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Stereoselective Metabolism of the (+)- and (−)-Enantiomers of *trans*-1,2-Dihydroxy-1,2-Dihydrochrysene to Bay-Region 1,2-Diol-3,4-Epoxide Diastereomers by Rat Liver Enzymes

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SUMMARY

Metabolism of the optically pure (+)- and (−)-enantiomers of chrysene 1,2-dihydrodiol by rat liver microsomes and by a reconstituted system has been examined. Microsomes prepared from control, phenobarbital-treated, or 3-methylcholanthrene-treated rats as well as a reconstituted system containing highly purified cytochrome P-450*c* stereoselectively metabolized (+)- and (−)-chrysene 1,2-dihydrodiols to its diastereomerically related bay-region 1,2-diol-3,4-epoxides. Bay-region diol epoxides constituted a higher percentage of total metabolites from (−)-chrysene (1R,2R)-dihydrodiol than from (+)-chrysene (1S,2S)-dihydrodiol. Microsomes from 3-methylcholanthrene-treated rats and the reconstituted system were the most regiospecific in that diol epoxides accounted for 65% and >80%, respectively, of the total metabolites whereas with microsomes from control and phenobarbital-treated rats, diol epoxides accounted for only 30% and 50%, respectively, of the total metabolites formed from (−)-chrysene (1R,2R)-dihydrodiol. In general, diol epoxide-1, in which the benzylic hydroxyl group and epoxide oxygen are *cis* to each other, was the major metabolite formed from (+)-chrysene (1S,2S)-dihydrodiol whereas (−)-chrysene (1R,2R)-dihydrodiol was predominantly metabolized to the diol epoxide-2 diastereomer, in which the benzylic hydroxyl group and epoxide oxygen have *trans* stereochemistry. The degree of stereoselectivity was dependent on the treatment of the rats. Microsomes from 3-methylcholanthrene-treated rats displayed the highest degree of stereoselectivity compared with microsomes from control or phenobarbital-treated rats. The ratio of diol epoxide-1 to diol epoxide-2 formed from the (+)-(1S,2S)-dihydrodiol by microsomes from 3-methylcholanthrene-treated rats was 6:1 and from the (−)-(1R,2R)-dihydrodiol was 1:20. The reconstituted system displayed even higher stereoselectivity. In addition to diol epoxides, a phenolic metabolite was identified from both the (+)- and (−)-dihydrodiols. This phenolic dihydrodiol is formed in significant amounts with microsomes from control and phenobarbital-treated rats, whereas microsomes from 3-methylcholanthrene-treated rats or the reconstituted system containing cytochrome P-450*c* produced very little or none of this metabolite. Addition of the phenolic dihydrodiol to incubations containing microsomes from 3-methylcholanthrene-treated rats caused marked inhibition of metabolism of (−)-chrysene (1R,2R)-dihydrodiol. Rates of metabolism of (+)-(1S,2S)-dihydrodiol compared with (−)-(1R,2R)-dihydrodiol were essentially similar for all enzyme systems used with the exception of control microsomes, which metabolized (+)-(1S,2S)-dihydrodiol at a greater rate (37%) than (−)-(1R,2R)-dihydrodiol. Both phenobarbital and 3-methylcholanthrene treatment of rats failed to induce metabolism of the enantiomeric dihydrodiols when the data were expressed per nanomole of cytochrome P-450. Phenobarbital treatment decreased the rate of metabolism by 45–59%, and 3-methylcholanthrene treatment decreased the rate by 13–43%. The reduction in rate of metabolism by 3-methylcholanthrene treatment is unusual for polycyclic aromatic hydrocarbon substrates.

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INTRODUCTION

Metabolism of benzo-ring *trans*-dihydrodiols with a bay-region double bond to diol epoxides is well established as the major pathway by which ultimate carcinogenic metabolites of polycyclic aromatic hydrocarbons are formed (1). The cytochrome P-450-dependent mixed-function oxidase system located in the endoplasmic reticulum of liver is primarily responsible for this often highly stereoselective biotransformation. Previous results from these and other laboratories have shown that metabolically formed B[*a*]P[3] 7,8-dihydrodiol, a proximate carcinogenic metabolite of the environmental carcinogen B[*a*]P, is metabolized predominantly to one of the four metabolically possible diol epoxides with enzyme preparations from rats (2–4), rabbits (5), humans (6), and fungi (7) as well as by prostaglandin synthetase (8, 9). Metabolism of pure enantiomers of B[*a*]P 7,8-dihydrodiol by liver microsomes isolated from 3-methylcholanthrene-treated rats indicated that (−)-(7R,8R)-dihydrodiol was metabolized to (+)-diol epoxide-2 and (−)-diol epoxide-1 in a ratio of 6:1. The degree of stereoselectivity was even higher for metabolism of the (+)-(7S,8S)-enantiomer; a (+)-diol epoxide-1 to (−)-diol epoxide-2 ratio of 19:1 was obtained (2). A reconstituted enzyme system containing highly purified cytochrome P-450*c* showed a similar degree of stereoselectivity (2). Recently, we demonstrated that phenanthrene 1,2-dihydrodiol is also metabolized to bay-region 1,2-diol-3,4-epoxides with a high degree of stereoselectivity (10). The relative amounts of diol epoxide-1 compared with diol epoxide-2 formed from (+)-(1S,2S)-dihydrodiol and (−)-(1R,2R)-dihydrodiol by liver microsomes from 3-methylcholanthrene-treated rats were 5.6:1 and 1:5.3, respectively. Biosynthetic chrysene 1,2-dihydrodiol [90% (−)-(1R,2R)-enantiomer] and (−)-BA (3R,4R)-dihydrodiol are also stereoselectively converted to diol epoxide-2 by liver microsomes from 3-methylcholanthrene-treated rats (11, 12). All of these studies indicate that (−)-(R,R)-dihydrodiols are predominantly metabolized to the (+)-diol epoxide-2 diastereomer. Interestingly, tumorigenicity studies have revealed that the (+)-diol epoxide-2 isomer is the most tumorigenic of the four isomers for both B[*a*]P (13) and chrysene.[4] The present study of metabolism of the (+)- and (−)-chrysene 1,2-dihydrodiols was undertaken to establish whether the stereospecificity of the cytochrome P-450 system toward these dihydrodiols parallels that observed for the corresponding phenanthrene 1,2- (10) and B[*a*]P 7,8-dihydrodiols (2). These results are of particular importance for our understanding of the steric requirements of the catalytic site of cytochrome P-450*c* (14), the predominant cytochrome P-450 in the livers of rats treated with 3-methylcholanthrene (15, 16). Structures of (+)- and (−)-enantiomers of chrysene 1,2-dihydrodiol are illustrated in Fig. 1.

