Stereoselectivity of Rat Liver Microsomal Enzymes in the Metabolism of 7-Fluorobenz(a)anthracene and Mutagenicity of Metabolites

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ABSTRACT

7-Fluorobenz(a)anthracene (7-FBA) was metabolized by rat liver microsomes predominantly to 4-hydroxy-7-FBA and 7-FBA trans-3,4-, 5,6-, 8,9-, and 10,11-dihydrodiols. Proton nuclear magnetic resonance spectral analyses indicated that the fluoro substituent causes 7-FBA trans-5,6- and trans-8,9-dihydrodiols to adopt preferentially quasidiaxial conformations (Chiu, P.-L., Fu, P. P., and Yang, S. K. Biochem. Biophys. Res. Commun., 106: 1405–1411, 1982). The major enantiomers of the quasidiaxial trans-3,4- and trans-8,9-dihydrodiols have been determined by the exciton chirality method to have R,R absolute stereochemistry. By comparing with the circular dichroism spectra of BA 3R,4R- and 10R,11R-dihydrodiols, the major enantiomers of the quasidiequatorial 7-FBA trans-3,4- and trans-10,11-dihydrodiols were also found to have R,R absolute configurations. All four 7-FBA trans-dihydrodiol metabolites obtained from incubations of 7-FBA with liver microsomes prepared from untreated and 3-methylcholanthrene- or phenobarbital-, and polychlorinated biphenyl-treated male Sprague-Dawley rats were enriched in R,R enantiomers, differing only in optical purities. Pretreatment of rats with phenobarbital, 3-methylcholanthrene, and polychlorinated biphenyls changed the rate of 7-FBA metabolism by liver microsomes predominantly to 4-hydroxy-7-FBA and 7-FBA trans-5,6- and trans-8,9-dihydrodiols by 0.47-, 1.14-, and 1.70-fold, respectively. Pretreatment of rats with enzyme inducers also altered the quantitative distribution of metabolites formed. The relative mutagenic activities of metabolites toward Salmonella typhimurium TA 100 were: 7-FBA trans-5,6-dihydrodiol > 7-FBA trans-10,11-dihydrodiol > 7-methyl-BA, 4-hydroxy-7-FBA > 7-FBA trans-8,9-dihydrodiol > 7-methyl-BA trans-10,11-dihydrodiol > 7-FBA trans-5,6-dihydrodiol ≈ 4-hydroxy-7-FBA. The relatively high mutagenic activities of 7-FBA trans-3,4- and trans-10,11-dihydrodiols suggest that both 7-FBA trans-3,4-dihydrodiol 1,2-epoxide(s) and 7-FBA trans-10,11-dihydrodiol 8,9-epoxide(s) may be the major metabolites which contribute to the carcinogenic properties of 7-FBA.

INTRODUCTION

Recent studies on the metabolic activation pathways of polycyclic aromatic hydrocarbons PAHs indicated that the conformation and absolute stereochemistry of metabolites in the epoxide → dihydrodiol → bay-region dihydrodiol-epoxide pathway are important factors in determining the carcinogenic potency of a PAH (10). The regio- and stereoselective properties of the cytochrome P-450-containing mixed-function oxidases and epoxide hydrolase in liver microsomes of rats have been extensively studied using BAP as the substrate (47, 48, 56). Pretreatment of rats with enzyme inducers may change the regioselectivity of liver microsomal enzymes in the metabolism of most unsubstituted and methyl-substituted PAHs (8, 27). In most of the PAHs studied, pretreatment of rats with enzyme inducers does not change the regioselective properties of the liver microsomes toward the substrate molecules (7, 46, 47). For example, dihydrodiols enriched in R,R enantiomers are formed from the metabolism of phenanthrene (39), chrysene (39), BA (46, 52), 11-methylbenz(a)anthracene (52), BAP (7, 47, 56), and 6-bromobenz(o)pyrene (18). However, dihydrodiol metabolites enriched in S,S enantiomers are also formed in the metabolism of 12-methylbenz(a)anthracene (15), 7-methylbenz(o)pyrene (7), and 8-methylbenz(a)anthracene (54).

