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STEREOSELECTIVE METABOLISM OF 7-NITROBENZ(A)ANTHRACENE TO 3,4- AND 8,9- trans-DIHYDRODIOLS

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SUMMARY: Metabolism of 7-nitrobenz(a)anthracene (7-NO_2-BA) by rat liver microsomes yielded 7-NO_2-BA trans-3,4-dihydrodiol and 7-NO_2-BA trans-8,9-dihydrodiol as major metabolites. Proton NMR spectral analyses indicate that 7-NO_2-BA trans-3,4-dihydrodiol preferentially adopts a quasidiequatorial conformation and that 7-NO_2-BA trans-8,9-dihydrodiol adopts a mixture of quasidiequatorial and quasidiaxial conformations. Circular dichroism spectral analyses of these compounds and their diacetoxy derivatives indicated that the major enantiomers of both dihydrodiols have R,R absolute stereochemistries. The identification of 7-NO_2-BA trans-8,9-dihydrodiol as a metabolite of 7-NO_2-BA indicates that oxidative metabolism can occur at position peri to the nitro substituent.

Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) have recently been found as mutagenic components in fly ash, diesel emissions, photocopier toners, cigarette smoke, and environmental samples (1-8). Since many nitro-PAHs are carcinogenic in experimental animals (8-12), a major concern now is the possible hazard of these compounds to human health. Published results indicate that both reduction of the nitro substituent (13-15) and ring oxidation (16-19) can be involved in the metabolic activation. However, little is known on the effects of the nitro substituent on the regio- and stereoselective metabolism of PAHs. We reported that the 6-nitro substitution of 6-nitrobenzo(a)pyrene effectively blocked metabolism at regions (i.e., 4,5- and 7,8-) peri to the nitro substituent, presumably because of steric and electronic constraints (16,17). A recent report on the metabolism of 1-nitropyrene also indicated that metabolism at the region (i.e., 9,10-positions) peri to the nitro substituent did not occur

ABBREVIATIONS: nitro-PAH, nitro-polycyclic aromatic hydrocarbon; BA, benz(a)-anthracene; 7-NO_2-BA, 7-nitrobenz(a)anthracene; HPLC, high performance liquid chromatography; NMR, nuclear magnetic resonance; CD, circular dichroism.

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(19). It is important to ascertain whether or not inhibition of PAH metabolism at positions peri to a nitro substituent is a general phenomenon. In this report, we described the rat liver microsomal metabolism of 7-NO_2-BA. We found that 7-NO_2-BA trans-8,9-dihydrodiol was one of the two major metabolites. This finding indicates that the nitro substituent of a nitro-PAH does not always inhibit the metabolism at the region(s) peri to it.

MATERIALS AND METHODS

Materials: BA was purchased from Aldrich Chemical Co., Milwaukee, WI. 7-NO_2-BA was synthesized according to a known procedure (20). BA 3R,4R-dihydrodiol and 8R,9R-dihydrodiol were prepared as described (21). 8R,9R-diacetoxy-8,9-dihydro-BA was synthesized by acetylation of BA 8R,9R-dihydrodiol with acetic anhydride and pyridine at ambient temperature.

In Vitro Incubations: Liver microsomes of 3-methylcholanthrene-treated immature male Sprague-Dawley rats (80-100 g body weight) were prepared as previously described (22). Incubation mixtures contained 50 mmol Tris-HCl, pH 7.5, 3 mmol $MgCl_2$, 1mmol $NADP^+$, 2 mmol glucose-6-phosphate, 100 units glucose-6-phosphate dehydrogenase, 1 g microsomal protein and 40 μmol 7-NO_2-BA (dissolved in 20 ml acetone) in a total incubation volume of 1 liter. Incubations were conducted aerobically with shaking for 60 min at 37° and then quenched by the addition of 1 liter acetone. The metabolites and residual substrate were partitioned into 2 liters ethyl acetate, and the organic phase was dried with anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residue was dissolved in 2 ml methanol/tetrahydrofuran (1:1, v/v) for analysis by HPLC.

HPLC Separation of Metabolites: Reversed-phase HPLC was performed with a Waters Associates system consisting of two 6000A pumps, a 660 solvent programmer, a U6K injector, and a 440 u.v. detector (254 nm). Metabolites were separated by using a Dupont Zorbax ODS column (9.4 mm x 25 cm) and eluting with a 40-min linear gradient of 75-100% methanol in water at a flow rate of 1.1 ml/min.

