Biochem. J. (1984) **223**, 775–782
Printed in Great Britain

The effect of the bay-region 12-methyl group on the stereoselective metabolism at the K-region of 7,12-dimethylbenz[*a*]anthracene by rat liver microsomes

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(*Received 11 May 1984/Accepted 6 July 1984*)

The enantiomers of a *trans*-5,6-dihydrodiol formed in the metabolism of 7,12-dimethylbenz[*a*]anthracene by rat liver microsomes (microsomal fractions) were resolved by chiral stationary-phase high-performance liquid chromatography. The major 7,12-dimethylbenz[*a*]anthracene *trans*-5,6-dihydrodiol enantiomer and its hydrogenation product 5,6,8,9,10,11-hexahydro-*trans*-5,6-diol were found to have 5*S*,6*S* absolute configurations by the exciton chirality c.d. method. The *R*,*R*/*S*,*S* enantiomer ratios of 7,12-dimethylbenz[*a*]anthracene *trans*-5,6-dihydrodiol formed in the metabolism of 7,12-dimethylbenz[*a*]anthracene by liver microsomes from untreated, 3-methylcholanthrene-treated and phenobarbital-treated male Sprague–Dawley rats were found to be 11:89, 6:94, and 5:95 respectively. These findings and those reported previously on the metabolic formations of *trans*-5,6-dihydrodiols from 7-methylbenz[*a*]anthracene and 12-methylbenz[*a*]anthracene suggest that the 12-methyl group in 7,12-dimethylbenz[*a*]anthracene plays an important role in determining the stereoselective metabolism at the K-region 5,6-double bond. Furthermore, the finding that formation of 5*S*,6*S*-dihydrodiol as the predominant enantiomer was not significantly affected by the isoenzymic composition of cytochrome *P*-450 present in microsomes prepared from the livers of the rats pretreated with the different inducing agents indicates that the stereoselectivity depends on the substrate metabolized rather than on the precise nature of the metabolizing-enzyme system.

Polycyclic aromatic hydrocarbons (PAHs) are stereoselectively oxygenated by mammalian drug-metabolizing enzyme systems to form optically active intermediates such as epoxides, dihydrodiols and dihydrodiol-epoxides (Sims & Grover, 1974; Thakker *et al.*, 1977, 1979, 1982; Yang *et al.*, 1978; Chou *et al.*, 1983; Yang & Fu, 1984). Some carcinogenic PAH metabolites with different absolute stereochemistries exhibit different mutagenic and carcinogenic activities (Huberman *et al.*, 1977; Slaga *et al.*, 1979; Levin *et al.*, 1984). Epoxidation is an important cytochrome *P*-450-isoenzyme-catalysed reaction in xenobiotic metabolism (Jerina & Daly, 1974). An understanding of the detailed stereoselective mechanisms of product formation can shed light on the three-dimensional interactions of the PAH substrates with cytochrome *P*-450 isoenzymes in the microsomal drug-metabolizing-enzyme complex.

Benz[*a*]anthracene (BA) is metabolized by liver microsomes from 3-methylcholanthrene (3-MC)-treated and phenobarbital (PB)-treated rats to form a *trans*-5,6-dihydrodiol with *R*,*R*/*S*,*S* enantiomer ratios of 81:19 and 68:32 respectively (Thakker *et al.*, 1979). The *trans*-5,6-dihydrodiols formed in the metabolism of 8-methylbenz[*a*]-anthracene (Yang *et al.*, 1982) and of 11-methyl-

Abbreviations used: PAH(s), polycyclic aromatic hydrocarbon(s); BaP, benzo[*a*]pyrene; BA, benz[*a*]-anthracene; PB, phenobarbital; 3-MC, 3-methylcholanthrene; 7,12-DMBA, 7,12-dimethylbenz[*a*]anthracene; 7,12-DMBA *trans*-5,6-dihydrodiol, *trans*-5,6-dihydroxy-5,6-dihydro-7,12-dimethylbenz[*a*]anthracene; 7-MBA, 7-methylbenz[*a*]anthracene; other monomethylbenz[*a*]anthracenes are similarly designated; c.s.p., chiral stationary phase; h.p.l.c., high-performance liquid chromatography; c.i., chemical ionization; e.i., electron impact.

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benz[*a*]anthracene (Yang, 1982) by rat liver microsomes were also highly enriched in *R,R* enantiomers. However, 7-methylbenz[*a*]anthracene (Yang & Fu, 1984) and 12-methylbenz[*a*]-anthracene (Fu *et al.*, 1982) were stereoselectively metabolized to *trans*-5,6-dihydrodiols with *R,R*/*S,S* enantiomer ratios of 53:47 and 5:95 respectively. These results indicate that a methyl substitution at either the C-7 or the C-12 position of BA increases the metabolic formation of the 5*S*,6*S*-dihydrodiol enantiomer.