MATERIALS AND METHODS

Dihydrodiol substrates. Enantiomerically pure (+)- and (−)-isomers of chrysene 1,2-dihydrodiol were synthesized as described (17); $[\alpha]_D$ −105°, +109° (c = 0.5, tetrahydrofuran). Both the (+)- and (−)-enantiomers were >99% chemically pure when analyzed by reverse-phase HPLC as described for incubated samples.

Synthetic metabolite standards. Diastereomeric bay-region diol epoxides-1 and -2 of chrysene in which the benzylic hydroxyl group and the epoxide oxygen are either *cis* (isomer-1 series) or *trans* (isomer-2 series) to each other were synthesized as described (18). Tetraols, which arise by *cis* and *trans* addition of water at benzylic carbon 4 of the epoxide (Fig. 2) were assigned relative stereochemistry by NMR and mass spectroscopy of their corresponding tetraacetates (18). A mixture of diol epoxide-1, 0.5 ml of dioxane, and 4.5 ml of Tris-perchlorate (0.1 M, pH 7.5) was maintained at 25° for 16 hr under argon to form keto-diol (18). After acidification to destroy residual diol epoxide, the keto-diol was separated from other products by HPLC (Fig. 3A). Keto-diol was reduced to a pair of triols on treatment with sodium borohydride for 10 min in water-methanol.

Enzyme preparations. Liver microsomes were prepared from control and treated male, immature (50–60 g) rats of the Long-Evans strain as described previously (19). The animals were treated daily with either phenobarbital (75 mg/kg, 3 days) or 3-methylcholanthrene (25 mg/kg, 4 days) by i.p. injection. Cytochrome P-450 contents were determined according to the method of Omura and Sato (20). Specific contents of cytochrome P-450 (nanomoles of P-450 per milligram of protein) were 0.67

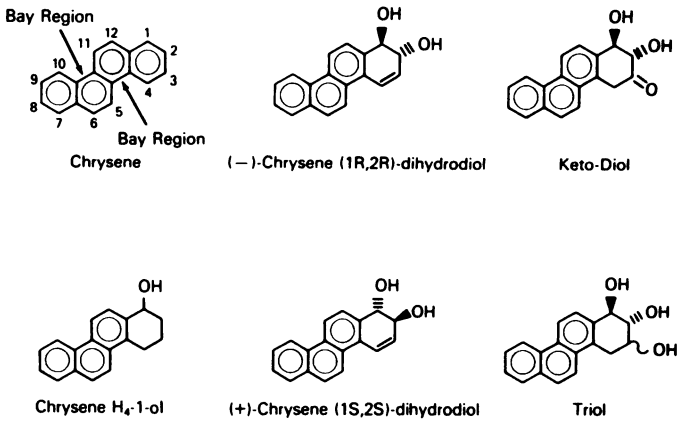

FIG. 1. *Absolute stereochemistry of (+)- and (−)-chrysene 1,2-dihydrodiols*

The keto-diol and -triols shown are potential metabolites. Chrysene H_4-1-ol was used as an internal standard.

[3] The abbreviations used are: B[*a*]P, benzo[*a*]pyrene; B[*e*]P, benzo[*e*]pyrene; BA, benz[*a*]anthracene; chrysene 1,2-dihydrodiol, *trans*-1,2-dihydroxy-1,2-dihydrochrysene; B[*a*]P 7,8-dihydrodiol, phenanthrene 1,2-dihydrodiol, BA 3,4-dihydrodiol, and chrysene 3,4-dihydrodiol are similarly abbreviated; chrysene 1,2-diol-3,4-epoxide-1 in which the benzylic hydroxyl group at carbon 1 and the epoxide oxygen are *cis*, (±)-1β,2α-dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydrochrysene; chrysene 1,2-diol-3,4-epoxide-2 in which the benzylic hydroxyl group at carbon 1 and the epoxide oxygen are *trans*, (±)-1β,2α-dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydrochrysene; similar abbreviations are used for bay-region diol epoxides of B[*a*]P and phenanthrene; chrysene H_4-1-ol, 1-hydroxy-1,2,3,4-tetrahydrochrysene; HPLC, high-pressure liquid chromatography.