Fluorine-substituted PAHs have been extensively used to probe the site(s) responsible for the carcinogenic properties of PAHs (2, 3, 5, 12, 22–26, 28, 37, 38). A fluoro substituent is believed to prevent the oxidative metabolism at the fluoro-substituted positions (5, 24–26, 38) and may also inhibit the oxidative metabolism at positions peri to the fluoro substituent (25). Although oxidative metabolism at the fluoro-substituted positions has not been reported, many examples indicate that a methyl group on the terminal benzo-ring of BAP and BA does not prevent the oxidative metabolism at the methyl-substituted aromatic double bond (30, 53, 55). A fluoro substituent is more electron-negative than a hydrogen or a methyl group and has a smaller single bond radius than a methyl group. It is known that the 5,6- and 8,9-dihydrodiols of 7-FBA are preferentially in quasidiaxial conformations due to electronic repulsion between the fluorne and the peri hydroxyl oxygen (6). Formation of relatively large amounts of 7-FBA, 5,6- and 8,9-dihydrodiols as metabolites of 7-FBA (Ref. 6 and this report) indicates that a methyl group on the terminal benzo-ring of BAP and BA does not prevent the oxidative metabolism at the methyl-substituted aromatic double bond (30, 53, 55). A fluoro substituent is more electron-negative than a hydrogen or a methyl group and has a smaller single bond radius than a methyl group. It is known that the 5,6- and 8,9-dihydrodiols of 7-FBA are preferentially in quasidiaxial conformations due to electronic repulsion between the fluorne and the peri hydroxyl oxygen (6). Formation of relatively large amounts of 7-FBA, 5,6- and 8,9-dihydrodiols as metabolites of 7-FBA (Ref. 6 and this report) indicates that a fluoro substituent at the C7-position of BA reduces, but does not prevent, the metabolism at positions peri to the fluoro group. BA is a very weak carcinogen. The tumor-initiating activity of BA is enhanced about 3-fold by a fluoro substituent and about 20-fold by a methyl substituent at the C7-position of BA (51). In an effort to understand the effects of a substituent on the metabolic activation and detoxification pathways of PAHs, we report in this paper the effects of a fluoro substituent at the C7-position of BA on the metabolic formations of various metabolites by rat liver microsomes. The effects of enzyme inductions of rat liver microsomal enzymes by PB, MC, and PCBs on the regioselective and stereoselective metabolism of 7-FBA have been studied. The absolute configurations of the dihydrodiol metabo-
lites of 7-FBA and the mutagenicity of 7-FBA metabolites toward Salmonella typhimurium TA100 are reported.

**MATERIALS AND METHODS**

**Materials.** 7-FBA was kindly given by Dr. Melvin Newman of the Ohio State University. 7-MBA was purchased from ICN Pharmaceuticals, Inc. (Cleveland, OH). PCBs were purchased from Analab (New Haven, CT). Sodium phenobarbital was purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). BA dihydrodiols and monohydroxybena(2)anthracenes were obtained from the Chemical Repository of the National Cancer Institute. 

**Procedure.** 7-MBA was synthesized according to the method of Harada et al. (20). Racemic 7-MBA trans-10,11-dihydrodiol was synthesized as described previously (16).

Immature male Sprague-Dawley rats (100 to 120 g) were given i.p. injections once a day of either sodium phenobarbital (80 mg/kg of body weight for 3 days), MC (25 mg/kg body weight for 4 days), or PCB (50 mg/kg body weight for 4 days), and were killed 1 day after the last injection. Livers were homogenized, and microsomes were prepared as described (9, 50).

**Preparation of Microsomes.** Liver microsomes were stored at -65°C before use. The protein content of liver microsomes was determined (35) with bovine serum albumin as the standard. Cytchrome P-450 content of liver microsomes was determined from the reduced carbon monoxide difference spectrum \((\Delta A_{600-550})\) by the method of Omura and Sato (40) using an extinction coefficient of 91 \(\text{mm}^{-1} \cdot \text{cm}^{-1}\). The cytochrome P-450 contents of liver microsomes from untreated, PB-, MC-, and PCB-treated rats were 0.45, 1.48, 1.52, and 1.63 \(\text{nmol/mg protein}\), respectively.

**Incubation of 7-FBA with Rat Liver Microsomes.** For circular dichroism spectral studies, dihydrodiol metabolites were prepared by incubation of 7-FBA in 100-ml incubation mixtures containing liver microsomes from untreated, PB-, MC-, or PCB-treated rats similarly as described (6). Large-scale incubations of 7-FBA with liver microsomes from PB-treated rats were carried out as described previously (6).