Experimental

Materials

7,12-DMBA *trans*-5,6-dihydrodiol metabolite was prepared by incubation of 7,12-DMBA with liver microsomes from untreated, 3-MC-treated and PB-treated male Sprague–Dawley rats and was subsequently purified by reversed-phase and normal-phase h.p.l.c. as described previously (Chou & Yang, 1979). BA *trans*-5,6-dihydrodiol with an *R,R*/*S,S* enantiomer ratio of 81:19 was prepared by incubation of BA with liver microsomes of 3-MC-treated male Sprague–Dawley rats and purified by h.p.l.c. (Yang, 1982). *p-NN*-Dimethylaminobenzoyl chloride was prepared as described by Harada *et al.* (1975). The *trans*-5,6-dihydrodiols of 7,12-DMBA and BA were converted into the respective 5,6,8,9,10,11-hexahydro-*trans*-5,6-diols by catalytic hydrogenation [tetrahydrofuran, PtO_2/H_2, 60lbf/in^2 (414kPa), 16h] and were each purified by reversed-phase h.p.l.c. Bis-*p-NN*-dimethylaminobenzoates of the *trans*-5,6-dihydrodiols and the 5,6,8,9,10,11-hexahydro-*trans*-5,6-diols of BA and 7,12-DMBA were prepared and subsequently purified by h.p.l.c. as described by Chiu *et al.* (1984) and Yang & Fu (1984). Diacetate derivatives were prepared by reaction of the diols with acetic anhydride in pyridine (70°C, 30min) and were purified by reversed-phase h.p.l.c.

Resolution of enantiomers

Direct resolution of 7,12-DMBA *trans*-5,6-dihydrodiol enantiomers by c.s.p.-h.p.l.c. was performed as described by Weems & Yang (1982), with a column [Pirkle type 1-A, 4.6mm i.d. (internal diameter) × 25cm; Regis Chemical Co., Morton Grove, IL, U.S.A.] packed with (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine ionically bonded to γ-aminopropylsilylated silica. The enantiomers were eluted with hexane/acetonitrile (17:2:1, by vol.) at 2ml/min.

Reversed-phase h.p.l.c.

All BA and 7,12-DMBA derivatives described in the present paper were purified by reversed-phase h.p.l.c. before u.v./visible, mass, c.d., and n.m.r. spectral analyses. A Waters Associates model 204 liquid chromatograph fitted with a Du Pont Zorbax ODS column (6.2mm i.d. × 25cm) was used. Compounds were eluted with a 15min linear gradient of methanol/water (13:7, by vol.) to methanol at a flow rate of 1.5ml/min.

Spectral properties

U.v./visible absorption spectra of chemicals dissolved in methanol were measured on a Varian Cary model 118C spectrophotometer. Mass-spectral analysis was performed on a Finnigan model 4000 gas chromatograph–mass spectrometer–data system with a solid probe by electron impact (e.i.) or by chemical ionization (c.i.) with methane as the ionization gas at 70eV and 250°C ionizer temperature. C.d. spectra of chemicals dissolved in methanol were measured in a quartz cell of 1cm path-length on a Jasco model 500A spectropolarimeter equipped with a model DP-500 data processor. C.d. spectra are expressed as ellipticity (Φ_λ in millidegrees) as described by Fu & Yang (1982) and Chiu *et al.* (1984). Proton n.m.r. spectra were obtained on a Fourier-transform high-resolution Bruker WM270 spectrometer.

Results and discussion

The K-region *trans*-5,6-dihydrodiol metabolite of 7,12-DMBA, previously identified by its h.p.l.c. retention time, u.v./visible absorption and mass-spectral analyses (Chou & Yang, 1979), is further confirmed by 270MHz proton n.m.r. spectroscopy. The proton assignments [($^2H_6$)acetone with a trace amount of ^2H_2O] are as follows: 2.80 (s, 3, CH_3 at C-7), 2.90 (s, 3, CH_3 at C-12), 4.83 (d, 1, H-5), 5.25 (d, 1, H-6), and 7.42–8.17 p.p.m. (m, 8, aromatic); $J_{5,6}$ 3.5Hz. The small coupling constant between the carbinol protons ($J_{5,6}$ 3.5Hz) indicates that the dihydrodiol is a *trans* isomer and preferentially adopts a quasidiaxial conformation (Zacharias *et al.*, 1979). In the absence of the steric hindrance of the 7-methyl group, BA *trans*-5,6-dihydrodiol adopts a quasidiequatorial conformation (Harvey *et al.*, 1975).

The enantiomers of the 7,12-DMBA *trans*-5,6-dihydrodiol formed in the metabolism of 7,12-DMBA by liver microsomes from 3-MC-treated male Sprague–Dawley rats were resolved by c.s.p.-h.p.l.c. (Fig. 1). The major enantiomer has previously been established to be a (+)-enantiomer by optical-rotation measurement (Chou & Yang, 1979). A milligram quantity of optically pure (+)-enantiomer, as shown in Fig. 1, was obtained by repetitive chromatography.