[4] R. L. Chang, W. Levin, A. W. Wood, H. Yagi, M. Tada, K. P. Vyas, D. M. Jerina, and A. H. Conney. Tumorigenicity of enantiomers of chrysene 1,2-dihydriol and of the diastereomeric bay-region chrysene 1,2-diol-3,4-epoxides on mouse skin and in newborn mice. Submitted for publication.

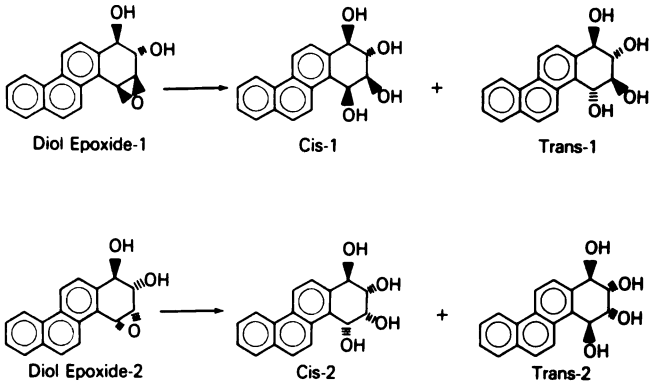

FIG. 2. *Tetraols formed from the diastereomeric diol epoxides-1 and -2 by cis (cis-1 and cis-2) and trans (trans-1 and trans-2) addition of water at benzylic carbon 4 of the diol epoxides*

The absolute stereochemistries shown are based on recent synthetic studies

for microsomes from control rats, 1.89 for microsomes from phenobarbital-treated rats, and 2.21 for microsomes from 3-methylcholanthrene-treated rats. Highly purified cytochrome P-450*c* (21), cytochrome P-450*b* (21), and NADPH-cytochrome *c* reductase (22) were prepared by previously described methods.

Incubations. Standard incubation mixtures contained 200 μmoles of potassium phosphate buffer (pH 7.4), 6 μmoles of $MgCl_2$, 2 μmoles of NADPH, 100 nmoles of substrate dissolved in 0.1 ml of acetone, and 0.25–8.0 mg of microsomal protein. Incubations with the reconstituted system contained 0.05–0.8 nmole of cytochrome P-450*c* or cytochrome P-450*b*, 3000 units of NADPH-cytochrome *c* reductase, 60 μg of lipid, and 200 μmoles of potassium phosphate buffer (pH 7.2) in addition to $MgCl_2$, NADPH, and substrate as above. Final incubation volumes were 2.0 ml. All reaction mixtures were incubated for 10 min at 37° with gentle shaking. Enzymatic reactions were terminated by the addition of 140 μl of 1.5 M $HClO_4$ (final pH ~ 2.0). Reaction mixtures were stored in the dark at room temperature for 2 hr before neutralization with 100 μl of saturated Tris base (final pH ≃ 7.0). Acid treatment was required to convert residual diol epoxides to tetraols as described (11). A solution of 10 nmoles of chrysene H_4-1-ol in 50 μl of methanol was added to the mixture as an internal standard, and the mixture was extracted with 6 ml of 2:1 ethyl acetate/acetone. After centrifugation, 5 ml of organic phase were carefully removed, dried over anhydrous sodium sulfate, and concentrated under a stream of nitrogen. Controls consisted of zero-time incubations and were treated in a similar manner.

Analysis of metabolites by HPLC. A Spectra Physics model 3500B HPLC system was used to analyze incubation samples. Concentrated extracts of incubation samples were dissolved in 100 μl of methanol, and aliquots were injected on a DuPont Zorbax ODS column (6.2 mm × 25 cm) eluted with a linear gradient of 50%–90% methanol in water at a rate of change of 1%/min after an initial 1-min delay. The column was eluted at a flow rate of 1.2 ml/min, and the eluent was monitored at 255 nm with a Schoeffel Instruments Model SF-770 UV-VIS variable wavelength detector. The value of 255 nm represents the approximate λ_{max} of the tetraols in the water-methanol compositions at which they emerge from the column, thus optimizing the detection of tetraols. Area under peaks was integrated with an Autolab System IV. UV spectra were recorded with a Hewlett-Packard 8450A UV/VIS spectrophotometer.

Quantitation of dihydrodiol metabolism. The quantitation of dihydrodiol metabolism was carried out using chrysene H_4-1-ol as an internal standard. Two calibration curves were constructed. One curve, used to determine the amount of dihydrodiol metabolized, was constructed by plotting ratios of the area under the dihydrodiol peak to that of internal standard peak versus nanomoles of dihydrodiol. A second curve, used to determine the amount of tetraols formed, was constructed by plotting ratios of area under tetraol peak to that of internal standard peak versus nanomoles of tetraol. Plotted in this way, the results are independent of the sample size injected. All samples for both calibration curves are corrected for recovery since they are obtained from zero-time incubations. Linear regression analysis indicated a correlation of 99.9% for both curves.

