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Enantiomeric Composition of *Trans*-Dihydrodiols Formed from *Meso*-K-Region Arene Oxides by Microsomal Epoxide Hydrolase

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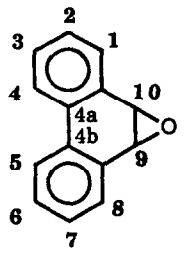
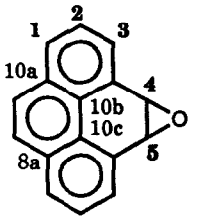
Abstract: Absolute configurations for the enantiomers of *trans*-4,5-dihydroxy-4,5-dihydrobenzo[e]pyrene were determined by the exciton chirality method and by correlation of physical properties of their (-)-(menthyloxy)acetyl diesters. Microsomal epoxide hydrolase catalyzed the hydrolysis of K-region arene oxides of benzo[e]pyrene, pyrene, and phenanthrene to *trans*-dihydrodiols containing 83%, 86%, and 42% of the *R,R* enantiomer, respectively.

Polycyclic aromatic hydrocarbons are activated to carcinogenic and mutagenic metabolites by the action of microsomal P450 monooxygenases and xenobiotic microsomal epoxide hydrolase (MEH).[1] MEH catalyzes the hydrolysis of *cis*-1,2-disubstituted epoxides and arene oxides to the corresponding diols by trans addition of water with inversion of configuration at the position of attack. The enzyme displays considerable enantio- and regioselectivity in the hydrolysis of chiral arene oxides.[2] Products of MEH-catalyzed hydrolysis of *meso*-K-region arene oxides are optically active, indicating preferential reaction at one of the two chiral centers. For example, MEH-catalyzed hydrolysis of phenanthrene 9,10-oxide[3] (Phe-O, Figure 1) produces K-region *trans*-dihydrodiol (*trans*-DHD) with little configurational preference (a slight excess of the (9*S*,10*S*)-enantiomer). In contrast, hydrolysis of pyrene 4,5-oxide[4] (Pyr-O) produces an excess of the *trans*-(4*R*,5*R*)-DHD, indicating preferential attack at the (*S*)-oxirane carbon. In this study, we assign absolute configurations to the enantiomers of *trans*-4,5-dihydroxy-4,5-dihydrobenzo[e]pyrene (BeP-DHD), the *trans* hydration product of benzo[e]pyrene 4,5-oxide (BeP-O), and compare enantiomeric compositions of the *trans*-DHDs formed from these three *meso*-arene oxides upon MEH-catalyzed hydrolysis under identical conditions.

Although BeP-DHD is a major microsomal metabolite of the parent hydrocarbon,[5] its enantiomeric



Phenanthrene 9,10-oxide (Phe-O)



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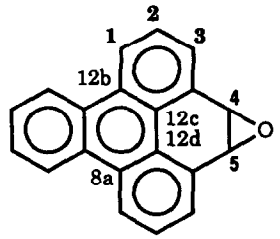

Benzo[e]pyrene 4,5-oxide (BeP-O)

Figure 1. Structures of *meso*-K-region arene oxides, abbreviations shown in parentheses.

composition and absolute configuration have not been reported. Resolution of BeP-DHD[6] was achieved by chiral HPLC and by HPLC separation of its diasteriomeric (-)-(menthyloxy)acetyl ((-)-MOA) diesters[7a] on a Du Pont Zorbax silica column (0.94 x 25 cm) eluted with 7% ether in cyclohexane. *Early*-eluting (k'_{early} = 12.8) and *late*-eluting (k'_{late} = 14.2) (-)-MOA diesters on the silica column had $[\alpha]_D$ -229° (c 1.1, THF) and $[\alpha]_D$ +94° (c 1.3, THF), respectively. HRMS (FAB) calculated for $C_{44}H_{54}O_6$, 678.3920; found 678.3917 and 678.3928 for *early*- and *late*-eluting diesters, respectively. The diasteriomeric diesters displayed nearly identical NMR spectra[8] (C_6D_6) in the aromatic region: δ 7.82 (d, J = 7.0, $H_{3,6}$), 6.75 (s, $H_{4,5}$) for *early*; δ 7.81 (d, J = 6.7, $H_{3,6}$), 6.73 (s, $H_{4,5}$) for *late*. The NMR signals for the -O-CH_AH_B-CO_2- group of the *early*-eluting (less polar) diester displayed two unsymmetrical doublets with nearly identical chemical shifts (δ 3.64 and 3.67, J = 16.5), whereas the resonances for the same two protons of the *late*-eluting (more polar) diester were well resolved (δ 3.57 and 3.78, J = 16.3). The separated diesters were converted to the enantiomers of BeP-DHD by saponification (0.3 M NaOH:methanol:THF (2:5:5), 1 h under N_2 at rt). Free *trans*-DHD enantiomers derived from *early*- and *late*-eluting (-)-MOA diesters eluted *early* (k'_{early} = 19.7) and *late* (k'_{late} = 20.8), respectively, on the chiral column.[9] *Early* BeP-DHD had $[\alpha]_D$ -25° (c 0.44, THF) and -85° (c 0.28, methanol), whereas the *late* enantiomer had $[\alpha]_D$ +21° (c 0.4, THF) and +81° (c 0.36, methanol). CD spectra of the *early*-(-)-BeP-DHD and its (-)-MOA diester are shown in Figure 2A.