The optically pure (+)-7,12-DMBA *trans*-5,6-dihydrodiol was converted into 7,12-DMBA

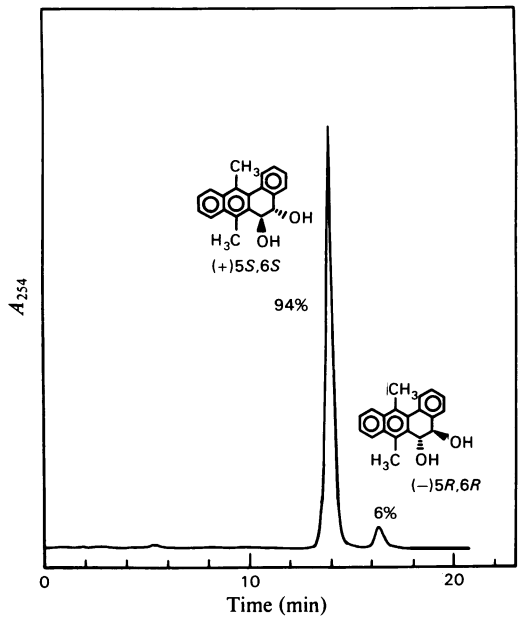

Fig. 1. *C.s.p.-h.p.l.c. resolution of the enantiomers of the trans-5,6-dihydrodiol formed from the metabolism of 7,12-DMBA by liver microsomes from 3-methylcholanthrene-treated rats*

The signs of optical rotation and the assignments of absolute configurations of the enantiomers are discussed in the text.

5,6,8,9,10,11-hexahydro-*trans*-5,6-diol (M^+ at m/z 294, e.i.) by catalytic hydrogenation. The u.v.-absorption spectra of these two diols are shown in Fig. 2. The (+)-BA *trans*-5,6-dihydrodiol with an *R,R*/*S,S* enantiomer ratio of 81:19 formed in the metabolism of BA by liver microsomes from 3-MC-treated rats (Thakker *et al.*, 1979; Yang, 1982) was also converted into the 5,6,8,9,10,11-hexahydro-*trans*-5,6-diol (M^+ at m/z 266, e.i.) by catalytic hydrogenation. The c.d. spectra of these diols are shown in Fig. 3. The c.d. Cotton effects of (+)-7,12-DMBA *trans*-5,6-dihydrodiol and its hexahydro hydrogenation product are each similar to those of (+)-BA 5*R*,6*R*-dihydrodiol and its hexahydro derivative, respectively. Based on these simple comparisons of c.d. Cotton effects, one might conclude that the major enantiomer of the *trans*-5,6-dihydrodiol formed *in vitro* in the metabolism of 7,12-DMBA has a 5*R*,6*R* absolute stereochemistry. Further investigation (see below) proved that this conclusion would have been incorrect.

Simple comparisons of the c.d. spectra discussed above did not take into consideration the conformational differences between the 7,12-DMBA *trans*-5,6-dihydrodiol (quasidiaxial) and the BA *trans*-5,6-dihydrodiol (quasidiequatorial). C.d. Cotton effects of PAH dihydrodiol enantiomers are dependent on the conformational state of the hydroxyl groups (Ahktar *et al.*, 1975; Buhler *et al.*, 1983; Chiu *et al.*, 1984; Fu & Yang, 1982, 1983; Yang & Fu, 1984). If the c.d. spectral patterns of Fig. 3 could be altered by changing the conformation of the diol enantiomers, a better insight into the absolute configuration of the (+)-7,12-DMBA *trans*-5,6-dihydrodiol enantiomer could be obtained. With this purpose in mind, diacetate derivatives were prepared from the dihydrodiols and hexahydrodiols.

Acetylation of the quasidiaxial (+)-7,12-DMBA *trans*-5,6-dihydrodiol or its hexahydro derivative did not significantly change the c.d. Cotton effects other than by altering intensities (Figs. 3 and 4). However, changes in the c.d. Cotton effects were observed (Figs. 3 and 4) when the quasidiequatorial BA 5*R*,6*R*-dihydriol ($J_{5,6}$ 10.3Hz; Fu *et al.*, 1983) and its hexahydro derivatives were converted into the predominantly quasidiaxial BA 5*R*,6*R*-diacetate ($J_{5,6}$ 4.4Hz; Harvey *et al.*, 1975) and BA 5,6,8,9,10,11-hexahydro-*trans*-5,6-diacetate. Acetylation of the quasidiequatorial K-region phenanthrene 9*S*,10*S*-dihydrodiol is known to result in a conformational change toward a predominantly quasidiaxial conformation (Jerina *et al.*, 1976; Zacharias *et al.*, 1979) which brought about changes in c.d. Cotton effects (Miura *et al.*, 1968). The results (Figs. 3 and 4) indicate that (i) on acetylation the c.d. Cotton effects of an enantiomeric quasidiequatorial K-region *trans*-diol enantiomer are changed toward those of the corresponding K-region quasidiaxial *trans*-diol enantiomer, and (ii) the characteristic c.d. Cotton effects and conformation are not significantly changed by acetylation for quasidiaxial PAH dihydrodiol and hexahydrodiol enantiomers.