Physicochemical Properties of Metabolites: Ultraviolet-visible absorption spectra of metabolites in methanol were measured on a Beckman model 25 spectrophotometer. Mass spectral analyses were performed on a Finnigan 4000 mass spectrometer by electron impact with a solid probe at 70 eV and 250°C ionizer temperature. Fourier transform proton NMR spectra of the dihydrodiols in acetone-d_6 with a trace of D_2O and the diacetoxy derivatives in acetone-d_6 were obtained with a Bruker WM 500 spectrometer. CD spectra of dihydrodiols were determined on a Jasco 500A spectropolarimeter and were expressed in ellipticity (in millidegrees) for methanol solutions that read 1.0 absorbance unit at the wavelength of maximum absorption (23).

RESULTS AND DISCUSSION

Metabolites formed by incubation of 7-NO_2-BA with rat liver microsomes were isolated by reversed-phase HPLC (Fig. 1). The uv-visible spectra of the two major metabolites were similar to that of BA trans-8,9-dihydrodiol and BA trans-3,4-dihydrodiol (24), respectively. Both of these metabolites had mass spectra with molecular ions at m/z 307 and characteristic ions at m/z 289 due to loss of a water molecule. The structures of these metabolites were determined by analysis of their high resolution 500 MHz proton NMR spectra (Fig. 2). The NMR reso-

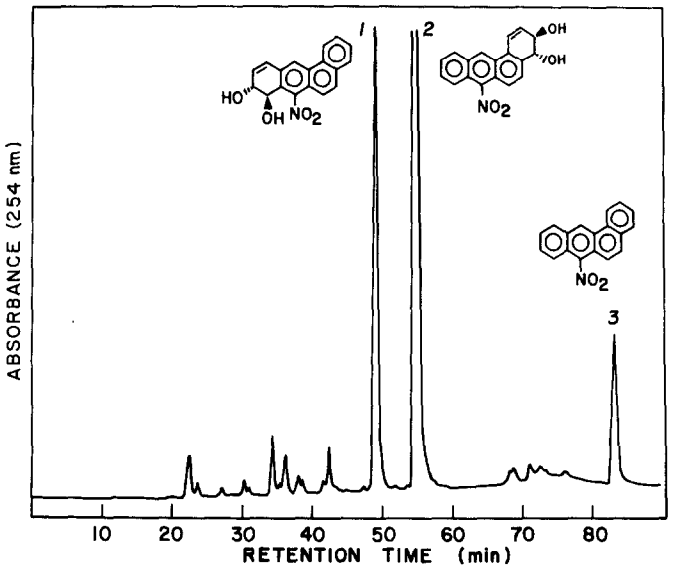

Fig. 1 Reversed-phase HPLC profile of ethyl acetate extractable metabolites obtained from incubation of 7-NO_2-BA with liver microsomes from 3-methylcholanthrene-treated male Sprague-Dawley rats.