Empirical correlation of physical properties of the (-)-MOA diesters of K-region *trans*-DHD's has indicated that the (*R,R*)-diesters ordinarily elute *early* on silica columns, exhibit a more negative rotation, and show a lesser degree of magnetic nonequivalence between the diastereotopic protons H_A and H_B of their -O-$CH_AH_BCO_2$- groups. By these criteria,[7b,10] the *early* and *late*-eluting (-)-MOA diastereomers derived from BeP-DHD have (4*R*,5*R*)- and (4*S*,5*S*)-absolute configuration, respectively. However, assignment of absolute configuration based on empirical correlation alone is ambiguous since the sign of optical rotation in THF[7b,10] and the order of elution on the chiral column[11] of the enantiomers of BeP-DHD were opposite to that expected for a presumably pseudodiequatorial K-region *trans*-DHD.

To eliminate any ambiguity in assignment of absolute configuration, the nonempirical exciton chirality method was used.[12] Both enantiomers of BeP-DHD were converted to their bis-(*p*-(dimethylamino)benzoate) and bis-(*p*-(dimethylamino)-*trans*-cinnamate) esters. A 40-fold excess of either acyl imidazole was heated with the BeP-DHD enantiomers under argon in pyridine containing a catalytic amount of *p*-(dimethylamino)pyridine at 70-85 °C for 4-5 days. Standard workup with ether and purification by reverse-phase HPLC provided the diesters.[13] Bis-(*p*-(dimethylamino)benzoate): NMR: δ 7.85 ($H_{benzoyl}$, d, J = 9), 6.84 ($H_{4,5}$, s), 6.69 ($H_{benzoyl}$, d, J = 9), 2.99 (CH_3, s). MS (CI-NH_3): 581 for M+1, HRMS (FAB) calculated for (M+1-H_2) $C_{38}H_{31}O_4N_2$ 579.2284, found 579.2272. UV (CH_3CN) λ_{max} 260, 315 nm. Bis-(*p*-(dimethylamino)cinnamate): NMR: δ 6.62 ($H_{4,5}$, s), 6.31 (H_{vinyl}, d, J = 16), 3.09 (CH_3, s) MS (CI-NH_3): 633 for M+1, HRMS (FAB) calculated for $C_{42}H_{36}O_4N_2$ 632.2675, found 632.2673. UV λ_{max} 261, 373 nm.

The CD spectrum of the bis-(*p*-(dimethylamino)benzoate) of (-)-BeP-DHD has a negative band at 323 nm ($\Delta\epsilon$ -54 M^{-1} cm^{-1}), passes through 0 at 308 nm, and has a positive band at 300 nm ($\Delta\epsilon$ +13, Figure 2B). The CD spectrum of the bis-(*p*-(dimethylamino)cinnamate) of (-)-BeP-DHD has a negative band at 385 nm ($\Delta\epsilon$ -15), passes through 0 at 360 nm, and has a positive band at 344 nm ($\Delta\epsilon$ +6.3) (Figure 2B, the corresponding diesters of (+)-BeP-DHD gave mirror image CD spectra). Although the magnitude of the shorter wavelength Cotton effect is small for both types of diester, the maxima are at the expected wavelengths for exciton interactions between the benzoates. The negative sign of the long wavelength band for the diesters of (-)-BeP-DHD (Figure 2B) indicates a left-hand skew sense between the two benzoate or

cinnamate chromophores, requiring (4*R*,5*R*)-absolute configuration for the (-)-BeP-DHD from *early*-eluting (-)-MOA diester. Thus, the tentative assignment based on the physical properties of the (-)-MOA diesters is correct.