RESULTS

The percentage conversion and rates of metabolism of (+)- and (−)-chrysene 1,2-dihydrodiols by liver microsomes from control and induced rats are shown in Table 1. Microsomes from control rats metabolized the (+)- and (−)-enantiomers of chrysene 1,2-dihydrodiol at rates of 3.58 and 2.60 nmoles/min/nmole of cytochrome P-450, respectively. Prior treatment of rats with inducers of the cytochrome P-450 enzyme system failed to enhance rates of metabolism. Although prior treatment of rats with 3-methylcholanthrene increased the total amount of chrysene 1,2-dihydrodiol metabolized per milligram of protein when compared with microsomes from control rats, it actually decreased the nanomoles of dihydrodiol metabolized per nanomole of cytochrome P-450 per minute by 13–43% compared with control microsomes. Phenobarbital treatment decreased the rate of metabolism by 45–59% when calculated per nanomole of cytochrome P-450. Although microsomes from control rats metabolized the (+)-dihydrodiol at a slightly higher rate than the (−)-dihydrodiol, both enantiomers were metabolized at the same rate after either phenobarbital or 3-methylcholanthrene treatment.

Relative amounts of diol epoxides-1 and -2 formed from (+)- and (−)-chrysene 1,2-dihydrodiols by the three rat liver microsomal preparations are shown in Table 2. HPLC profile of metabolites formed from (+)- and (−)-chrysene 1,2-dihydrodiol by liver microsomes from 3-methylcholanthrene-treated rats are shown in Fig. 3. Diol epoxides were detected and quantitated as their solvolysis products, the *cis* and *trans* tetraols. The ratio of bay-region diol epoxides formed was dependent on both the enzyme preparation and the enantiomer used. Bay-region diol epoxides represented as little as 15% of the total metabolites when the (+)-(1S,2S)-enantiomer was metabolized by control microsomes, and represented as much as 66% of total metabolites with the (−)-(1R,2R)-enantiomer as substrate and microsomes from 3-methylcholanthrene-treated rats.