The rate of 7-FBA metabolism by various rat liver microsomes was studied in a 5-ml reaction mixture (pH 7.4) containing 0.25 mmol of Tris-HCl, 15 \(\mu\)mol of MgCl\(_2\), 3.25 mg of glucose-6-phosphate, 0.5 unit of glucose-6-phosphate dehydrogenase, 0.5 mg of NADP\(^+\), and 1.5 mg protein of liver microsomes from untreated or PB-treated rats, or 0.5 mg protein of liver microsomes from MC- or PCB-treated rats. The reaction mixture was preincubated at 37°C for 3 min, followed by the addition of 7-FBA (0.34 \(\mu\)mol in 0.2 ml methanol). The reaction mixture was further incubated at 37°C for 10 min. The reaction was stopped by the addition of acetone (10 ml), then 58 nmol (in 0.25 ml methanol) of 7,8,9,10-tetrahydro-BAP (\(\text{t}_{\text{R}}\) 79257) was added as an internal standard for chromatography. The internal standard, the unreacted substrate, and its metabolites were extracted with 20 ml of ethyl acetate. The resulting organic layer was evaporated under reduced pressure, and the residue was dissolved in 0.15 ml of methanol for HPLC analysis.

**HPLC.** 7-FBA and its metabolites were separated on a Waters Associates liquid chromatograph fitted with a Waters Associates CRC-100 Radial Compression Module containing a Radial-Pak 8C1805 cartridge. The metabolites were eluted with a linear gradient of methanol:water (3:2 \(v/v\)) to methanol over a period of 40 min at a solvent flow rate of 1.2 ml/min. Metabolites were detected by a Waters Associates Model 440 absorbance (254 nm) detector. The areas under the chromatographic peaks were recorded with a Hewlett Packard Model 3390A integrator.

**Quantification of the Metabolites Formed.** Since the same amount (58 nmol) of the internal standard was added to all the reaction mixtures, the relative amounts of each metabolite formed under different incubation conditions can be compared by determining the ratio of areas under the chromatographic peaks after they are normalized against the area under the chromatographic peak of the internal standard. For example, the ratio of a metabolite formed by PB microsomes and control microsomes is:

\[
\frac{(A_{\text{PB}}/A_{\text{C}})}{(A_{\text{IC}}/A_{\text{C}})} = \frac{A_{\text{PB}}}{A_{\text{IC}}}
\]

where \(A_{\text{PB}}, A_{\text{C}}, \) and \(A_{\text{IC}}\) are the areas under the chromatographic peaks of a metabolite formed by PB microsomes, control microsomes, and the internal standard, respectively. Thus, the effects of enzyme induction for the formation of different metabolites can be compared by determining the ratio of the area under the chromatographic peak against that of an internal standard.

The amount of each metabolite formed was determined by collecting all the metabolite peaks and the internal standard from an HPLC run in volumetric conical tubes, so that the total volume can be accurately determined. The \(\epsilon_{\text{max}}\) (extinction coefficient at maximal absorption) values of the dihydrodiol and phenolic metabolites of 7-FBA were assumed to be the same as those of the corresponding BA derivatives. They are: BA trans-3,4-dihydrodiol, \(\epsilon_{\text{max}}\) 10450 (34); BA trans-5,6-dihydrodiol, \(\epsilon_{\text{max}}\) 41800 (33, 46); BA trans-8,9-dihydrodiol, \(\epsilon_{\text{max}}\) 71950 (33); BA trans-10,11-dihydrodiol, \(\epsilon_{\text{max}}\) 67280 (33); and 4-OH-BA, \(\epsilon_{\text{max}}\) 48688 \(\text{M}^{-1} \cdot \text{cm}^{-1}\). The total amounts of each of the metabolites and the internal standard can then be determined from the value of the \(\epsilon_{\text{max}}\) and by measuring the UV absorbance at the absorption maximum. Since the amount of internal standard in each ml of reaction mixture is known, the amount of each metabolite formed in a 1-ml reaction mixture can be calculated. The \(\epsilon_{\text{max}}\) values of BA dihydrodiols were also used to determine the \(\epsilon\) values of the CD spectra of the 7-FBA dihydrodiol metabolites.

**Catalytic Hydrogenation of 7-FBA**

**Catalytic Hydrogenation of 7-FBA 8,9-Dihydrodiol.** 7-FBA trans-8,9-dihydrodiol metabolite (−0.1 mg) and PtO\(_2\) (−1.5 mg) in 2 ml THF were bubbled with hydrogen gas (1 atmosphere) for 1 hr at ambient temperature. After centrifugation, the solid material was removed, and the THF solution was evaporated. The residue was dissolved in 0.2 ml THF:methanol (1:1 \(v/v\)), and the products were isolated by HPLC on a Zorbax Sil column (6.2-mm inside diameter \(\times\) 25 cm), eluted with THF:hexane (3:7 \(v/v\)) at 2 ml/min. 7-FBA 8,9,10,11-tetrahydro-8,9-diol (retention time, 17 min; \(m/z\) of \(M^+\) at 282) and BA 8,9,10,11-tetrahydro-8,9-diol (retention time, 21 min; identical to an authentic compound) were obtained in a ratio of 30:1.

