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Metabolism of benzo[*a*]pyrene: Conversion of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to highly mutagenic 7,8-diol-9,10-epoxides*

(benzo[*a*]pyrene 7,8-dihydrodiol metabolism/chemical carcinogenesis/high-pressure liquid chromatography/liver microsomes/cytochrome *P*-450)

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ABSTRACT **Metabolites of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene formed by rat liver microsomes and by a highly purified monoxygenase system were analyzed by high-pressure liquid chromatography. Four stereoisomeric tetraols of 7,8,9,10-tetrahydrobenzo[*a*]pyrene, known solvolysis products of the two highly mutagenic stereoisomers of the 9,10-epoxide of the 7,8-dihydrodiol, were identified as products. The ratio of the two highly unstable diol epoxides formed (7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, diol epoxide 1; 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, diol epoxide 2) ranged from about 1.7 to 0.4. The diol epoxides are sufficiently reactive to alkylate phosphate buffer (pH 7.4) at 37°. Microsomes, particularly those from control animals, formed a substantial amount of an additional metabolite that appears to be phenolic. In analogy to benzo[*a*]pyrene, the metabolism of the 7,8-dihydrodiol shows similar induction after pretreatment of rats with phenobarbital or 3-methylcholanthrene. *Neither diol epoxide appears to be a substrate for epoxide hydrase* based on the ratios of tetraols formed in the presence or absence of epoxide hydrase. In view of the known carcinogenicity of benzo[*a*]pyrene 7,8-oxide and 7,8-dihydrodiol and of the marked mutagenicity of the stereoisomeric diol epoxides, both of these diol epoxides qualify for consideration as "ultimate carcinogen(s)" of benzo[*a*]pyrene.**

The polycyclic aromatic hydrocarbon benzo[*a*]pyrene (BP) is generally considered to be one of the most prevalent environmental carcinogens to which man is exposed (1, 2). Although the carcinogenicity of BP is widely accepted as resulting from covalent interaction of BP metabolite(s) with some critical cellular macromolecule(s), such as DNA (3–5), both the nature of this metabolite and the target molecule within the cell are unknown. Since the metabolite(s) from BP responsible for inducing neoplasia is probably of high chemical reactivity, direct isolation of such a species may not be possible. Thus, study of urinary, biliary, fecal, or *in vitro* metabolites could conceivably result in the identification of breakdown products or fail to detect the key metabolite due to extensive binding to components of an *in vitro* incubation medium. In addition, all primary oxidative metabolites of BP are known to form secondary oxidative metabolites (6–8), any of which may be candidates for the ultimate carcinogen.

Experiments over the past few years have provided substantial insight into the possible mechanism of action of BP. Borgen *et al.* (9) found that the liver microsomal metabolism of *trans*-7,8-dihydroxy-7,8-dihydro BP (BP 7,8-dihydrodiol) *in vitro* resulted in more extensive binding to added DNA than did metabolism of BP or other metabolites of BP, such as BP 4,5- or BP 9,10-dihydrodiol. Sims *et al.* (10) have provided evidence that a 7,8-diol-9,10-epoxide was a reasonable candidate for the active metabolite by showing that metabolism of BP 7,8-dihydrodiol by rat liver microsomes in the presence of DNA led to nucleoside adducts similar to those produced when primary cultures of Syrian hamster embryo cells were exposed to BP. A similar result has been obtained with mouse liver microsomes (11). The 8,9-dihydrodiol of benz[*a*]anthracene is also converted to a diol epoxide which binds to DNA (12) and is converted to a tetraol (13). BP 7,8-dihydrodiol is activated to a potent mutagen(s) by intact cells (14), liver microsomes (15), and by a highly purified monoxygenase system containing cytochrome *P*-448 (16). Although a direct demonstration of the nature of the active metabolite(s) has not been possible, tetraols have been identified as metabolites from BP 7,8-dihydrodiol (14, 17). Recently these laboratories described (18) the unequivocal assignment of relative stereochemistry to the two possible stereoisomers of BP 7,8-diol-9,10-epoxide: (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro BP (diol epoxide 1)§ and (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro BP (diol epoxide 2), both of which are potent mutagens toward bacterial (15, 16, 19, 20) and mammalian (14, 19, 20) cells.