Formation of diol epoxides was found to be stereose-

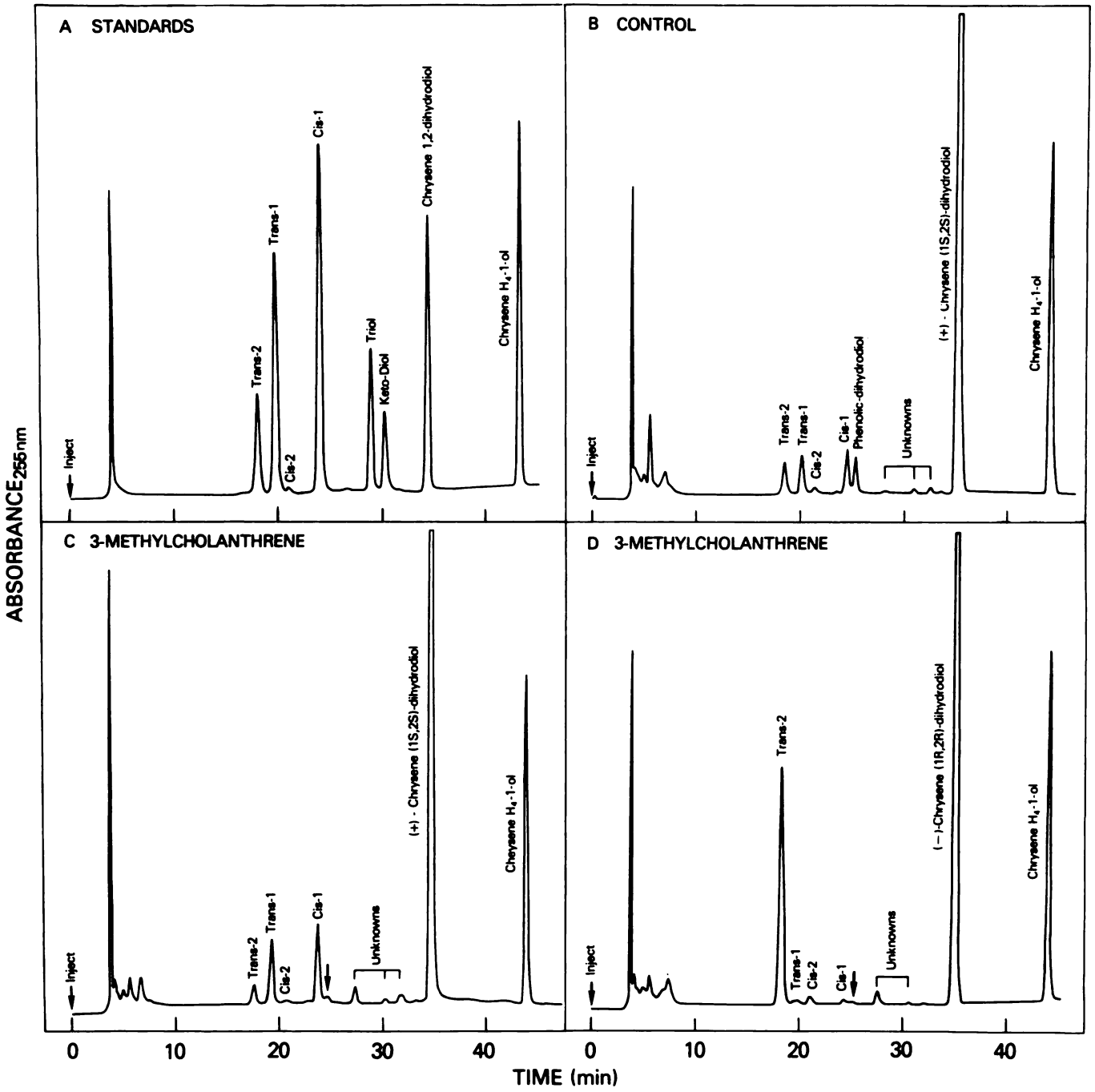

FIG. 3. *Chromatographic separations by HPLC*

A. Synthetic standards of the tetraols, derived from acid-catalyzed hydrolysis of the chrysene 1,2-diol-3,4-epoxides-1 and -2, the keto-diol formed by spontaneous isomerization of chrysene 1,2-diol-3,4-epoxide-1 at neutral pH, triols which result upon reduction of the above keto-diol, chrysene 1,2-dihydrodiol, and an internal standard chrysene H_4-1-ol.

B. Metabolites of (+)-chrysene 1,2-dihydrodiol formed by liver microsomes from control rats.

C and D. Metabolites of (+)- and (−)-chrysene 1,2-dihydrodiol, respectively, formed by liver microsomes from 3-methylcholanthrene-treated rats. The *arrow* in C and D designates the point of elution of the phenolic dihydrodiol. The chromatographic system used for the separation of synthetic standards as well as metabolites is as described under Materials and Methods.

lective. In general, the diol epoxide-1 diastereomer was the major metabolite of (+)-(1S,2S)-dihydrodiol, whereas the diol epoxide-2 diastereomer was the major metabolite of (−)-(1R,2R)-dihydrodiol. The degree of stereoselectivity depended on the type of enzyme preparation used. Microsomes from 3-methylcholanthrene-treated rats showed a higher degree of stereoselectivity when compared with microsomes from control or phenobarbital-treated rats. Microsomes from 3-methylcholanthrene-treated rats metabolized (+)-chrysene (1S,2S)-dihydrodiol to (+)-diol epoxide-1 and (−)-diol epoxide-2 in a ratio of 6:1, whereas the relative amount of (+)-diol epoxide-2 and (−)-diol epoxide-1 formed from (−)-chrysene (1R,2R)-dihydrodiol was 20:1.

TABLE 1

Metabolism of (+)- and (−)-chrysene 1,2-dihydrodiols by liver microsomes from control and induced rats

Experimental conditions were as described under Materials and Methods. The substrate concentration was 100 nmoles/incubation and the protein concentration was 0.5 mg/incubation in a final volume of 2.0 ml.

Dihydrodiol enantiomer	Treatment	% Dihydrodiol metabolized	Rate[a]
(+)-(1S,2S)	Control	12.0	3.58
	Phenobarbital	14.0	1.47
	3-Methylcholanthrene	22.6	2.05
(−)-(1R,2R)	Control	8.7	2.60
	Phenobarbital	13.5	1.42
	3-Methylcholanthrene	24.9	2.25

[a] Rate of metabolism is expressed as nanomoles of dihydrodiol metabolized per nanomole of cytochrome P-450 per minute.

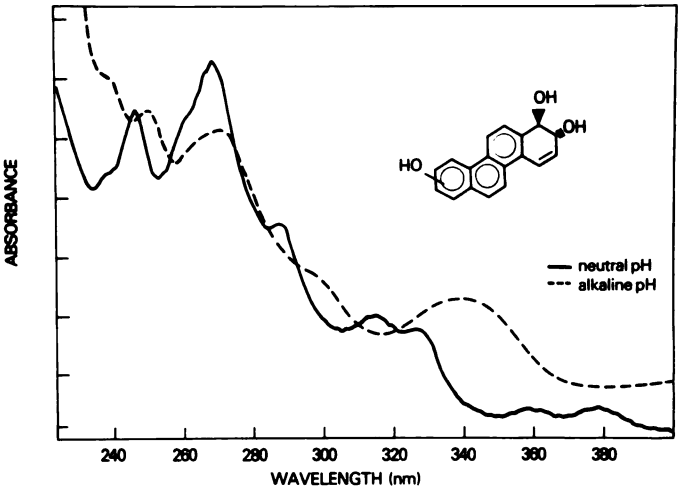

FIG. 4. *UV spectra of the phenolic dihydrodiol in water-methanol (3:7) before and after the addition of sodium hydroxide*

In addition to diol epoxides, several unknown metabolites were detected. One of these was formed in a sufficient amount to allow its characterization. This metabolite, which eluted immediately following *cis*-1 tetraol on HPLC (Fig. 3B), is tentatively identified as a phenolic dihydrodiol. The UV spectrum of the phenolic dihydrodiol (Fig. 4) underwent a red shift characteristic of phenols when measured in an alkaline medium. Neutralization restored the original spectrum. The chemical ionization mass spectrum of the metabolite with NO-N_2 gas gave a molecular ion at m/e 278 [M^+] with a base peak at m/e 260 [M^+-H_2O]. The molecular weight of 278 is 16 mass units above that of the dihydrodiol. The combination of the UV and mass spectral data indicate that the metabolite is a phenolic dihydrodiol. Although the exact position of the introduced hydroxyl group is presently unknown, the benzo-ring remote from the dihydrodiol ring seems most probable since the vast majority of the metabolism of chrysene occurs on the benzo-rings (11). Both the (+)- and (−)-dihydrodiols serve as precursors to the phenolic dihydrodiol. Although only trace amounts of this metabolite were formed by microsomes from 3-methylcholanthrene-treated rats, significant amounts of the phenolic dihydrodiol were formed by microsomes from phenobarbital-treated rats. Detectable amounts of keto-diol or triol metabolites were not observed.