**Catalytic Hydrogenolysis of 7-FBA**

**Catalytic Hydrogenolysis of 7-FBA 5,6-Dihydrodiol.** 7-FBA trans-5,6-dihydrodiol metabolite (−0.5 mg) dissolved in THF (7 ml) was partially defluorinated by bubbling with hydrogen gas (1 atmosphere) in the presence of 10% palladium on activated charcoal (−1.5 mg) overnight at ambient temperature. Pd-C powder was removed by centrifugation. The THF solution was evaporated, and the residue was dissolved in 0.2 ml THF:methanol (1:1 \(v/v\)) for HPLC analysis. HPLC analysis was performed with a Zorbax ODS column (4.6-mm inside diameter \(\times\) 25 cm) with a 15-min linear gradient from methanol:water (11:9 \(v/v\)) to methanol at a solvent flow rate of 1.5 ml/min. 7-FBA 5,6-dihydrodiol and BA 5,6-dihydrodiol were eluted at 8.5 and 12 min, respectively. Under the conditions described, approximately 10% of the 7-FBA 5,6-dihydrodiol were defluorinated to form BA 5,6-dihydrodiol.

**Preparation of bis-p-N,N-Dimethylanilinozoate.** 7-FBA trans-5,6-dihydrodiol (−0.5 mg) was dissolved in 1 ml THF that had been dried by treating with Na\(_2\)H. Na\(_2\)H (−7 mg) was added, followed by p-N,N-dimethylanilinozoic acid (−6 mg). The solution was stirred at ambient temperature for 4 hr. Solid material was removed by centrifugation, and the supernatant was injected onto a DuPont Zorbax ODS column (4.6-mm inside diameter \(\times\) 25 cm). The column was eluted with a linear gradient of methanol:water (13:7 \(v/v\)) to methanol over a period of 15 min at a flow rate of 1.4 ml/min. The bis-p-N,N-dimethylanilinozoate was eluted at 20 min. The bis-p-N,N-dimethylanilinozoate of 7-FBA 8,9,10,11-tetrahydro-trans-5,6-diol was similarly prepared.

**Physicochemical Properties of Metabolites.** UV-visible absorption spectra of metabolites in methanol were measured on a Cary 118C spectrophotometer. Mass spectral analysis was performed on a Finnigan Model 4000 gas chromatograph-mass spectrometer-data system by electron impact ionization with a solid probe at 70 eV and 250°C ionizer temperature. Optical
rotations of dihydrodiol metabolites were measured in a Perkin-Elmer 241-MC polarimeter. Circular dichroism spectra of dihydrodiol metabolites in methanol were measured with a cell of 1-cm path length at room temperature on a Jasco Model 500A spectropolarimeter equipped with a Jasco Model DP-500 data processor. In cases where the extinction coefficient of a compound is not known, CD spectra are expressed by ellipticity ($[\theta]$ in m°) for methanol solutions that read 1.0 absorbance in a UV-vis spectrophotometer at the wavelength of maximum absorption in a quartz cell of 1-cm path length. The ellipticity and molar ellipticity ($[\theta]_m$ in degrees • sq cm/dmol) are related to molar extinction coefficient ($\epsilon_{\text{max}}$ in cm$^{-1}$ M$^{-1}$) as:

$$[\theta]_m = 0.1 \epsilon_{\text{max}} \phi,$$

CD spectral data obtained from different laboratories are more conveniently compared if they are expressed by $\phi$, when the $\epsilon_{\text{max}}$ value of a compound is not known.

**Mutagenicity Test.** *S. typhimurium* tester strain TA100 was obtained from Dr. Bruce Ames of the University of California, Berkeley, CA. S9 mixture containing NADPH-regenerating system was prepared according to the method of Ames et al. (1). Bacteria reaching late log phase were grown in oxoid broth at 37° in a water bath for 4.5 hr, and kept in ice bath throughout the experiment for not more than 3 hr.