A possible scheme for the metabolic formation of an ultimate carcinogen from BP thus emerges; metabolism of BP to BP 7,8-oxide by the cytochrome *P*-450 monoxygenase system, hydration of BP 7,8-oxide to BP 7,8-dihydrodiol by epoxide hydrase, further oxidative metabolism by the cytochrome *P*-450 system to diol epoxides 1 and 2, and subsequent binding of these diol epoxides to critical tissue constituents. In direct support of this postulate are the findings that BP 7,8-oxide (20) and BP 7,8-dihydrodiol¶ are potent skin carcinogens. The present study establishes that diol epoxides 1 and 2 spontaneously hydrate to stereoisomeric pairs of tetraols *via cis* and *trans* addition of water and that all four tetraols (compare Figs. 1 and 2) are formed by liver microsomes and highly purified monoxygenase systems.

Abbreviations: BP, benzo[*a*]pyrene; BP 7,8-dihydrodiol, (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene; diol epoxide 1, (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro BP; diol epoxide 2, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro BP.

* Part IV of a series. For preceding papers see refs. 6 and 27.

§ In this notation, the 7-hydroxy group is designated on the top or β-face of the racemic molecule. The notations (±) 7α,8β-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene used in ref. 14 and (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]-pyrene used in our present and earlier studies (16, 18–20) both refer to the same racemic molecule.

¶ W. Levin, A. W. Wood, H. Yagi, D. M. Jerina, and A. H. Conney, (1976) *Proc. Natl. Acad. Sci. USA* **73**, in press.

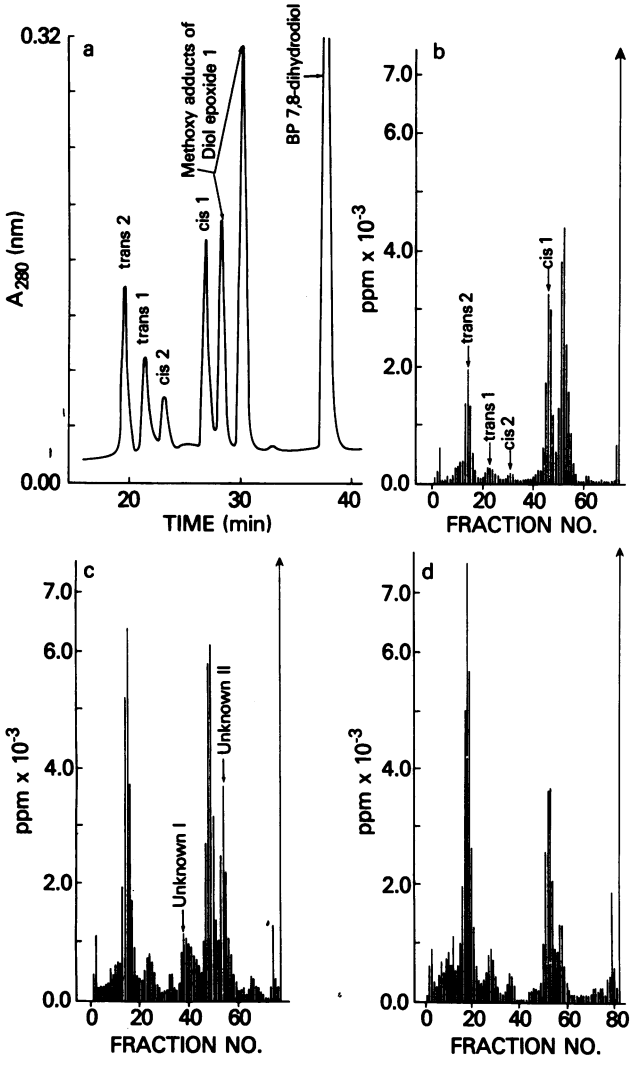

FIG. 1. High-pressure liquid chromatographic profile of BP 7,8-dihydrodiol metabolites: UV trace (a) obtained with reference compounds; and chromatographic distribution of radioactive metabolites obtained with microsomes from control animals (b), with microsomes from 3-methylcholanthrene-treated animals (c), and with the purified cytochrome *P*-448 system (d). Conversion is indicated as ppm relative to incubated substrate. The regions before and after the metabolite peaks are compressed in the plots of radioactivity due to collection of larger fractions as described in *Materials and Methods*. The three large peaks in each of the radioactive traces correspond to *trans* 2, *cis* 1, and Unknown II, respectively (Tables 1 and 2).