The data in Figs. 3 and 4 provided additional understanding of the relationships between c.d. Cotton effects and the conformation of PAH K-region dihydro and hexahydro *trans*-diol enantiomers. They do not, however, provide evidence for determining the absolute configurations of the (+)-7,12-DMBA *trans*-5,6-dihydrodiol or its hexahydro derivative. The absolute configuration of (+)-7,12-DMBA *trans*-5,6-dihydrodiol was obtained by applying the exciton-chirality method of Harada & Nakanishi (1972) and is described below.

The absolute configuration of a 5,6,8,9,10,11-hexahydro-*trans*-5,6-diol derived from catalytic hydrogenation of (−)-BA *trans*-5,6-dihydrodiol was elucidated by the exciton-chirality method (Thakker *et al.*, 1979). The hexahydro derivative with a biphenyl chromophore was chosen so that

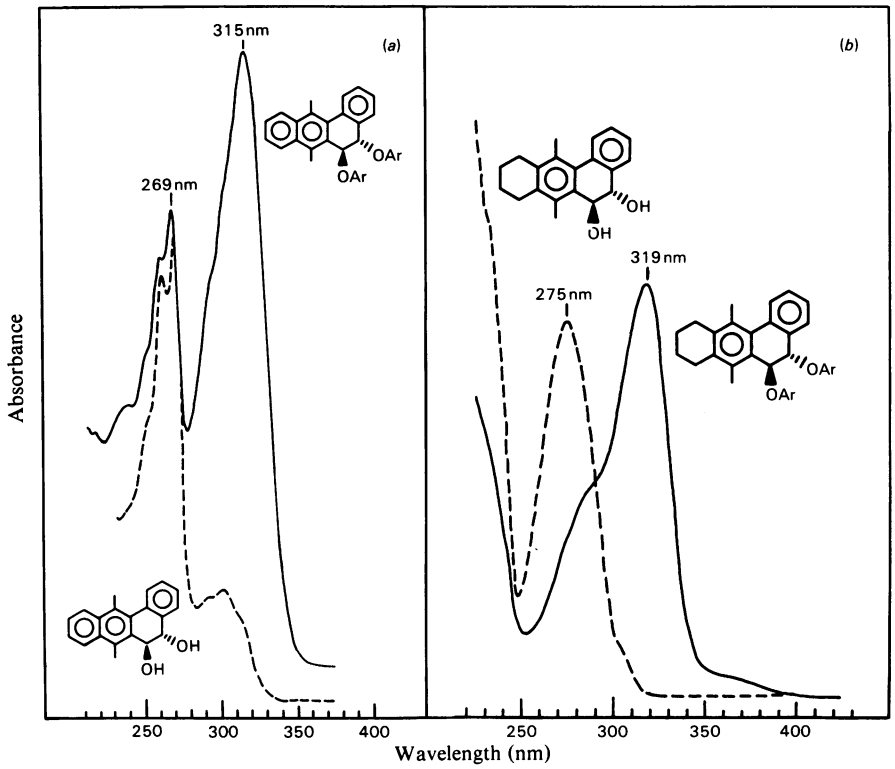

Fig. 2. *U.v. absorption spectra of 7,12-DMBA trans-5,6-dihydrodiol (a, ----) and its bis-p-NN-dimethylaminobenzoate derivative (a, ——), and of 7,12-DMBA 5,6,8,9,10,11-hexahydro-trans-5,6-diol (b, ----) and its bis-p-NN-dimethylaminobenzoate derivative (b, ——)*
Abbreviations used: Ar, *p-NN*-dimethylaminobenzoyl.

the longer-wavelength absorptions are decreased, thereby minimizing the electronic transition dipole interactions between the benzoyloxy groups and the 5,6-dihydro-BA chromophore. A symmetrical exciton splitting was expected, which would reveal the chirality of the two benzoyloxy groups and hence the absolute configuration of the hexahydrodiol. However, only a positive c.d. band at 322nm was observed (Thakker *et al.*, 1979), but was sufficient for determining the 5*S*,6*S* absolute stereochemistry of the BA 5,6,8,9,10,11-hexahydro-*trans*-5,6-diol derived from the (−)-BA *trans*-5,6-dihydrodiol. The evidence for the absolute configuration of phenanthrene 9*S*,10*S*-dihydrodiol was also based on a positive c.d. band at 322nm observed for its bis-*p-NN*-dimethylaminobenzoate derivative (Thakker *et al.*, 1979).