Metabolites were purified by HPLC from a metabolite mixture obtained from the incubation of 7-FBA with liver microsomes from PCB-treated rats. Testing compound, dissolved in 0.1 ml dimethyl sulfoxide, was added to a 13 x 100-mm test tube containing 0.5 ml of S9 mixtures and 0.1 ml testing bacteria (8 x 10$^7$ cells). The reaction mixture was incubated in a 37° dry bath for 10 min prior to the addition of 2 ml top agar. The total mixture was then poured on a minimum glucose agar plate. The plate was incubated in a 37° incubator for 40 to 48 hr before the histidine revertant colonies on the plate were counted. The number of revertant colonies for each data point was an average of triplicate plates.

**RESULTS**

**HPLC Separation and Identification of Metabolites.** A reversed-phase HPLC separation of the metabolites obtained from an incubation of 7-FBA with MC-microsomes is shown in Chart 1. The major metabolites were dihydrodiols and phenols. The most abundant phenolic metabolite was identified as 4-OH-7-FBA. Its UV absorption spectrum (Chart 2) was similar to that of 4-OH-BA and was different from all the other isomeric monohydroxy-BA. Mass spectral analysis (Chart 3A) indicated molecular ions at m/z 262 and a fragment ion at m/z 233 which was characteristic of a phenolic compound. By comparing the UV-vis absorption spectra (Chart 4) with those of BA dihydrodiols (33), the metabolites eluted with retention times of 8, 12, 21, and 23 min (Chart 1) were identified to be 5,6-, 8,9-, 3,4-, and 10,11-dihydrodiols, respectively. Mass spectral analyses (Chart 3) were consistent with these structural assignments. $^1$H nuclear magnetic resonance analyses (6) indicated that the dihydrodiols are all trans isomers; the 5,6- and 8,9-dihydrodiols preferentially adopt quasidiaxial conformation, whereas the 3,4- and 10,11-dihydrodiols preferentially adopt quasiequatorial conformations.

**Absolute Configurations of 7-FBA 3,4- and 10,11-Dihydrodiols.** The CD spectra of the 7-FBA 3,4- and 10,11-dihydrodiols formed from the metabolism of 7-FBA by MC-microsomes are similar to those of the BA 3R,4R- and 10R,11R-dihydrodiols, respectively (Chart 4, A and D). The fluoro substituent is remote from the chiral centers and does not alter the quasiequatorial...
conformations of 7-FBA 3,4- and 10,11-dihydrodiols (6). We conclude that the major enantiomers of both 7-FBA 3,4- and 10,11-dihydrodiols have R,R absolute stereochemistries. Comparisons of the intensities of the Cotton effects in the CD spectra, shown in Chart 4, A and D, indicate that the metabolically formed 7-FBA 3,4- and 10,11-dihydrodiols are highly enriched in R,R enantiomers.

**Absolute Configuration of 7-FBA 5,6-Dihydrodiol.** The 7-FBA 5,6-dihydrodiol formed from the metabolism of 7-FBA by PCB-microsomes is optically active ([α]_D^25 -132° at 6.2 mg/ml, THF; -126° at 9.1 mg/ml, methanol). Most of the CD Cotton effects are opposite in sign to those of BA 5R,6R-dihydrodiol (Chart 4B). Comparison of the CD Cotton effects may suggest that the major enantiomer of the enzymatically formed 7-FBA 5,6-dihydrodiol has $S_S$ absolute configuration. Further investigation described below proved that this conclusion would have been incorrect.

In order to determine the absolute configuration, the enzymatically formed (−)-7-FBA trans-5,6-dihydrodiol in dried THF was treated with 10% Pd-C and was bubbled with hydrogen gas (1 atmosphere) for 15 hr at ambient temperature. 7-FBA trans-5,6-dihydrodiol was partially defluorinated to form a BA trans-5,6-dihydrodiol which had a CD spectrum (not shown) identical to that of BA 5R,6R-dihydrodiol ([R,R,S,S enantiomer ratio, 81:19; Refs. 46 and 52] obtained from the metabolism of BA by MCMicrosomes (Chart 4B). Thus, these results provide unequivocal proof that the enzymatically formed (−)-7-FBA trans-5,6-dihydrodiol has 5R,6R absolute configuration, and has an optical purity of 62%.