MATERIALS AND METHODS

Chemicals. Synthetic (±)-*trans*-7,8-dihydroxy-7,8-dihydro-[*9,10*-^{3}H]BP ([^{3}H]BP 7,8-dihydrodiol) was prepared by catalytic reduction of the dibenzoate of BP 7,8-dihydrodiol in the presence of tritium gas to the dibenzoate of (±)-*trans*-7,8-dihydroxy-7,8,9,10-[*9,10*-^{3}H]tetrahydro BP, which was then used as a precursor (11, 22) in the synthesis of labeled BP 7,8-dihydrodiol; specific activity was 26.8 mCi/mmol [by weight and by UV absorbance, ϵ_{368} = 48,200 M^{-1} cm^{-1} (22)]. The sample was spectroscopically (nuclear magnetic resonance and UV) pure and >98% radiochemically pure as determined by thin-layer chromatography on silica gel [acetone:$CHCl_3$ (1:4), R_F 0.3]. On high-pressure liquid chromatography, about 0.3% of the radioactivity emerged from the column in the metabolite region described later. Synthetic diol epoxides were obtained as previously described (18). Tritium-labeled diol epoxides were prepared from [^{3}H]BP 7,8-dihydrodiol. Diol epoxides were stored under argon at −80° in dimethylsulfoxide distilled from CaH_2.

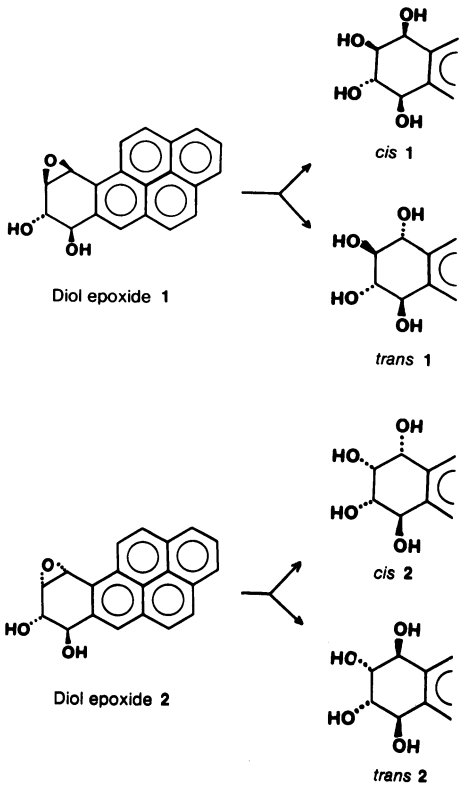

FIG. 2. Hydrolysis products from the 7,8-diol-9,10-epoxides of BP.

High-Pressure Liquid Chromatography. Samples were chromatographed on a Du Pont Zorbax octadecyltrimethoxysilane (ODS) column (7.9 mm × 0.25 m) which was monitored at 280 nm. One minute after injection, the column was eluted with a linear gradient of 60–98% methanol in water for 38 min at a constant flow rate of 1.2 ml/min. A Spectra Physics, model 3500B, liquid chromatograph with two coupled pressure restrictors [3000 pounds/inch^2 (21 MPa) total back pressure at 4 ml of water per min] on each pump was used to construct reproducibly the gradient. Fractions were collected every 1.2 min before and after the metabolite region and every 0.2 min (>20 drops) in the metabolite region (Fig. 1a).