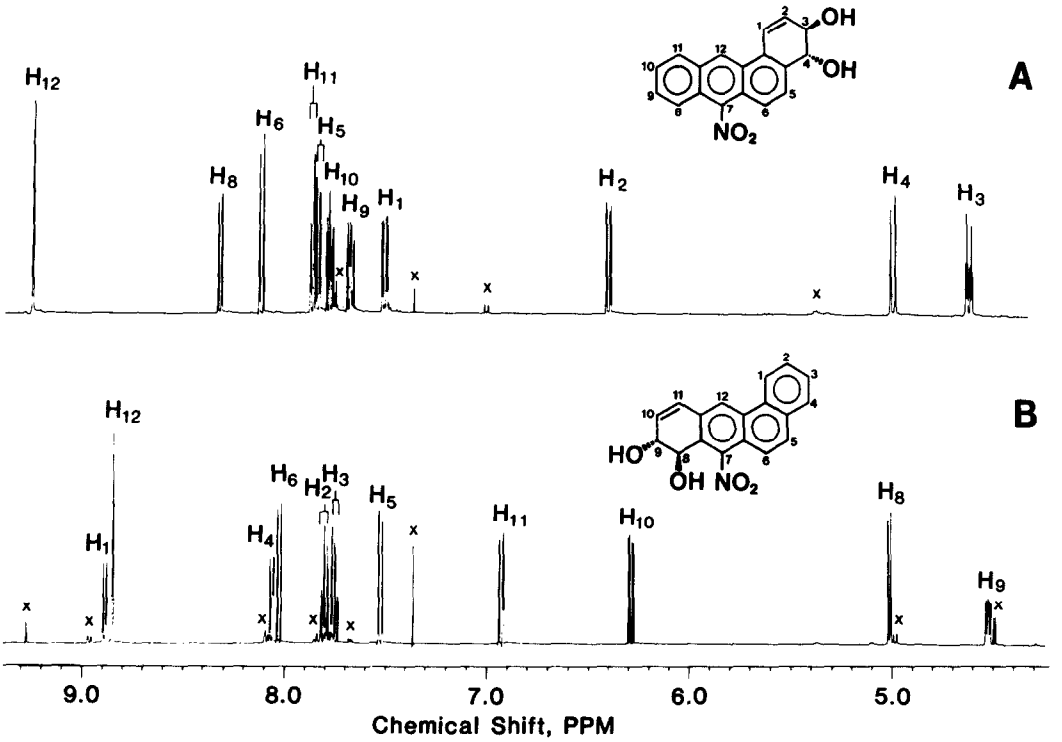

Fig. 2 500 MHz proton NMR spectra of metabolites identified as (A) 7-NO_2-BA trans-3,4-dihydrodiol and (B) 7-NO_2-BA trans-8,9-dihydrodiol. Chemical shifts are in ppm relative to tetramethylsilane. The resonances marked by "x" are derived from impurities.

nance assignments were determined both by comparison to their respective BA dihydrodiol analogues (24) and by extensive homonuclear decoupling experiments. The identified metabolites labeled as peaks 1 and 2 in Fig. 1 were identified as 7-NO_2-BA trans-8,9-dihydrodiol and 7-NO_2-BA trans-3,4-dihydrodiol, respectively. The proton NMR assignments are as follows:

7-NO_2-BA trans-8,9-dihydrodiol: 4.52 (m,1,H_9), 5.00 (d,1,H_8), 6.28 (dd,1, H_{10}), 6.92 (dd,1,H_{11}), 7.52 (d,1,H_5), 7.75 (apparent t,1,H_3), 7.80 (apparent t, 1,H_2), 8.02 (d,1,H_6), 8.06 (d,1,H_4), 8.85 (s,1,H_{12}), and 8.89 ppm (d,1,H_1); $J_{1,2}$ =8.6, $J_{3,4}$=7.7, $J_{5,6}$=9.0, $J_{8,9}$=6.5, $J_{9,10}$=3.9, $J_{9,11}$=1.3, and $J_{10,11}$=9.9 Hz.

7-NO_2-BA trans-3,4-dihydrodiol: 4.61 (dt,1,H_3), 4.98 (d,1,H_4), 6.39 (dd,1, H_2), 7.51 (dd,1,H_1), 7.68 (apparent t,1,H_9), 7.78 (apparent t,1,H_{10}), 7.84 (d, 1,H_5), 7.86 (d,1,H_{11}), 8.11 (d,1,H_6), 8.33 (d,1,H_8), and 9.26 ppm (s,1,H_{12}); $J_{1,2}$=11.7, $J_{1,3}$=2.4, $J_{2,3}$=2.3, $J_{3,4}$=10.2, $J_{5,6}$=9.1, and $J_{8,9}$=8.6 Hz.

The large coupling constant between the carbinol protons ($J_{3,4}$=10.2 Hz) of 7-NO_2-BA trans-3,4-dihydrodiol indicates that this dihydrodiol preferentially adopts a quasidiequatorial conformation. However, the coupling constant between the carbinol protons of 7-NO_2-BA trans-8,9-dihydrodiol is relatively small ($J_{8,9}$ = 6.5 Hz). These results indicate that this 8,9-dihydrodiol adopts a mixture of