The enzymatically formed 7-FBA 5,6-dihydrodiol was also converted to a bis-p-N,N-dimethylethionictozone by reaction with p-N,N-dimethylethionictozone chloride in dried THF in the presence of NaH. The bis-p,N,N-dimethylethionictozone of 7-FBA 5,6-dihydrodiol, purified by reversed-phase HPLC, showed an intense UV absorption at 315 nm (Chart 5) and a negative CD band at 322 nm which was due to electronic transition dipole-dipole interactions between the benzoate groups (Chart 5) (21). The characteristic negative CD band at 322 nm of the exciton chirality spectrum (Chart 5) indicated that the (−)-7-FBA trans-5,6-dihydrodiol enantiomer formed from the metabolism of 7-FBA by PCB-microsomes has a 5R,6R absolute configuration (20, 21, 54). The Cotton effects between 280 and 315 nm in the exciton chirality spectrum (Chart 5) may be eliminated if the 8,9,10,11-ring of the 7-FBA 5,6-dihydrodiol is saturated. However, such an approach would not have changed the conclusion. For example, the bis-p-N,N-dimethylethionictozone of both phenanthrene 9S,10S-dihydrodiol and BA 5,6,8,9,10,11-hexahydro-5S,6S-diol showed a positive CD band at 322 nm (46) which is not significantly different (except the sign) from that shown in Chart 5. The above results indicate that the fluoro substituent at the C7-position of BA does not alter the stereoselective properties of the rat liver microsomal mixed-function oxidases and epoxide hydrolase in the metabolic formation of a 5,6-dihydrodiol.

As shown in Chart 4B, the CD Cotton effects of the quasidiauxial 7-FBA 5R,6R-dihydrodiol are different from those of the quasidiequatorial BA 5R,6R-dihydrodiol. These results provide evidence that the CD Cotton effects of an enantiomeric dihydrodiol are highly dependent on the conformation of the dihydrodiol. This finding is consistent with an earlier observation that the CD spectrum of quasidiaxial 6-bromobenzo(a)pyrene 4R,5R-dihydrodiol is different from that of quasidiequatorial benzo(a)pyrene 4R,5R-dihydrodiol (18).

**Absolute Configuration of 7-FBA 8,9-Dihydrodiol.** Several CD Cotton effects of the enzymatically formed 7-FBA 8,9-dihydrodiol ([α]_D^25 -21° at 0.44 mg/ml, methanol; -29° at 0.69 mg/ml, THF) are opposite in sign to those of BA 8R,9R-dihydrodiol (optical purity, 96%; Ref. 46) (Chart 4C). However, the 2 CD spectra are not mirror images of each other (Chart 4C). Therefore, the absolute configuration of the (−)-7-FBA trans-8,9-dihydrodiol cannot be determined by simple comparison of its CD spectrum with that of BA 8R,9R-dihydrodiol. Due to the strong CD Cotton effects between 300 and 330 nm of the 7-FBA 8,9-dihydrodiol (Chart 4C), the CD chirality spectrum of its bis-p-N,N-dimethylethionictozone derivative cannot be used to determine the absolute configuration of 7-FBA 8,9-dihydrodiol enantiomer. The strong CD Cotton effects between 300 and 330 nm (Chart 4C) were largely eliminated by catalytic hydrogenation of the dihydrodiol to 7-FBA 8,9,10,11-tetrahydro-trans-8,9-diol. The bis-p-N,N-dimethylethionictozone of the 8,9-tetrahydrodiol showed a pair of strong, symmetric Cotton effects; negative at 324 nm with $\phi_{224}$ 6.8° and positive at 303 nm with $\phi_{303}$ 9.4° which passed through zero at 314 nm (Chart 6). This exciton chirality spectrum indicates that the 7-FBA 8,9,10,11-tetrahydro-trans-8,9-diol has an 8R,9R absolute configuration (20, 21, 54). The predominant enantiomer of the enzymatically formed (−)-7-FBA-trans-8,9-dihydrodiol is, therefore, deduced to have an 8R,9R absolute stereochemistry. Comparison of the CD spectra of the 8R,9R-dihydrodiol isomers of BA and 7-FBA (Chart 4C) further corroborate the observation (Chart 4B; Refs. 4 and 18) that conformational change can drastically alter the CD Cotton effects of a dihydrodiol enantiomer of polycyclic aromatic hydrocarbons.

**Steroselectivity of Various Rat Liver Microsomal Preparations.** The effects of pretreatment of immature male Sprague-Dawley rats with enzyme inducers on the stereoselective prop-
properties of liver microsomes in the metabolism of 7-FBA were studied by determining the optical activities of dihydrodiol metabolites. As indicated by the results in Table 1, different rat liver microsomal preparations exhibit similar stereoselective preference with regard to the formation of dihydrodiol metabolites that are enriched in R,R enantiomers. However, the optical purities of the dihydrodiol metabolites differ, depending on the source of liver microsomes. With liver microsomes from untreated rats, the 3,4- and 8,9-dihydrodiols had the lowest optical purity, whereas the 10,11-dihydrodiol had the highest optical purity (Table 1). PB-microsomes had the highest stereoselectivity in catalyzing the formation of 3,4- and 5,6-dihydrodiols. The stereoselective properties of MC- and PB-microsomes were similar in catalyzing the formation of all 4 dihydrodiols. Except for the 5,6-dihydrodiol, the exact optical purities of the 3,4-, 8,9-, and 10,11-dihydrodiol metabolites of 7-FBA have not been determined.