The rate of metabolism of (−)-chrysene (1R,2R)-dihydrodiol by liver microsomes from 3-methylcholanthrene-treated rats was independent of protein concentration from 0.25 mg to 1.0 mg of protein per 2.0-ml incubation (Table 3). At a protein concentration of 2.0 mg/2.0-ml incubation (57% metabolism), the rate of metabolism began to decrease. Linearity with protein concentration for the metabolism of (−)-chrysene (1R,2R)-dihydrodiol was not observed with microsomes from control and phenobarbital-treated rats even at very low protein concentrations (Table 3). Parallel results were obtained with (+)-chrysene (1S,2S)-dihydrodiol as substrate for the three microsomal preparations (data not shown). The formation of substantial amounts of the phenolic dihydrodiol with microsomes from control and phenobarbital-treated rats suggested to us that it may be an inhibitor of the cytochrome P-450 system. Microsomes from 3-methylcholanthrene-treated rats formed only trace amounts of this metabolite. Addition of the phenolic dihydrodiol to incubation mixtures containing microsomes from 3-methylcholanthrene-treated rats caused inhibition of the metabolism of (−)-chrysene (1R,2R)-dihydrodiol (Table 4). Concentrations of the phenolic dihydrodiol were selected such that they bracketed the

TABLE 2

Relative amounts of diol epoxides (DE) formed from (+)- and (−)-chrysene 1,2-dihydrodiols by liver microsomes from control and induced rats

Experimental conditions were as described under Materials and Methods. The substrate concentration was 100 nmoles/incubation and the protein concentration was 0.5 mg/incubation in a final volume of 2.0 ml.

Isomer	Treatment	Individual tetraols as % of total tetroals				DE as % of total metabolites[a]	DE-1/DE-2
		trans-2	*cis*-2	*trans*-1	*cis*-1		
(+)-(1S,2S)	Control	23.0	7.5	34.1	35.4	15.4	2.3/1
	Phenobarbital	32.1	8.6	29.3	29.8	23.6	1.4/1
	3-Methylcholanthrene	12.0	2.4	39.2	46.5	38.2	6.0/1
(−)-(1R,2R)	Control	79.1	5.2	10.5	5.2	29.5	1/5.3
	Phenobarbital	82.8	4.7	7.6	4.9	49.8	1/7.0
	3-Methylcholanthrene	91.7	3.5	3.0	1.8	65.8	1/20.0

[a] Values were obtained by dividing total nanomoles of tetraols formed by nanomoles of dihydrodiol metabolized based on the two calibration curves.

TABLE 3

Effect of protein concentration on the metabolism of (−)-chrysene (1R,2R)-dihydrodiol by liver microsomes from control and induced rats

Experimental conditions were as described under Materials and Methods. The substrate concentration was 100 nmoles/2.0-ml incubation. DE, Diol epoxide.

Treatment	Protein concentration (mg/2.0-ml incubation)	% Dihydrodiol metabolized	Rate[a]	DE-1/DE-2
Control	0.5	8.7	2.60	1/5.3
	1.0	9.3	1.39	1/6.5
	2.0	16.7	1.24	1/7.0
	4.0	16.7	0.62	1/9.4
	8.0	19.3	0.36	1/9.3
Phenobarbital	0.5	13.5	1.42	1/7.0
	1.0	21.1	1.12	1/8.6
	2.0	23.1	0.61	1/9.4
	4.0	29.8	0.39	1/11
	8.0	21.8[b]	0.14	1/5.9
3-Methylcholanthrene	0.25	10.4	1.90	1/20
	0.5	24.9	2.25	1/20
	1.0	39.6	1.79	1/30
	2.0	56.3	1.27	1/32

[a] Rate of metabolism is expressed as nanomoles of dihydrodiol metabolized per nanomole of cytochrome P-450 per minute.

[b] At a protein concentration of 8.0 mg/2.0-ml incubation, the percentage conversion actually decreased in two separate experiments. Similar data were obtained for (+)-(1S,2S) enantiomer.

amounts isolated from similar incubations with liver microsomes from control rats. The data clearly indicate that the phenolic dihydrodiol produces a dose-dependent inhibition of the metabolism of the (−)-(1R,2R)-dihydrodiol by liver microsomes from 3-methylcholanthrene-treated rats. The phenolic dihydrodiol used in this experiment was isolated from a large-scale (50 ml) incubation containing 2.5 μmoles of substrate and 100 mg of microsomal protein from untreated rats. The data in Table 3 also show the relative amounts of the diastereomeric diol epoxides formed from (−)-chrysene (1R,2R)-dihydrodiol by all three microsomal preparations at several protein concentrations. The stereoselectivity of the formation of the (+)-diol epoxide-2 diastereomer increased to some extent as the protein concentration was increased for all three microsomal preparations.