Enzyme Preparations. Immature (50–60 g) male rats of the Long-Evans strain were treated with phenobarbital (75 mg/kg per day) or 3-methyl-cholanthrene (25 mg/kg per day in trioctanoin) for 4 days. Microsomes were prepared and stored at −90° prior to use (cf. ref. 6). Cytochrome *P*-450 contents for microsomes from control rats (0.88 nmol/mg of protein) or from phenobarbital- (2.25 nmol/mg of protein) or 3-methylcholanthrene- (1.89 nmol/mg of protein) induced animals were determined as described (23). Highly purified cytochrome *P*-450 and *P*-448 (24) as well as NADPH-cytochrome *c* reductase (25), and homogeneous epoxide hydrase (26), were prepared as described.

Incubation mixtures with liver microsomes contained 500–2000 μg of microsomal protein, 200 μmol of potassium phosphate buffer (pH 7.4), 6 μmol of $MgCl_2$, 1 μmol of NADPH, and 80 nmol of [^{3}H]BP 7,8-dihydrodiol (in 100 μl acetone:NH_4OH, 1000:1), in a final volume of 2.0 ml.

Incubation mixtures with the reconstituted system (24, 27) contained 200 μmol of potassium phosphate buffer (pH 6.8),

Table 1. Metabolism of BP 7,8-dihydrodiol by rat liver microsomes

Protein	Incubation conditions, μg/ml	min	Proportion of products* and specific activities† *trans* 1	*cis* 1	*trans* 2	*cis* 2	Unknown I	Unknown II	Total	% Recovery
Control microsomes	500	10	4.0 0.01	29.9 0.09	17.5 0.05	2.4 0.007	3.9 0.01	42.3 0.13	3.4 0.33	91
	1000	10	9.4 0.01	24.8 0.04	28.4 0.04	5.5 0.008	14.3 0.02	17.6 0.03	3.4 0.21	71
Phenobarbital microsomes	500	10	8.4 0.02	22.4 0.04	23.2 0.04	3.1 0.005	10.4 0.02	32.7 0.06	5.0 0.23	77
	1000	10	11.1 0.009	21.7 0.02	27.2 0.02	4.9 0.004	15.1 0.01	20.0 0.01	4.5 0.11	72
Methylcholanthrene microsomes	250	5	5.5 0.06	31.9 0.36	30.3 0.34	2.5 0.03	12.4 0.14	17.4 0.20	6.7 1.39	81
	500	10	8.1 0.06	24.0 0.17	46.0 0.32	3.7 0.03	10.0 0.07	8.2 0.06	16.9 0.85	83

Incubation mixtures contained the indicated amounts of microsomal protein. Radioactivity not extractable from the aqueous phase is not considered in these calculations. Recovery is the percentage of radioactivity emerging before BP 7,8-dihydrodiol in defined metabolite peaks, while total is the amount of radioactivity above blank which emerges from the column prior to BP 7,8-dihydrodiol.

* The upper row of numbers for each experiment is the percent of each metabolite expressed as proportion of total radioactivity eluting before BP 7,8-dihydrodiol.

† The lower row of numbers is the specific activity of preparation, in nmol of product formed per nmol of *P*-450 or *P*-448 per min.

6 μmol of $MgCl_2$, 1 μmol of NADPH, 0.40–0.80 nmol of cytochrome *P*-450 or *P*-448, 450–900 units of cytochrome *c* reductase, 60 μg of dilauroyl phosphatidylcholine, and 80 nmol of [^{3}H]BP 7,8-dihydrodiol in a final volume of 2.0 ml. Experiments with epoxide hydrase contained 68 units of enzyme in the 2.0 ml incubation mixture.

One unit of epoxide hydrase activity (26) is defined as 1 nmol of styrene glycol formed per min, and 1 unit of NADPH-cytochrome *c* reductase (25) is equal to 1 nmol of cytochrome *c* reduced per min. All incubations were at 37°.

BP 7,8-dihydrodiol and its metabolites were extracted into ethyl acetate/acetone, dried, and concentrated as described for BP and its metabolites (6). Dried samples were dissolved in 100 μl of methanol and UV-detectable quantities of *trans* **2**, and *cis* **2**, and diol epoxide **1** were added, and an aliquot was injected onto the column. Diol epoxide **1** gives rise to four UV-detectable peaks (*cis* **1**, *trans* **1**, and two methoxy adducts at C-10 of diol epoxide **1**; Figs. 1a and 2).