Effects of Enzyme Induction on the Rate of 7-FBA Metabolism. Due to the lack of radiolabeled substrate, the rate of 7-FBA metabolism by various rat liver microsomal preparations was determined by the use of 7,8,9,10-tetrahydro-BAP as an internal standard for HPLC (Chart 1). The details of quantification

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Chart 4. A, UV absorption (---) and CD (——) spectra of the metabolically formed 7-FBA trans-3,4-dihydrodiol and the CD spectrum of an optically pure BA 3R,4R-dihydrodiol (—-; Refs. 46 and 52). B, UV absorption (---) and CD (——) spectra of the metabolically formed 7-FBA trans-5,6-dihydrodiol and the CD spectrum of BA 5R,6R-dihydrodiol (——; optical purity, 82%; Refs. 46 and 52). C, UV absorption (---) and CD (——) spectra of the metabolically formed 7-FBA trans-8,9-dihydrodiol and the CD spectrum of BA 8R,9R-dihydrodiol (——; optical purity, 96%; Refs. 46 and 52). D, UV absorption (---) and CD (——) spectra of the metabolically formed 7-FBA trans-10,11-dihydrodiol and the CD spectrum of BA 10R,11R-dihydrodiol (——; optical purity, 96%; Refs. 46 and 52). All compounds were dissolved in methanol for spectral measurement.
are described in “Materials and Methods.”

The specific activities for the formation of various metabolites in the metabolism of 7-FBA by 4 different rat liver microsomal preparations were determined and are summarized in Table 2. When compared with that of control microsomes, the rates of overall metabolism were approximately 0.47-, 1.14-, and 1.70-fold, due to pretreatment of the rats with PB, MC, and PCB, respectively. 4-OH-7-FBA and 7-FBA 8,9-dihydrodiol together constitute 64 and 72% of all the metabolites formed by PCB- and MC-microsomes, respectively. 7-FBA 10,11-dihydrodiol constitutes 52% of all the metabolites formed by liver microsomes from untreated rats. The K-region (5,6-double bond) of 7-FBA was the most favored site of metabolism by PB-microsomes. These results indicate that different rat liver microsomal preparations have different regioselective properties in the metabolism of 7-FBA. These regioselective properties of rat liver microsomal

Charts. UV-vis absorption and CD spectra of the bis-p-N,N-dimethylamino-benzoate of the metabolically formed 7-FBA trans-5,6-dihydrodiol. The ellipticity (m°) is expressed for a methanol solution of 1.0 absorbance unit/ml at 315 nm.

Table 1
Circular dichroism spectral data of the 7-FBA-dihydrodiol metabolites obtained by incubation of 7-FBA with 4 rat liver microsomal preparations.

<table>
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<th></th>
<th>3,4-Dihydrodial (251 nm)</th>
<th>5,6-Dihydrodial (242 nm)</th>
<th>8,9-Dihydrodial (316 nm)</th>
<th>10,11-Dihydrodial (307 nm)</th>
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<td>+2.36 (62)</td>
<td>+3.34 (62)</td>
<td>+2.70 (62)</td>
<td>-1.35 (62)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of optical purity (% of R,R enantiomer - % of S,S enantiomer), determined by defluorination as described in the text.

Table 2
Comparison of the metabolites formed from the metabolism of 7-FBA and BA by rat liver microsomes.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control-microsomes</th>
<th>PB-microsomes</th>
<th>MC-microsomes</th>
<th>PCB-microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-Dihydrodial</td>
<td>224</td>
<td>99</td>
<td>271</td>
<td>423</td>
</tr>
<tr>
<td>5,6-Dihydrodial</td>
<td>233</td>
<td>374</td>
<td>215</td>
<td>479</td>
</tr>
<tr>
<td>8,9-Dihydrodial</td>
<td>531</td>
<td>283</td>
<td>854</td>
<td>1157</td>
</tr>
<tr>
<td>10,11-Dihydrodial</td>
<td>1269</td>
<td>243</td>
<td>272</td>
<td>575</td>
</tr>
<tr>
<td>4-Hydroxy</td>
<td>52.4 (4.0)</td>
<td>21.4 (4.6)</td>
<td>9.8 (3.8)</td>
<td>14.0</td>
</tr>
<tr>
<td>Total metabolites identified</td>
<td>2419</td>
<td>1133</td>
<td>2767</td>
<td>4104</td>
</tr>
</tbody>
</table>

* Values are the average of duplicate samples which agree within 10% of the values shown. The experimental conditions are described in “Materials and Methods.”

