (1982).
2. H. S. Rosenkranz and R. Mermelstein, *Mutation Res.* **114**, 217 (1983).
3. F. A. Beland, R. H. Heflich, P. C. Howard and P. P. Fu, in *Polycyclic Hydrocarbons and Carcinogenesis* (Ed. R. G. Harvey), p. 371. American Chemical Society, Washington, DC (1985).
4. M. W. Chou, R. H. Heflich, D. A. Casciano, D. W. Miller, J. P. Freeman, F. E. Evans and P. P. Fu, *J. med. Chem.* **27**, 1156 (1984).
5. M. S. Newman and J. A. Cathcart, *J. org. Chem.* **5**, 618 (1940).
6. L. F. Fieser and E. B. Hershberg, *J. Am. chem. Soc.* **60**, 1893 (1938).
7. P. M. G. Bavin, *Can. J. Chem.* **37**, 1614 (1959).
8. P. C. Howard, R. H. Heflich, F. E. Evans and F. A. Beland, *Cancer Res.* **43**, 2052 (1983).
9. H. Cho and R. G. Harvey, *Tetrahedron Lett.* 1491 (1974).
10. R. G. Harvey, S. H. Goh and C. Cortez, *J. Am. chem. Soc.* **97**, 3468 (1975).
11. M. W. Chou and S. K. Yang, *J. Chromat.* **185**, 635 (1979).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
13. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **243**, 6242 (1968).
14. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
15. T. Matsubara, A. Touch and Y. Tochino, *Jap. J. Pharmac.* **27**, 127 (1977).
16. W. F. Greenlee and A. J. Poland, *J. Pharmac. exp. Ther.* **205**, 596 (1978).
17. P. M. Dansette, G. C. Dubois and D. M. Jerina, *Analyt. Biochem.* **97**, 340 (1979).
18. A. H. Phillips and R. G. Langdon, *J. biol. Chem.* **237**, 2652 (1962).
19. K. Saito, T. Kamataki and R. Kato, *Cancer Res.* **44**, 3169 (1984).
20. G. G. Simpson, A. Roe and R. C. Lewontin, *Quantitative Zoology*, p. 176. Harcourt & Brace, New York (1960).
21. I. B. Tsyrlov, N. E. Zakharova, O. A. Gromova and V. V. Lyakhovich, *Biochim. biophys. Acta* **421**, 44 (1976).

22. P. Asokan, M. Das, H. S. Rosenkranz, D. R. Bickers and H. Mukhtar, *Biochem. biophys. Res. Commun.* **129**, 134 (1985).
23. F. F. Kadlubar and G. J. Hammons, in *Mammalian Cytochromes P-450*, Vol. II (Ed. F. P. Guengerich), pp. 81–130. CRC Press, Boca Raton (1986).
24. A. J. Conney, E. C. Miller and J. A. Miller, *Cancer Res.* **16**, 450 (1956).
25. P. P. Fu, M. W. Chou, D. W. Miller, G. L. White, R. H. Heflich and F. A. Beland, *Mutation Res.* **143**, 173 (1985).
26. H. S. Rosenkranz and R. Mermelstein, *J. environ. Sci. Hlth* **3**, 221 (1985).
27. P. G. Wislocki, E. S. Bagan, A. Y. H. Lu, K. L. Dooley, P. P. Fu, H. Han-Hsu, F. A. Beland and F. F. Kadlubar, *Carcinogenesis* **7**, 1317 (1986).
28. R. H. Heflich, P. C. Howard and F. A. Beland, *Mutation Res.* **149**, 25 (1985).
29. R. H. Heflich, E. K. Fifer, Z. Djurić and F. A. Beland, *Environ. Hlth Perspect.* **62**, 135 (1985).
30. P. J. Andrews, M. A. Quilliam, B. E. McCarry, D. W. Bryant and D. R. McCalla, *Carcinogenesis* **7**, 105 (1986).
31. C. A. Stanton, F. L. Chow, D. H. Phillips, P. L. Grover, R. C. Garner and C. N. Martin, *Carcinogenesis* **6**, 535 (1985).
32. Y. Hashimoto and K. Shudo, *Jap. J. Cancer Res. (Gann)* **76**, 253 (1985).