RESULTS

Hydrolysis of Diol Epoxides. The half-lives of diol epoxides **1** and **2**, as measured by their mutagenic activity, range from 0.5 to 2 min at 37° in pH 7 buffer (20). As such, it was deemed impractical to identify either of these compounds directly. Consequently, both compounds were incubated at 37° for 10 min in 0.1 M phosphate buffer, and the solvolysis products were examined. Both diol epoxides undergo *cis* and *trans* addition of water at the 10 position of the oxirane to form stereoisomeric pairs of (±)-7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydro BP derivatives (Fig. 2). Structures of these tetraols as their tetraacetates have been unequivocally assigned from their NMR spectra.‖ Radioactive diol epoxides **1** and **2** were carried through the above 10 min incubation procedure in buffer with the following results: diol epoxide **1** forms 85% *cis* **1** and 15% *trans* **1** while diol epoxide **2** forms 7% *cis* **2** and 93% *trans* **2** (Fig. 2) by respective *cis* and *trans* addition of water. Samples incubated with enzyme give slightly different *cis*:*trans* ratios, presumably due to the hydrophobic environment of the microsomal membrane. Solvolysis in dimethyl sulfoxide/water, for example, gave *cis*:*trans* ratios approaching 1:1. No other significant solvolysis products were detected in the metabolite region. Recovery of the tetraols was approximately 85% for the metabolized samples. Exhaustive extraction of aqueous solutions of radioactive diol epoxides **1** and **2** that had undergone solvolysis in pure water or in 0.05 M Tris·HCl buffer (pH 7.4) removed almost all radioactivity. In contrast, greater than 10% of the added radioactivity was not extractable into the organic solvent when 0.1 M phosphate buffer (pH 7.4) was used. The nonextractability of radioactivity is presumably due to alkylation of phosphate, since heating the buffer at 100° for 1 hr causes this radioactivity to become extractable.

Direct high-pressure liquid chromatographic analysis of diol epoxide **1** in dimethyl sulfoxide results in four sharp peaks corresponding to *trans* **1**, *cis* **1**, and two more strongly retained peaks which result from *cis* and *trans* addition of methanol at the 10 position (Fig. 1a). In contrast, the less reactive (18) diol epoxide **2** seems to partially survive high-pressure liquid chromatography in that a predominant broad peak (not shown), indicative of change on the column, emerges shortly after *cis* **2** along with a minor amount of broadened *trans* **2**, the major solvolysis product. In summary, diol epoxide **1** does not survive the incubation conditions, but if it did it would be detectable in part as its methanol adducts, while traces of diol epoxide **2** might survive the incubation and workup procedure and would be detectable as a broadened peak between *cis* **2** and *cis* **1** (Fig. 1a). Very little, if any, diol epoxide **2** was detected in metabolized samples, and had any of it survived chromatography, it would have appeared in the region designated as Unknown I.

Metabolism of [^{3}H]BP 7,8-Dihydrodiol by Liver Microsomes. Samples were incubated with 250–1000 μg of micro-

‖ H. Yagi, D. R. Thakker, O. Hernandez, M. Koreeda, and D. M. Jerina, manuscript submitted.

Table 2. Metabolism of BP 7,8-dihydrodiol by purified *P*-450 and *P*-448

Protein	Incubation conditions, nmol/ml	min	Proportion of products and specific activities							
			trans 1	*cis* 1	*trans* 2	*cis* 2	Unknown I	Unknown II	Total	% Recovery
P-448	0.2	5	6.8 0.14	30.0 0.61	50.9 1.03	3.9 0.08	2.2 0.05	6.2 0.13	5.1 2.69	75
P-448 + epoxide hydrase	0.2	5	7.9 0.17	28.3 0.60	47.2 1.00	3.8 0.08	1.3 0.03	11.6 0.25	5.4 2.77	77
P-450	0.4	5	5.7 0.005	21.6 0.02	62.6 0.06	4.8 0.004	0.0 0.00	5.4 0.005	0.46 0.10	88
P-450 + epoxide hydrase	0.4	5	3.9 0.004	32.1 0.03	58.5 0.06	5.6 0.005	0.0 0.00	0.0 0.00	0.47 0.10	90