7,12-DMBA 5,6,8,9,10,11-hexahydro-*trans*-5,6-diol derived by hydrogenation of the optically pure (+)-7,12-DMBA *trans*-5,6-dihydrodiol was converted into a bis-*p-NN*-dimethylaminobenzoate derivative. The bis-*p-NN*-dimethylaminobenzoate of this hexahydro derivative [$(M+1)^+$ at *m/z* 589 and $(M+29)^+$ at *m/z* 617; c.i., methane], purified by reversed-phase h.p.l.c., gave a positive c.d. band at 317nm which was due to electronic transition dipole–dipole interactions between the benzoate groups (Fig. 5). The characteristic positive c.d. band at 317nm of the exciton chirality spectrum (Fig. 5) indicates that the 7,12-DMBA 5,6,8,9,10,11-hexahydro-*trans*-5,6-diol has a 5*S*,6*S* absolute stereochemistry. The predominant (+)-enantiomer of the enzymically formed 7,12-DMBA *trans*-5,6-dihydrodiol is, therefore, deduced to have a 5*S*,6*S* absolute stereochemistry. At wavelengths greater than 300nm, the 5,6,8,9,10,11-hexahydro derivative of the optically pure (+)-7,12-DMBA *trans*-5,6-dihydrodiol had u.v. absorptions (Fig. 2*a*), although it exhibited weak c.d. Cotton effects (Fig. 3*b*). The electronic transition dipoles of the u.v.-absorption bands between 250 and 330nm apparently interact with the electronic transition dipoles of the benzoate groups (Fig. 2*b*) to yield a chirality c.d. spectrum with unsymmetrical exciton splitting, so that the maximum of the positive c.d. band was at a wavelength lower than expected (Fig. 5).

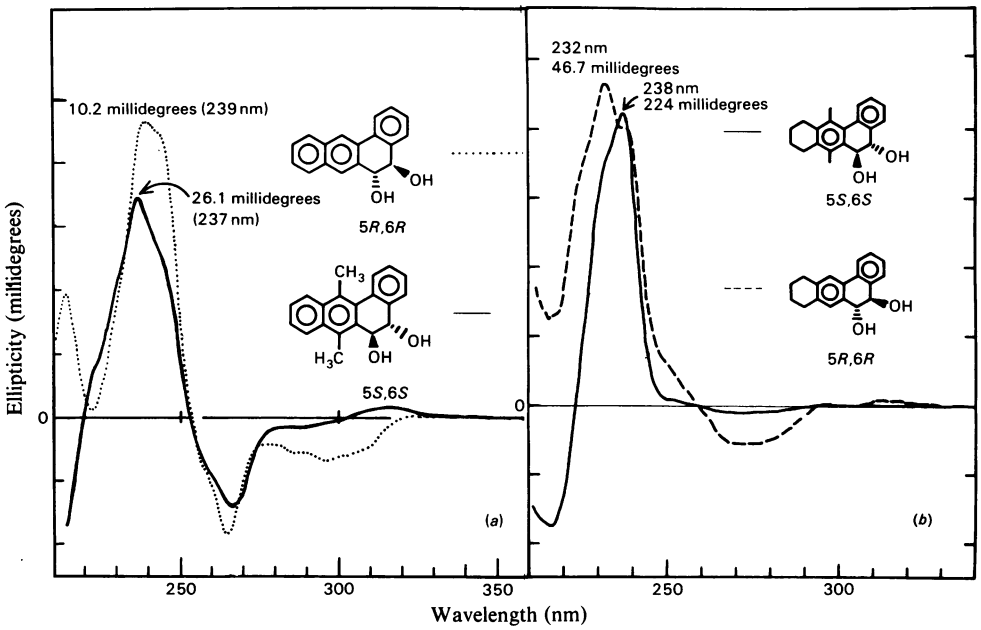

Fig. 3. *C.d. spectra of optically pure (+)-7,12-DMBA trans-5,6-dihydrodiol (a, ——; conc. 1.0 A_{269}/ml) and its hexahydro derivative (b, ——; conc. 1.0 A_{276}/ml), and of (+)-BA trans-5,6-dihydrodiol with an R,R/S,S enantiomer ratio of 4:1 (a, ····; conc. 1.0 A_{266}/ml) and its hexahydro derivative (b, ----; conc. 1.0 A_{271}/ml)*