TABLE 4

Effect of the phenolic dihydrodiol on metabolism of (−)-chrysene (1R,2R)-dihydrodiol by liver microsomes from 3-methylcholanthrene-treated rats

The phenolic dihydrodiol was isolated and purified by reverse-phase HPLC (see Materials and Methods and legend to Fig. 3) from a large-scale (50-ml) incubation (15 min) containing 2.5 μmoles of substrate and 100 mg of microsomal protein from untreated rats. The phenolic dihydrodiol was dissolved in methanol and added to the 2.0-ml incubation mixtures. Methanol (0–20 μl) was added to incubation samples such that all samples contained 20 μl of methanol.

Phenolic dihydrodiol ($A_{269\ nm}$ units)	% Dihydrodiol metabolized	% Inhibition
0.0	23.0[a]	0
0.02	20.8	10
0.05	20.0	13
0.10	18.8	18
0.20	15.2	34

[a] This corresponds to a rate of metabolism 1.95 nmoles of dihydrodiol consumed per nanomole of cytochrome P-450 per minute. The protein concentration was 1.0 mg/2.0-ml incubation. A comparable incubation with 1.0 mg of microsomal protein from control rats would have led to the isolation of about 0.05 $A_{269\ nm}$ units of the phenolic dihydrodiol.

Metabolism of enantiomeric dihydrodiols was also examined with the reconstituted system containing cytochrome P-450*c* and cytochrome P-450*b*. Cytochrome P-450*c* is the major form of cytochrome P-450 induced by 3-methylcholanthrene, and cytochrome P-450*b* is the major cytochrome P-450 induced by phenobarbital treatment (15, 16). Metabolism of (+)- and (−)-chrysene 1,2-dihydrodiols by the reconstituted system containing different concentrations of cytochrome P-450*c* is shown in Table 5. The rate of metabolism of both enantiomers was constant at all concentrations of cytochrome P-450*c* tested. Cytochrome P-450*c* metabolizes the (+)- and (−)-dihydrodiols very efficiently and essentially at similar rates. Bay-region diol epoxides were major metabolites formed from both dihydrodiols. Formation of the diastereomeric diol epoxides was highly stereoselective. The (−)-(1R,2R)-dihydrodiol was exclusively metabolized to (+)-diol epoxide-2, whereas the (+)-(1S,2S)-dihydrodiol was predominantly converted to the (+)-diol epoxide-1 diastereomer. The degree of stereoselectivity is even higher than that obtained with liver microsomes from 3-methylcholanthrene-treated rats. Interestingly, not even trace amounts of the phenolic dihydrodiol were detected. Both enantiomeric dihydrodiols were found to be very poor substrates for the highly purified cytochrome P-450*b* system. Even at a concentration of 0.8 nmole of cytochrome P-450*b* per 2-ml incubation, metabolism of either enantiomer could not be detected.

DISCUSSION

In parallel with previous studies on the formation of bay-region diol epoxides from the (+)- and (−)-enantiomers of B[*a*]P 7,8- (2) and phenanthrene 1,2-dihydrodiols (10), formation of the diastereomeric chrysene 1,2-diol-3,4-epoxides-1 and -2 has been found to be highly ster-

TABLE 5

Metabolism of (+)- and (−)-chrysene 1,2-dihydrodiol by the reconstituted enzyme system containing highly purified cytochrome P-450c

Experimental conditions were as described under Materials and Methods. The substrate concentration was 100 nmoles/2.0 ml incubation.

Isomer	Cytochrome P-450*c* (nmoles/2.0-ml incubation)	% Dihydrodiol metabolized	Rate[a]	Relative amounts of diol epoxides formed as % of total metabolites[b]	
				DE-1	DE-2
(−)-(1R,2R)	0.05	8.4	16.7	ND[c]	100
	0.10	15.4	15.4	ND	>99
	0.20	27.5	13.8	ND	80[d]
(+)-(1S,2S)	0.05	7.5	15.0	58	5.2
	0.10	14.2	14.2	63	6.1
	0.20	30.3	15.2	42	3.5

[a] Rate of metabolism is expressed as nanomoles of dihydrodiol metabolized per nanomole of cytochrome P-450*c* per minute.

[b] See Table 2, footnote *a*.

[c] Not detected.

[d] In this and the following entries, diol epoxides were not the sole metabolites based on the amount of dihydrodiol consumed and the amount of tetraols formed. Several other peaks were present on the HPLC profiles.

eoselective and dependent on the microsomal preparation. With microsomes from 3-methylcholanthrene-treated rats, (−)-chrysene (1R,2R)-dihydrodiol was metabolized predominantly to (+)-diol epoxide-2, whereas (+)-chrysene (1S,2S)-dihydrodiol was metabolized mainly to (+)-diol epoxide-1. The degree of stereoselectivity was even higher with the cytochrome P-450*c*-dependent reconstituted system to the extent that only diol epoxide-2 was detected from the (−)-dihydrodiol (Table 5). Cytochrome P-450*c* constitutes >70% of cytochromes P-450 present in liver microsomes from 3-methylcholanthrene-treated rats (15, 16). Of the remaining 20–30%, cytochromes P-450*a* and P-450*b* make up 11% and <3% of the total cytochrome P-450, respectively, and are low in catalytic activity toward most polycyclic hydrocarbon substrates (21). Thus for B[*a*]P, phenanthrene, and chrysene, cytochrome P-450*c* stereoselectively metabolizes the (−)-R,R-enantiomer to (+)-diol epoxide-2 and the (+)-S,S-enantiomer to (+)-diol epoxide-1. These results are in good accord with the predictions of a stereochemical model for the catalytic binding site of cytochrome P-450*c* (14). Microsomes from either control or phenobarbital-treated rats were much less stereoselective in forming the diol epoxide diastereomers from the chrysene 1,2-dihydrodiols, as was previously found to be the case for the (+)- and (−)-enantiomers of phenanthrene 1,2- (10) and B[*a*]P 7,8-dihydrodiols (2). Cytochromes P-450*a*, P-450*b*, and P-450*c* constitute 7%, 4%, and 4%, respectively, of the cytochromes P-450 in liver microsomes from control rats and 7%, 57%, and 2%, respectively, of the cytochromes P-450 in liver microsomes from phenobarbital-treated rats (15). Thus, the decreased stereospecificity with these microsomes is probably due to metabolism by presently uncharacterized cytochromes P-450 in these preparations.