We considered the possibility that the anomalous optical rotation and elution order on the chiral column for the enantiomeric *trans*-DHD's might result from an unexpected preference for a pseudodiaxial, rather than a pseudodiequatorial, conformation for the hydroxyl groups. NMR data show that this is not the case. Since the two carbinol protons ($H_{4,5}$) of BeP-DHD and its diester derivatives are magnetically equivalent, the conformational preference cannot be determined from their ^1H spectra; however, the ^{13}C satellites[14] are easily observed (THF-d_8) on both sides of the $H_{4,5}$ resonance at δ 4.89 ($J_{C\text{-}H}$ = 141.3) for the *trans*-DHD and at δ 6.44 ($J_{C\text{-}H}$ = 153.3) for both its *early* and *late*-eluting (-)-MOA diesters. The coupling constant between the non-equivalent carbinol protons on ^{13}C and ^{12}C of BeP-DHD is 10.7 Hz, indicating a preference for the pseudodiequatorial conformation of the hydroxyl groups.[15] In contrast, the *early* and *late*-eluting (-)-MOA diesters display ^{13}C satellites for $H_{4,5}$ as doublets with coupling constants of 4.4 and 4.5 Hz, respectively, indicative of a preference for the pseudodiaxial conformation of the acyloxy groups.

The K-region *trans*-DHD's of Phe, benz[a]anthracene (BA) and benzo[c]phenanthrene (BcPh) can all be thought of as "skew biphenyl" chromophores. Their CD spectra are dominated by strong bands which are related to the skew sense of these chromophores. Thus, CD spectra of the free DHD's (pseudodiequatorial hydroxyl groups in THF) and their diesters (pseudodiaxial acyloxy groups) are similar in shape but opposite in sign due to the change in skew sense with change in conformational preference.[7b,16] In contrast, the CD spectra of pyrene-derived K-region *trans*-DHD's and their diesters do not change in sign despite similar changes in conformational preference; e.g., the resolved *trans*-4,5-DHD of BeP and its (-)-MOA diester (Figure 2A), and the resolved *trans*-4,5-DHD of pyrene and its (-)-α-methoxy(trifluoromethyl)phenylacetic acid diester.[4,7b] Similarly, CD spectra of the K-region *trans*-4,5-DHD's of benzo[a]pyrene (BaP) and 6-bromo-BaP of identical absolute configuration but opposite conformation (pseudodiequatorial and pseudodiaxial hydroxyl groups, respectively) show maxima at different wavelengths but with the same sign.[17]

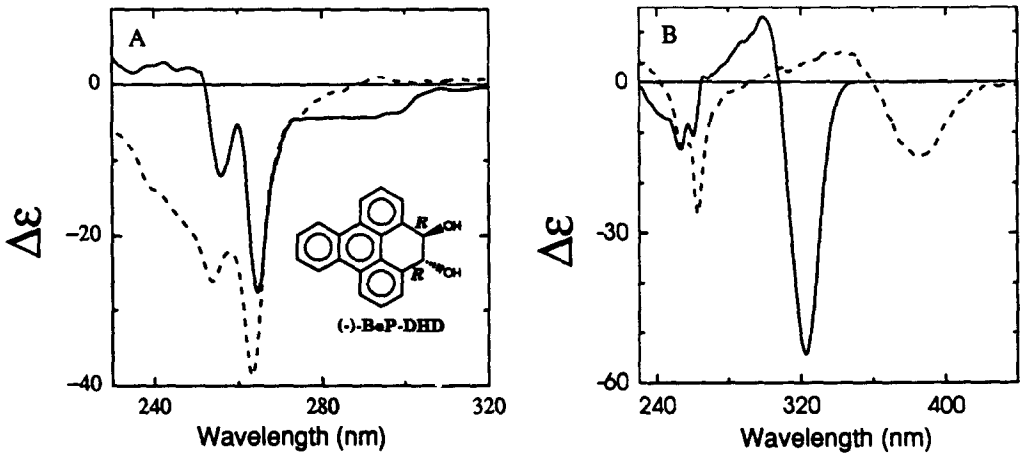

Figure 2. CD spectra of BeP (-)-(4*R*,5*R*)-DHD and its diesters. (A) Free DHD (solid line) and the *early*-eluting (-)-MOA diester (dashed line) in THF. (B) The *p*-(dimethylamino)benzoate (solid line) and *trans*-*p*-(dimethylamino)cinnamate (dashed line) diesters in CH_3CN.

The K-region *trans*-DHD's and diesters related to pyrene have twisted "phenanthrene-like" chromophores. As such, their CD spectra might be expected to reflect the skew sense of this twist. Resolved 4,5-dimethylphenanthrene, for example, has a very strong CD spectrum due to such a helical twist (27.9° angle between the mean planes of the outer rings).[18] Molecular modeling calculations (CHARM-QUANTA, Polygen, Waltham, MA) were used to obtain energy minimized structures for pseudodiequatorial conformations of the K-region *trans*-DHD's of BeP and Pyr (dihedral angles C_{8a}-C_{12d}-C_{12c}-C_{12b} of 12°, and C_{10a}-C_{10b}-C_{10c}-C_{8a} of 6°, respectively). The modest degree of twist calculated for the DHD's may account for the insensitivity of their CD spectra to the skew sense of this twist.