The ideal result of the exciton-chirality method is to generate a new symmetrical exciton splitting from the benzoate groups (Harada & Nakanishi, 1972). Absence of u.v. absorption, and hence the absence of c.d. Cotton effects at the wavelength range where the benzoate groups absorb, will minimize the interference of the dipole–dipole interactions between the two benzoate groups. Because of interference by the biphenyl chromophore, symmetrical exciton splitting was not observed for the bis-*p-NN*-dimethylaminobenzoates of the 5,6,8,9,10,11-hexahydro-*trans*-5,6-diols derived either from the (+)-7,12-DMBA *trans*-5,6-dihydrodiol (Fig. 5) or from the (−)-BA *trans*-5,6-dihydrodiol (Thakker *et al.*, 1979). Since the interference could not be eliminated even when the 8,9,10,11-benzo rings of the 5,6-dihydrodiols were saturated, we wondered whether the 5,6-dihydrodiol had to be converted into the hexahydro product before applying the exciton-chirality method. To answer this question, the bis-*p-NN*-dimethylaminobenzoate derivative of the (+)-BA 5*R*,6*R*-dihydrodiol (M^+ at *m/z* 556, e.i.) was prepared. A relatively strong negative c.d. band with Φ_{323} −13.3millidegrees [$\Delta\varepsilon$ −6.8 $M^{-1}\cdot cm^{-1}$, calculated on the basis of ε_{310} 17000 $M^{-1}\cdot cm^{-1}$ determined for the bis-*p-NN*-dimethylaminobenzoate of phenanthrene 9*S*,10*S*-dihydrodiol (Thakker *et al.*, 1979)] was observed for the bis-*p-NN*-dimethylbenzoate derivative of the (+)-BA *trans*-5,6-dihydrodiol, which has an *R,R/S,S* enantiomer ratio of 81:19 (Fig. 6). Thus, even in the absence of the knowledge that the BA *trans*-5,6-dihydrodiol used is enriched in *R,R* enantiomer, the c.d. chirality spectrum, as shown by Fig. 6, provides the evidence that the major enantiomer of the BA *trans*-5,6-dihydrodiol under study has a 5*R*,6*R* absolute stereochemistry. The result indicates that the absolute configuration of the BA K-region *trans*-5,6-dihydrodiol enantiomer can be determined by the exciton-chirality method without saturating the 8,9,10,11-benzo ring.

The bis-*p-NN*-dimethylaminobenzoate derivative of the optically pure (+)-7,12-DMBA *trans*-5,6-dihydrodiol [$(M+1)^+$ at *m/z* 585 with characteristic ions at *m/z* 613 and 625; c.i., methane) was also prepared. However, a weak c.d. band with Φ_{324} 2.4 millidegrees ($\Delta\varepsilon = +1.2 M^{-1}\cdot cm^{-1}$, calculated on the basis of ε_{315} 17000 $M^{-1}\cdot cm^{-1}$) was observed. Although the interference by the 5,6-dihydro-BA chromophore is again apparent, the positive c.d. band at 324nm nevertheless provides important evidence for the 5*S*,6*S* absolute configuration of (+)-7,12-DMBA *trans*-5,6-dihydrodiol. Although the dihydrodiol is optically pure, the intensity of the longer-wavelength c.d. extremum (at 324nm), due to the interacting transition moments of the benzoates, is very weak when it is