The regiospecificity of rat liver microsomes in the formation of bay-region diol epoxides from dihydrodiols of B[*a*]P, BA, phenanthrene, and chrysene is also dependent on prior treatment of the rats. In general, microsomes from 3-methylcholanthrene-treated rats show the highest regiospecificity. The lower regiospecificity, as well as previously mentioned diastereomer specificity, by microsomes from control and phenobarbital-treated rats is best explained by the presence of substantial amounts of presently uncharacterized cytochromes P-450 (15) with differing regiospecificities in these microsomes. Cytochrome P-450*b*, which constitutes more than one-half of the total P-450 in liver microsomes from phenobarbital-treated rats, actually failed to produce detectable metabolites from the chrysene 1,2-dihydrodiol despite the fact that microsomes from phenobarbital-treated rats effectively metabolize the dihydrodiol. Furthermore, with microsomes from 3-methylcholanthrene-treated rats in particular, the percentage of total metabolites represented by bay-region diol epoxides is highly substrate-dependent: as much as 95% from B[*a*]P 7,8-dihydrodiol (2) and as little as 16% for BA 3,4-dihydrodiol (12). Chrysene 1,2-dihydrodiol (38–65%) is intermediate.

Chrysene 1,2-diol-3,4-epoxides accounted for only 15–30% of the total metabolites when microsomes from control rats were used as the source of enzyme. However a phenolic dihydrodiol was formed from both enantiomers of chrysene 1,2-dihydrodiol. Whether this phenolic dihydrodiol arises via rearrangement of a labile arene oxide of the dihydrodiol or via dehydration of a labile *bis*-dihydrodiol is unknown. This metabolite was also produced by microsomes from phenobarbital-treated rats and in only trace amounts by microsomes from 3-methylcholanthrene-treated rats. Studies with the reconstituted system indicated that the phenolic dihydrodiol was not a product of either cytochrome P-450*b* or P-450*c*. Phenolic dihydrodiols have also been detected as metabolites of B[*a*]P 7,8-dihydrodiol (2) and B[*e*]P 9,10-dihydrodiol (23).

Although the rate at which the (−)-chrysene (1R,2R)-dihydrodiol was metabolized was proportional to protein concentration over a several-fold range with microsomes from 3-methylcholanthrene-treated rats, such was not the case for microsomes from control or phenobarbital-treated rats (Table 3). Sensitivity of the assay precluded study of lower protein concentrations. At least part of the nonlinearity with these microsomes may be due to their formation of significant amounts of the phenolic dihydrodiol, which was shown to be an effective inhibitor of metabolism of (−)-chrysene (1R,2R)-dihydrodiol by microsomes from 3-methylcholanthrene-treated rats

(Table 4). Several related examples where product phenols inhibit substrate metabolism are now known: e.g., oxyphenbutazone inhibits metabolism of phenylbutazone, and 4-hydroxy-antipyrine inhibits antipyrine metabolism (24); hydroxybenz[*a*]anthracenes inhibit metabolism of BA (25, 26), and quinones of B[*a*]P or possibly their related hydroquinones inhibit the metabolism of B[*a*]P and B[*a*]P 7,8-dihydrodiol (27, 28).

The rate of hepatic microsomal metabolism (nanomoles of product per nanomole of cytochrome P-450 per minute) of many polycyclic aromatic hydrocarbons is markedly stimulated (2- to 30-fold) by treatment of rats with 3-methylcholanthrene. With dihydrodiols as substrates, 3-methylcholanthrene treatment has varying results. Although metabolism of B[*a*]P 7,8- (2) and BA 3,4-dihydrodiol (12) is markedly stimulated, metabolism of phenanthrene 1,2- (10) and chrysene 3,4-dihydrodiols (29) is little affected. The rate of metabolism of (+)-chrysene (1S,2S)-dihydrodiol actually decreased by 43% upon treatment of rats with 3-methylcholanthrene in the present study. Notably, microsomes from control rats metabolized the (+)-1,2-dihydrodiol 4- to 20-fold better (nanomoles metabolized per minute per nanomole of cytochrome P-450) than other dihydrodiols with bay-region double bonds such as those from B[*a*]P, BA, and phenanthrene. The decrease in the rate of dihydrodiol metabolism by phenobarbital treatment clearly results from the fact that the dihydrodiol is a very poor substrate (if a substrate at all) for cytochrome P-450*b*, which constitutes 57% of the total cytochrome P-450 in these microsomes.

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