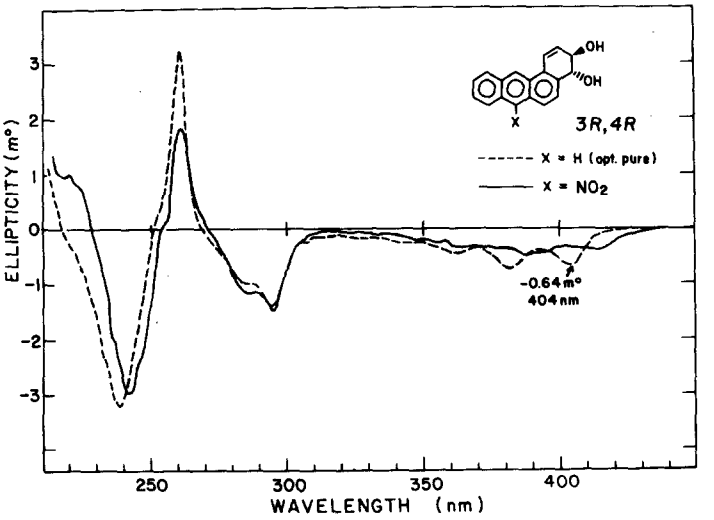

Fig. 3 CD spectra of optically pure BA 3R,4R-dihydrodiol (---, E_{max} at 261 nm) and the metabolite identified as 7-NO_2-BA trans-3,4-dihydrodiol (——, E_{max} at 262 nm).

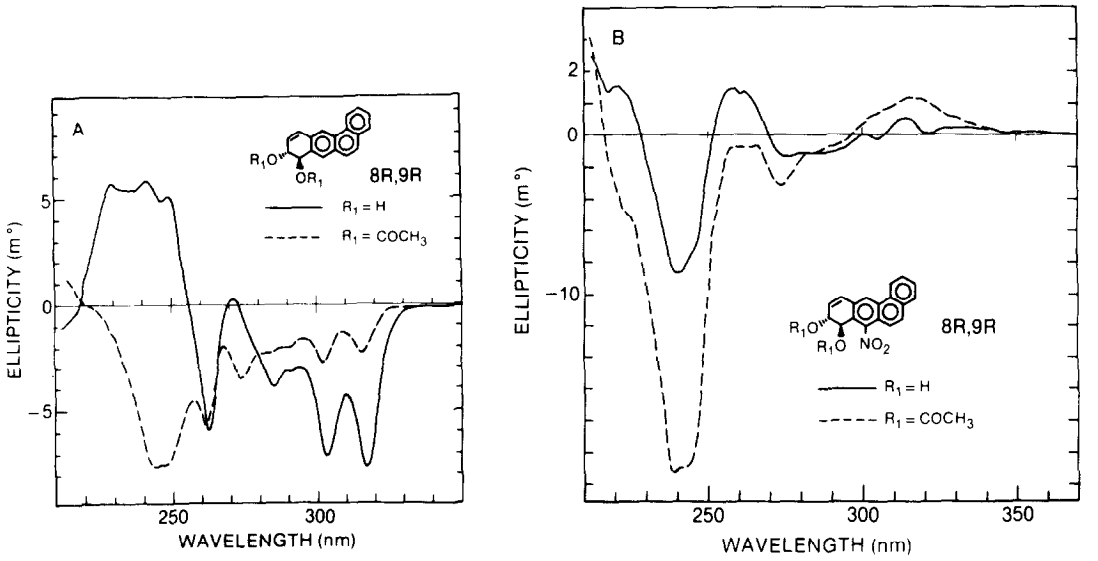

Fig. 4 CD spectra of (A) BA 8R,9R-dihydrodiol (—— E_{max} at 264 nm) and its diacetate (- - - - , E_{max} at 265 nm) and (B) the metabolite identified as 7-NO_2-BA trans-8,9-dihydrodiol (——, E_{max} at 264 nm) and its diacetate (- - - , E_{max} at 265 nm).

quasidiaxial and quasidiequatorial conformations (25). The conformational preference of 7-NO_2-BA trans-8,9-dihydrodiol is similar to that of trans-8,9-diacetoxy-8,9-dihydro-BA ($J_{8,9}$=6.5 Hz) which also adopts a mixture of quasidiequatorial and quasidiaxial conformations (24,25). The proton NMR assignments of trans-8,9-diacetoxy-8,9-dihydro-BA are: 2.03 (s,3,CH_3), 2.14 (s,3,CH_3), 5.69 (dd,1,H_9), 6.14 (dd,1,H_{10}), 6.35 (d,1,H_8), 7.07 (d,1,H_{11}), 7.68 (m,1,H_3), 7.74 (m,1,H_2), 7.87 (AB,2,$H_{5,6}$),7.94 (s,1,H_7), 8.01 (dd,1,H_4), 8.73 (s,1,H_{12}), and 8.85 ppm (d,1,H_1); $J_{1,2}$=8.2, $J_{3,4}$=8.6, $J_{5,6}$=8.2, $J_{8,9}$=6.5, $J_{9,10}$=3.9, and $J_{10,11}$=9.9 Hz.