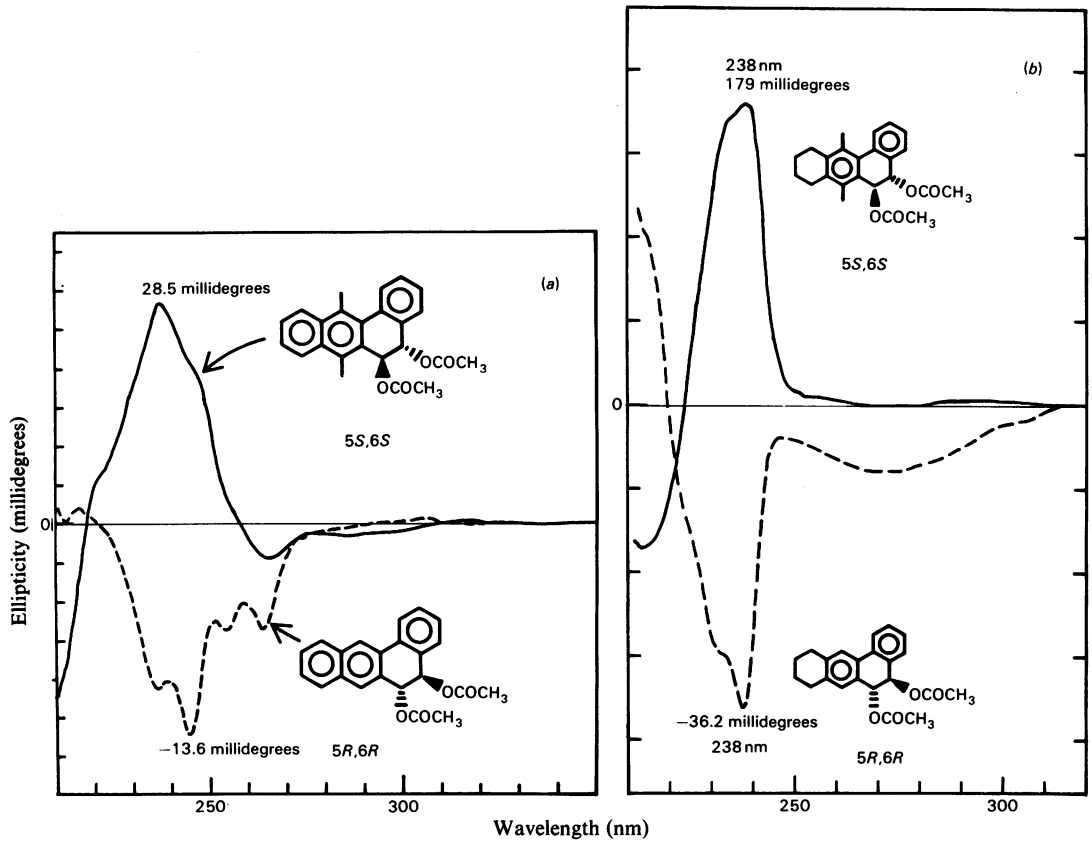

Fig. 4. *C.d. spectra of the optically pure (+)-7,12-DMBA trans-5,6-dihydrodiol diacetate [a, ——; conc. 1.0 A_{270}/ml; $(M+1)^+$ at m/z 375, c.i., methane], and its hexahydro derivative [b, ——; $(M+1)^+$ at m/z 379, c.i., methane], and of (+)-BA trans-5,6-dihydrodiol diacetate with an R,R/S,S enantiomer ratio of 4 : 1 (a, ----; conc. 1.0 A_{266}/ml; M^+ at m/z 346, e.i.) and its hexahydro derivative (b, ----; conc. 1.0 A_{278}/ml; M^+ at m/z 350, e.i.).*

compared with the strong c.d. band obtained for (+)-BA 5*R*,6*R*-dihydrodiol (Fig. 6). The exact reasons for the weak dipole–dipole interactions in the presence of the 7- and 12-methyl groups are not known. One possible explanation for the observed weak exciton splitting may be due to steric hindrance exerted by the 7-methyl group to result in a nearly 180° angle between the two alcoholic C–O bonds. The intensities of the positive c.d. bands observed for the bis-*p*-*NN*-dimethylaminobenzoate derivatives of the quasidiaxial 7-MBA 5*R*,6*R*-dihydrodiol (Yang & Fu, 1984), 7-bromo-BA 5*R*,6*R*-dihydrodiol (Yang & Fu, 1983), and 7-fluoro-BA 5*R*,6*R*-dihydrodiol (Chiu *et al.*, 1984) were also considerably weaker than that of the bis-*p*-*NN*-dimethylaminobenzoate derivative of (+)-BA 5*R*,6*R*-dihydrodiol (Fig. 6).

Our results established that a 5*S*,6*S*-dihydrodiol is the major enantiomer formed during the metabolism at the K-region of 7,12-DMBA by liver microsomes from 3-MC-treated rats. In contrast, a 5*R*,6*R*-dihydrodiol is the major enantiomer formed from the metabolism at the K-region of BA by similar rat liver microsomal preparations (Thakker *et al.*, 1979; Yang, 1982). Cytochrome *P*-448 is the major *P*-450 isoenzyme present in liver microsomes from 3-MC-treated rats (Lu & West, 1980). Since the stereoselective properties of various cytochrome *P*-450 isoenzymes may be different (Yang *et al.*, 1982), we have also examined the enantiomeric compositions of the *trans*-5,6-dihydrodiol formed from the metabolism of 7,12-DMBA by liver microsomes from untreated and PB-treated male Sprague–Dawley rats, which have different contents of cytochrome *P*-450 isoenzymes (Lu & West, 1980). By using the method that yielded Fig. 1, the *R*,*R*/*S*,*S* enantiomer ratios of the 7,12-DMBA *trans*-5,6-dihydrodiol metabolites formed by liver microsomes from untreated and PB-treated rats were found to be 11 : 89 and 6 : 94 respectively. These results indicate that the three different liver microsomal prepara-

tions used in the present study exhibit a similar stereoselective preference in catalysing the formation of the enantiomeric 7,12-DMBA *trans*-5,6-dihydrodiols.

The enantiomeric compositions of the *trans*-5,6-dihydrodiols formed in the metabolism of BA, 7-bromo-BA, 7-fluoro-BA, 7-MBA, 8-MBA, 11-MBA, 12-MBA, and 7,12-DMBA by liver microsomes from 3-MC-treated rats are shown in Table 1. The *R,R* enantiomer predominates in the *trans*-5,6-dihydrodiol metabolites of 8-MBA, 11-MBA, 7-fluoro-BA, and 7-bromo-BA. A methyl group at C-7 of BA increased the formation of the 5*S*,6*S*-dihydrodiol to not more than 50% of the two

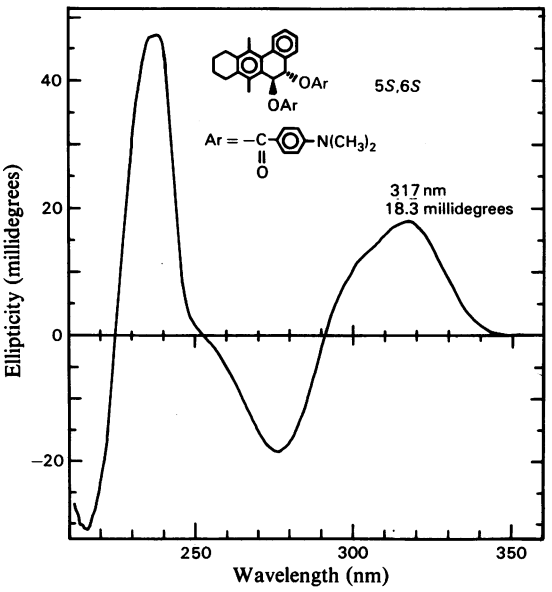

Fig. 5. *C.d. spectrum of the bis-p-NN-dimethylamino-benzoate of the 5,6,8,9,10,11-hexahydro-trans-5,6-diol (conc. 1.0 A_{319}/ml) derived by catalytic hydrogenation of the optically pure (+)-7,12-DMBA trans-5,6-dihydrodiol (see Fig. 2b for its u.v.-absorption spectrum)*