Incubation of BeP-O[19a], Pyr-O[19b], and Phe-O[19b] with liver microsomes[20] from untreated, male adult rats of the Long-Evans strain (50 mM Tris-HCl buffer, pH 8.4, 37 °C) provided enantiomerically enriched *trans*-DHD's (Table I).[21] The enantiomeric composition of the DHD from BeP-O was determined with the chiral column. DHD's from Pyr-O and Phe-O were converted to their (-)-MOA diesters and analyzed by HPLC as described previously.[7b] Relative amounts of the enantiomers and diastereomeric diesters were determined by integration (260 nm). Metabolism of Pyr to its *trans*-4,5-DHD with liver microsomes from

Table I. Enantiomeric composition of *trans*-DHD's formed from *meso*-arene oxides by MEH from rat liver.[a]

Meso-Arene Oxide	Enantiomer Composition	
	% (*R*,*R*)	% (*S*,*S*)
BeP-O	83	17
Pyr-O	86	14
Phe-O	42	58
Benzene oxide[b]	82	18

[a]50 mM Tris-HCl buffer, pH 8.4, at 37 °C. [b]Result from reference 22 using rabbit liver microsomes.

control and induced rats gave similar results, 78-79% (4*R*,5*R*)-enantiomer.[4] Liver microsomal metabolism of Phe also gave comparable results (42% *trans*-(9*R*,10*R*)-DHD)[3b] as did hydrolysis of Phe-O by rat liver microsomes[3a] and purified[3c] MEH (37-40% (9*R*,10*R*)-enantiomer). The results show that MEH preferentially catalyzes attack of water at (*S*)-oxirane carbons of *meso*-arene oxides (benzene oxide included) except in the case of Phe-O, although the differences in energy for attack at the (*S*)- and (*R*)-carbons of Phe-O are quite small (0.2 kcal/mol for a ratio of 42:58). In the case of *cis*-stilbene oxide, which closely resembles Phe-O in structure, MEH from rabbit liver preferentially catalyzes attack (>96%) at the (*S*)-oxirane carbon.[23,24] Only two other cases of predominant attack of water at the (*R*)-carbon of a *meso*-epoxide have been reported. Incubation of 10,11-dihydro-10,11-epoxy-5*H*-dibenzo[a,d]cycloheptene with rabbit liver microsomes gives

76% of the *trans*-(10*S*,11*S*)-enantiomer.[24] Patients taking 5*H*-dibenzo[b,f]azepine-5-carboxamide metabolize it to *trans*-dihydroxy-10,11-dihydro-5*H*-dibenzo[b,f]azepine-5-carboxamide (90% (10*S*,11*S*)-enantiomer), which presumably is formed *via* the corresponding epoxide.[25] These epoxides can be considered analogs of Phe-O in which the central six-membered ring is expanded to a seven-membered ring. In *meso*-substrates, the two epoxide centers have identical chemical reactivities and therefore intrinsic chemical reactivities are not a factor in the observed enantioselectivity. The enantioselectivity expressed in the product *trans*-DHD must be due to differential stabilization of the two chiral transition states for reaction of the epoxide and water. In the case of chiral arene oxides, the stereoselectivity of MEH is also highly substrate dependent and in addition is influenced by the relative reactivity of the two epoxide centers.

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21. (a) In a typical experiment, BeP-O (0.8 μmol in 0.40 mL of acetonitrile) was added to 1 mg of microsomal protein in 6 mL of Tris-HCl buffer (50 mM, pH 8.4) and incubated at 37 °C for 10 min. Reaction was stopped with 50 mL of 33% acetone in ethyl acetate and the organic layer washed with water. The product *trans*-DHD was purified by HPLC as described above in reference 6. Products from Pyr-O and Phe-O were purified by HPLC as described in reference 7b. Yields of *trans*-DHD ranged from 1-10% of the starting oxide depending upon the length of incubation and the amount of microsomes added. Results are the average of two or more determinations. Control incubations done in the presence of 400 μM 3,3,3-trichloropropene 1,2-oxide, a potent inhibitor of MEH described in reference 21b, established that spontaneous hydrolysis was not significant. (b) Oesch, F.; Kaubisch, N.; Jerina, D. M.; Daly, J. W. *Biochemistry* **1971**, *10*, 4858-4866.
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