Calculations are as described for Table 1.

somal protein per ml from control as well as phenobarbital- and 3-methylcholanthrene-treated rats for 5 or 10 min. All incubations were with 40 μM [^{3}H]BP 7,8-dihydrodiol, a saturating concentration. Radioactivity in the aqueous phases after extraction was found to increase as a function of the extent of metabolism. Very little of this radioactivity (10–15%) can be removed from the aqueous phase by further organic solvent extraction. Since the total aqueous radioactivity represents 30–50% of the metabolites found in the organic extracts (Tables 1 and 2), these aqueous phases (<15% as tritiated water) were further examined. After prolonged storage or heating, about half of this radioactivity becomes extractable into organic solvent. Analysis of these extracts by high-pressure liquid chromatography indicated the presence of tetraols and BP 7,8-dihydrodiol in quantities that would not markedly alter the ratios of the metabolites.

Results obtained with microsomes are given in Table 1. Controls consisted of incubated samples in which the microsomes were boiled. Predominant metabolites consist of *cis* 1 and *trans* 2, the major solvolysis products of their respective diol epoxides, along with an unknown metabolite(s) (Unknown II) which emerges after *cis* 1 but does not cochromatograph with the less retained methanol adduct of diol epoxide 1. Minor metabolites consist of *trans* 1 and *cis* 2, as expected, along with trace amounts of radioactivity between *cis* 2 and *cis* 1, (Unknown I), where diol epoxide 2 chromatographs as a broad peak. Small amounts of radioactivity due to tritiated water were generally observed with the solvent breakthrough. At low protein concentrations, control microsomes (Fig. 1b) produce approximately twice as much diol epoxide 1 (*cis* and *trans* 1) compared to diol epoxide 2 (*cis* and *trans* 2) and Unknown II predominates (42% of the total metabolism). Microsomes from 3-methylcholanthrene- or phenobarbital-treated rats produce comparable amounts of the two diol epoxides (Fig. 1c). Unknown metabolites (Unknown I and II) were also formed (18–46%). Diol epoxide 2 (*cis* and *trans* 2) was formed in higher amounts relative to diol epoxide 1 (*cis* and *trans* 1) at higher concentrations of all three microsomal proteins. In control microsomes, the ratio between diol epoxides 1 and 2 changed from 2:1 to 1:1 when the protein concentration was changed from 500 to 1000 μg/ml. The amounts of Unknown II decreased with increased microsomal protein concentrations.

Metabolism of [^{3}H]BP 7,8-Dihydrodiol by a Reconstituted System with and without Epoxide Hydrase. Metabolism of [^{3}H]BP 7,8-dihydrodiol was studied in a purified monoxygenase system containing either cytochrome *P*-450 or *P*-448 (Table 2). Both of these preparations are free of other microsomal proteins but contain more than one form of cytochrome *P*-450 or *P*-448 (28, 29). When the system was reconstituted with cytochrome *P*-448, diol epoxide 2 was the major metabolite, accounting for as much as 55% of the total metabolism; diol epoxide 1 was formed to the extent of 35–40% of the total metabolism and only a trace of unknown metabolite(s) was detected (Fig. 1d). Cytochrome *P*-450 has a much lower specific activity for metabolism of the dihydrodiol. With either cytochrome, excess epoxide hydrase (68 units), an enzyme which catalyzes the *trans* addition of water to epoxides, had little or no effect on the profile of metabolites. While an effect on diol epoxide 2 would be hard to detect since *trans* 2 greatly predominates on solvolysis, diol epoxide 1 would have been converted to *trans* 1, the minor solvolysis product, had diol epoxide 1 been a substrate for the enzyme. In addition, Unknown II was not significantly affected by epoxide hydrase. A substantial portion of this metabolite(s) was extractable from ethyl acetate into 4 M NaOH solution (>50%), but not into water alone, suggesting that the metabolite(s) may be phenolic.