Both 7-NO_2-BA trans-3,4-dihydrodiol and 7-NO_2-BA trans-8,9-dihydrodiol were found to be optically active (Figs. 3 and 4B). The 7-NO_2-BA trans-3,4-dihydrodiol has CD Cotton effects similar to those of BA 3R,4R-dihydrodiol (Fig. 3). Since the nitro substituent is away from the asymmetric centers and does not change the conformation of the dihydrodiol, we conclude that the major enantiomer of 7-NO_2-BA trans-3,4-dihydrodiol has 3R,4R absolute stereochemistry.

Because of the difference in their conformation and the dependence of the CD Cotton effects on the conformation of the dihydrodiols, the CD spectrum of 7-

NO_2-BA trans-8,9-dihydrodiol is different from that of BA 8R,9R-dihydrodiol (Fig. 4). Upon acetylation, the CD Cotton effects of BA 8R,9R-dihydrodiol changed sign between 210-255 nm (Fig. 4A) due to the change of the conformational preference. The characteristic Cotton effects of 8R,9R-dihydroxy-8,9-dihydro-BA were also observed in the CD spectrum of 7-NO_2-BA trans-8,9-dihydrodiol (Fig. 4B). These results suggest that the major enantiomer of 7-NO_2-BA trans-8,9-dihydrodiol metabolite has R,R absolute stereochemistry. The enhanced Cotton effects observed between 210-255 nm in the CD spectrum of the quasidiaxial trans-8,9-diacetoxy-8,9-dihydro-7-NO_2-BA (Fig. 4B) are consistent with the assignment of the 8R,9R absolute stereochemistry. The preference of quasidiaxial conformation of trans-8,9-diacetoxy-8,9-dihydro-7-NO_2-BA was determined by the NMR coupling constant between the carbinol protons ($J_{8,9}$= 3.9 Hz)(24,25). Its proton assignments are as follows: 2.01 (s,3,CH_3), 2.05 (s,3,CH_3), 5.52 (apparent t,1, H_9), 6.26 (d,1,H_8), 6.33 (dd,1,H_{10}), 7.26 (d,1, H_{11}), 7.59 (d,1, H_6), 7.81-7.86 (m,2,$H_{2,3}$), 8.11 (apparent d,1,H_4), 8.12 (d,1,H_5), 8.95 (d,1,H_1), and 9.08 ppm (s,1,H_{12}); $J_{1,2}$=8.2, $J_{3,4}$=7.3, $J_{5,6}$=9.0, $J_{8,9}$=3.9, $J_{9,10}$=5.2 and $J_{10,11}$=9.5 Hz.

We previously reported that the 6-nitro substituent in 6-nitrobenzo(a)pyrene can inhibit the metabolic formation of trans-dihydrodiols peri to the nitro substituent (16,17). The identification of 7-NO_2-BA trans-8,9-dihydrodiol as a major metabolite of 7-NO_2-BA clearly indicates that a nitro substituent does not always inhibit the microsomal oxidation of a nitro-PAH at the aromatic double bond peri to the nitro substituent. However, a nitro group can cause the peri trans-dihydrodiol to adopt a mixture of quasidiaxial and quasidiequatorial conformations. This effect on dihydrodiol conformation is different from those of a methyl (26), a fluoro (27), and a bromo substituent (23) which cause the peri trans-dihydrodiols preferentially to adopt the quasidiaxial conformations. Similar to the results in the metabolism of BA (21 and refs. therein), 7-methyl-BA (28), 7-bromo-BA (29) and 7-fluoro-BA (30) the predominant enantiomers of the trans-3,4- and 8,9-dihydrodiol metabolites of 7-NO_2-BA also have R,R absolute stereochemistries. This indicates that the presence of a nitro substituent at the 7-position of BA does not significantly alter the stereoselective properties

of rat liver microsomal mixed-function oxidases and epoxide hydrolase in the formation of 7-NO_2-BA trans-3,4- and 8,9-dihydrodiol metabolites.

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