Percentage of each metabolite formed.

Numbers in parentheses: dihydrodiol formed as the percentage of all the metabolites formed from the metabolism of BA. These data were taken from Tables 1 and 2 of Ref. 45 for comparison.
enzymes were also observed in the metabolism of other PAHs (8, 11, 27).

Mutagenic activities of 7-FBA Metabolites toward S. typhimurium TA100. Although 7-MBA is a much more potent carcinogen than 7-FBA or BA (11, 14, 44, 51), its mutagenicity toward S. typhimurium TA100 in the presence of rat liver S9 fraction is similar to that of 7-FBA or BA (Chart 7A). The mutagenic activities of the metabolites formed from the metabolism of 7-FBA by PCB-microsomes were each tested with S. typhimurium tester strain TA100. The relative mutagenic activity was: 7-FBA 3,4-dihydrodiol > 7-FBA 10,11-dihydrodiol > 7-MBA 10,11-dihydrodiol > 4-OH-7-FBA 5,6-dihydrodiol = 7-FBA 5,6-dihydrodiol.

DISCUSSION

The major metabolic sites differ between 7-FBA and BA (Table 2). For example, with liver microsomes from untreated rats, the 5,6-dihydrodiol was 44.4% of all the metabolites formed from BA metabolism, whereas it was 9.7% in the metabolism of 7-FBA. The 8,9-dihydrodiol was 41.5% of all the metabolites formed from BA metabolism, whereas it was 21.9% in the metabolism of 7-FBA. The 10,11-dihydrodiol was the most abundant metabolite (52.4%) formed from the metabolism of 7-FBA, but it was a minor metabolite of BA (45). The regioselective metabolism of 7-FBA and BA by PB- or MC-microsomes is also different (Table 2). Relatively more metabolism occurs at the 3,4- and 10,11-positions of 7-FBA than those of BA. The sum of 4-phenol and 3,4-dihydrodiol represents the total metabolism at the 3,4-double bond of BA or 7-FBA. About 16% of metabolism occurs at the 3,4-positions of 7-FBA (Table 2), whereas it was only about 3% in the metabolism of BA (45). Thus, a fluoro substituent at the 7-position of BA shifted the metabolism toward the 3,4-positions. Pretreatment of rats with PB or MC substantially enhanced the metabolism at the 3,4-positions of 7-FBA. When the percentages of metabolites formed from the metabolism of BA and 7-FBA are compared, it is apparent that the metabolic formation of the 5,6- and 8,9-dihydrodiol metabolites are reduced due to the presence of the peri 7-fluoro substituent. According to Sheikh et al. (42), the electronic effect of fluorine at C7 of BA should increase the monooxygenase reactions at the 3,4- and 10,11-positions and decrease at the 8,9-positions relative to the nonfluorinated parent hydrocarbon. The results of 7-FBA metabolism (Table 2) conform to the prediction made by Sheikh et al. (42). However, the proposed electronic effect of a fluoro substituent on metabolism (42) did not correctly predict the formations of 7,8- and 9,10-dihydrodiols from 6-F-BAP relative to that from BAP by rat liver microsomes (4).

BA is known to be stereoselectively metabolized by rat liver microsomes to 3,4-, 5,6-, 8,9-, and 10,11-dihydrodiols enriched in the R,R enantiomers (34, 46). In spite of the 7-fluoro substituent, 7-FBA is also metabolized to dihydrodiols that are enriched in R,R enantiomers. Thus, the fluoro substituent in 7-FBA does not significantly alter the stereoselective properties of the rat
liver microsomal mixed-function oxidases and epoxide hydrolase in the metabolic conversion of 7-FBA to the R,R dihydrodiols enantiomers. It remains to be established, however, if one or both of the 2 enzymatic reactions are responsible for the formation of dihydrodiols enriched in R,R enantiomers from the metabolism of 7-FBA.

REFERENCES

P-L. Chiu et al.


Stereoselectivity of Rat Liver Microsomal Enzymes in the Metabolism of 7-Fluorobenz(a)anthracene and Mutagenicity of Metabolites

Pei-Lu Chiu, Peter P. Fu and Shen K. Yang

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