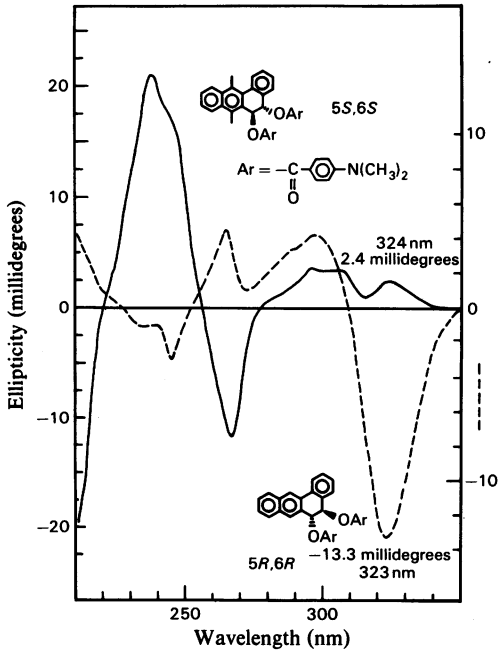

Fig. 6. *C.d. spectra of the bis-p-NN-dimethylamino-benzoate of the optically pure (+)-7,12-DMBA trans-5,6-dihydrodiol (——; conc. 1.0 A_{315}/ml) and of (+)-BA trans-5,6-dihydrodiol with an R,R/S,S enantiomer ratio of 4:1 (----; conc. 1.0 A_{310}/ml)*

Table 1. *Comparison of the enantiomeric composition of the trans-5,6-dihydrodiol metabolite formed in the metabolism of various substituted BAs by liver microsomes from 3-MC-treated rats*

PAH	Enantiomer (%) *R,R*	Enantiomer (%) *S,S*	Optical purity (%)*	Reference
BA	81	19	62	Thakker *et al.* (1979)
8-MBA†	90	10	80	Yang *et al.* (1982)
11-MBA†‡	75	25	46	Yang (1982)
7-Bromo-BA	98	2	96	Fu & Yang (1983)
7-Fluoro-BA	81	19	62	Chiu *et al.* (1984)
7-MBA	53	47	6	Yang & Fu (1984)
12-MBA†	5	95	90	Fu *et al.* (1982)
7,12-DMBA	6	94	88	The present paper

* The optical purity is defined as the difference between the percentage of the two enantiomers.

† The enantiomeric composition was determined as described in Fig. 1, with 5% ethanol/acetonitrile (2:1, v/v) in hexane.

‡ The 5,6-dihydrodiol formed from the metabolism of 11-MBA by liver microsomes from 3-MC-treated rats was isolated as described by Yang (1982).

enantiomers combined (Table 1). These results indicate that the 7-methyl group is not the major factor that alters the stereoselective metabolism at the K-region of 7,12-DMBA. The *trans*-5,6-dihydrodiol metabolite of 12-MBA is enriched in the 5*S*,6*S* enantiomer (Fu *et al.*, 1982, and Table 1). Taken together, the results suggest that it is the bay-region 12-methyl group that plays a crucial role in determining the major dihydrodiol enantiomer formed at the K-region double bond of 7,12-DMBA.

The mechanism of stereoselective epoxidation at the K-region 5,6-double bond of 7,12-DMBA catalysed by various cytochrome *P*-450 isoenzymes is currently unknown. The (+)-7,12-DMBA 5*S*,6*S*-dihydrodiol may be formed from epoxide hydrolase-catalysed hydration of either the 5*R*,6*S*- or the 5*S*,6*R*-epoxide intermediate. Thus the exact stereoselective pathways in the metabolic formation of (+)-7,12-DMBA 5*S*,6*S*-dihydrodiol from 7,12-DMBA cannot yet be established.

We thank Dr. Dwight W. Miller for n.m.r. spectral measurements and Mr. Henri Weems for mass-spectral analyses. This investigation was supported in part by U.S. Public Health Service grant CA29133. The opinions or assertions contained herein are our private ones and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences or the Food and Drug Administration. The experiments reported herein were conducted according to the principles set forth in the *Guide for the Care and Use of Laboratory Animals* [Institute of Animal Resources, National Research Council, Department of Health Education and Welfare Publication no. (NIH) 78-23].

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