DISCUSSION

Our laboratories have shown that: (*i*) BP 7,8-oxide and BP 7,8-dihydrodiol are very weak mutagens in bacterial and mammalian cells (30, 31); (*ii*) the diol in the presence of the cytochrome *P*-448 monoxygenase system or the arene oxide in the presence of the cytochrome *P*-448 monoxygenase system and epoxide hydrase is metabolized to a potent mutagen(s) (16); (*iii*) both diol epoxide 1 and 2 are very potent mutagens when tested at concentrations at which the increase in mutation frequency is linearly related to the applied dose (19, 20); and (*iv*) BP 7,8-oxide and BP 7,8-dihydrodiol are potent skin carcinogens (21)¶. Taken together, these observations strongly support the conclusion by Sims and coworkers that a diol epoxide of BP 7,8-dihydrodiol is formed and causes the major damage to DNA *in vivo* and to the DNA of cells in culture (10, 32).

The present study establishes that racemic BP 7,8-dihydrodiol is metabolized to both diol epoxides 1 and 2, based on the four tetraols identified**. These results are at variance with a recent study (14) which concluded that diol epoxide 2 is formed to the nearly complete exclusion of diol epoxide 1 (<5%). It is apparent from Tables 1 and 2 that both diol epoxides are formed in substantial amounts upon incubation of BP 7,8-dihydrodiol with microsomes from control or induced rats, or with purified cytochrome *P*-450 or *P*-448 as the source of the terminal oxi-

** Whether or not these metabolically formed diol epoxides and subsequent tetraols are optically active is unknown.

dase. Since the previous study (14) used biosynthetic BP 7,8-dihydrodiol diluted with racemic unlabeled diol, we examined the optical activity of the diol formed by microsomes from 3-methylcholanthrene-treated rats and found high optical purity (molar ellipticity $\theta_{367} = -58{,}900$ degrees·cm^2/dmol, 92–96% this enantiomer).

In a separate experiment with microsomes from 3-methylcholanthrene-treated rats, *the ratio of diol epoxide 2 to diol epoxide 1 was 9 to 1 for the optically active substrate and again 1.5 to 1 (see Table 1) for the racemic substrate.* Thus, the ratio of diol epoxide 1 to 2 is dependent on the source of the monoxygenase system, the conditions of the incubation, and the optical purity of the substrate.

Substantially more diol epoxide 2 is formed by the reconstituted cytochrome-*P*-450- or *P*-448-dependent monoxygenase system than by microsomes. Lack of a significant effect of epoxide hydrase on Unknown II, its elution time on the gradient and its extractability from organic solvent with alkali suggest a phenolic derivative of BP 7,8-dihydrodiol as a probable structure. The demonstration that diol epoxide 1 is a poor substrate for epoxide hydrase is consistent with our observation (16, 20) that epoxide hydrase had no effect on the mutagenicity of diol epoxide 1 and only slightly reduced the mutagenicity of diol epoxide 2.

The epoxide group in these diol epoxides resides in the hindered bay region of the hydrocarbon. We wish to draw attention to the facts that tetrahydrobenzo-ring epoxides in bay regions have been predicted to form benzylic carbonium ions with unusual ease (33) and that aromatic ring substituents which block metabolic formation of diol bay region epoxides also tend to inhibit carcinogenicity (34). This concept of epoxides at the bay region of saturated, angular benzo rings provides a unified theory of hydrocarbon-induced carcinogenicity which should be subjected to experimental test.

Note Added in Proof. In view of the above finding that the ratio of diol epoxides 1 and 2 formed from BP 7,8-dihydrodiol was dependent on the optical purity of the substrate, we have resolved BP 7,8-dihydrodiol into (+) and (−) enantiomers and have studied their metabolism with liver microsomes from 3-methylcholanthrene-treated rats. The (−) enantiomer gave results similar to those for the biosynthetic dihydrodiol. In contrast, the (+) enantiomer gave almost exclusively diol epoxide 1. Clearly, the ratio of diol epoxides 1 and 2 formed from BP will be dependent on the stereoselectivity of both the cytochrome *P*-450 system and epoxide hydrase in the organ of the species under study.

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