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ABSOLUTE CONFIGURATION OF THE K-REGION 4,5-DIHYDRODIOLS AND 4,5-OXIDE OF BENZO[a]PYRENE

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Abstract: Application of the exciton chirality rule has allowed assignment of absolute configuration to (+)-trans-(4R,5R)-dihydroxy-4,5-dihydrobenzo[a]pyrene, a mammalian metabolite of the hydrocarbon. The cis 4,5-dihydrodiol and 4,5-oxide are also assigned based on correlation with the trans dihydrodiol.

Because of the widespread interest in the metabolism of the environmental carcinogen benzo[a]pyrene (B[a]P) and in the biological activity of its metabolites[1], the present study sought to determine the absolute configuration of the trans 4,5-dihydrodiol (*1*) and other K-region derivatives of the hydrocarbon. Previous studies have shown that the proximally carcinogenic[2] trans 7,8-dihydrodiol of B[a]P formed either by mammalian[3,4] or fungal enzyme[5] systems consists predominantly of the (7R,8R) enantiomer ($[\alpha]_D$ -410°, THF). This dihydrodiol is formed by the epoxide hydrolase catalyzed trans addition of water to B[a]P (7R,8S)-oxide ($[\alpha]_D$ +100°, THF) at C-8[6]. The predominant mammalian enantiomers of the K-region trans dihydrodiols formed from phenanthrene[7] and benzo[a]anthracene[8] have also been assigned; phenanthrene (9S,10S)-dihydrodiol ($[\alpha]_D$ -170°, THF) and benzo[a]anthracene (5R,6R)-dihydrodiol ($[\alpha]_D$ +50°, THF).

Resolution of the (±)-trans-4,5-dihydrodiol of B[a]P, (±)-*1*, was achieved by chromatographic separation of its bis esters with (-)-menthoxyacetic acid. The bis esters (*2*) were obtained in quantitative yield by allowing the dihydrodiol to react with an excess of menthoxyacetyl chloride in pyridine at 4°C for 15 hrs. Usual workup followed by rapid filtration through silica gel with methylene chloride provided a solid consisting of equal amounts of the two diastereomers: HPLC on a Du Pont Zorbax SIL column (6.2 x 250 mm) eluted with 10% ether in cyclohexane; (-)-*2*, k_1' = 1.88 and (+)-*2*, k_2' = 2.17. Initial crystallization of the solid (212 mg) from hexane provided 41 mg of nearly pure more polar diastereomer, whereas recrystallization of the concentrated mother liquor from methanol provided 60 mg of nearly pure less polar diastereomer. Diastereomerically pure samples were obtained by passage of enriched samples (~50 mg/injection) through a 2.12 x 25 cm Du Pont Zorbax SIL column eluted with 5% ether in cyclohexane:

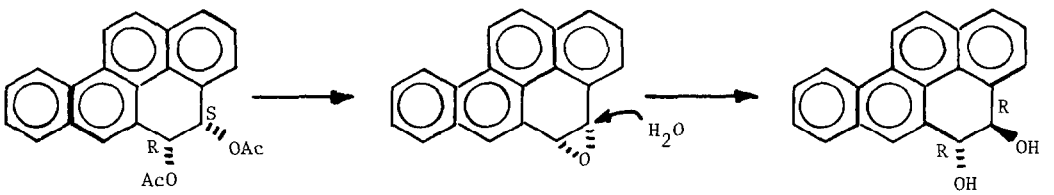
(-)-*2*; k_1' (less polar) = 1.88, mp 149-150°C, $[\alpha]_D$ -299° (c = 5 mg/ml, THF)
(+)-*2*; k_2' (more polar) = 2.17, mp 168-169°C, $[\alpha]_D$ +147° (c = 5 mg/ml, THF)

The nmr spectra (100 MHz, C_6D_6) of the diastereotopic protons of the pair of $-CO-CH_2-O-$ in the (-)-menthoxyacetyl groups in (+)-*2* and (-)-*2* are particularly diagnostic. In (-)-*2*, each of the $-CH_2-$ groups appears as a singlet at 3.64 δ and 3.60 δ (slightly broadened) whereas in (+)-*2* the $-CH_2-$ group in each of the ester groups appears as an AB quartet (3.48, 3.52, 3.76, and 3.80 δ with $J_{A,B} \sim J_{A',B'}$ = 8.1 Hz). Comparison of the nmr spectra, relative retention times, and values of their $[\alpha]_D$ with those of related vicinal trans bromomenthoxyacetates suggests that (-)-*2* may have (4R,5R) absolute configuration[9].

Hydrolysis of (-)-*2* (50% 1N NaOH in CH_3OH, 0°C, 90 min) provided (+)-*1*, $[\alpha]_D$ +39° (c = 5 mg/ml, THF). In THF solution, the (+)-dihydrodiol shows a negative rotation below 436 nm. The sign of rotation of (+)-*1* is also solvent dependent; $[\alpha]_D$ -61° (c = 4 mg/ml, CH_3OH). The ORD spectrum of (+)-*1* in methanol shows an increasingly negative rotation from 600 nm down to 350 nm while the CD spectrum shows a strong negative band at 271 nm in methanol. Metabolism of B[a]P by liver microsomes is known to produce (+)-*1* as the major enantiomer of the trans 4,5-dihydrodiol.[10]

Absolute configuration was assigned to (+)-*1* by application of the exciton chirality rule of Harada and Nakanishi[14] to the CD spectrum of the bis-(p-N,N-dimethylamino)benzoate of a derivative of *1*. In order to minimize undesired interactions between the electric transition dipoles of the two benzoate chromophores and the dihydrodiol chromophore, (+)-*1* (5 mg) was reduced with H_2 (45 psi, 5 days) in the presence of Pt (20 mg PtO_2 in 3 ml THF). The crude produce was acetylated (pyridine/acetic anhydride) and separated into a hexahydro (*3*, 70%) and an octahydro (*4*, 30%) derivative of *1*[15] (cf. Fig. 1); on a Du Pont Zorbax SIL column (6.2 mm x 25 cm) eluted with CH_2Cl_2 (6 ml/min), *3* emerged at 7.2 min and *4* at 10.4 min. Both *3* and *4* were hydrolyzed to the free diols and converted into their bis-(p-N,N-dimethylamino)benzoate esters in the usual manner. Their CD spectra (Fig. 1) show strong negative interaction bands ∿320-325 nm crossing through zero ∿310-313 nm which requires (4R,5R) absolute configuration[14]. The lack of complete symmetry for the positive bands at shorter wavelength is probably due to interaction between the p-N,N-dimethylaminobenzoates and the hydrocarbon residue as would be expected based upon comparison with the CD spectrum of the bis-(p-N,N-dimethylamino)dibenzoate of (-)-(9S,10S)-dihydroxy-9,10-dihydrophenanthrene[8,16]. Notably, there is fairly good symmetry between the Cotton effects centered at 313 nm for the octahydro derivative of *4*. Thus, the predominant enantiomer of the 4,5-dihydrodiol formed from B[a]P by rat liver microsomes has (4R,5R) absolute configuration in contrast to speculation[11] that the opposite enantiomer is formed.

Epoxide hydrolase has been shown to selectively hydrate (+)-B[a]P 4,5-oxide to (+)-*1* by attack of water at the 4-position[17]. Thus, (+)-B[a]P 4,5-oxide[18] and (+)-cis dihydrodiol diacetate from which it is derived must both have (4S,5R) absolute configuration. (+)-B[a]P (4S,5R)-oxide appears to be the predominent enantiomer formed from B[a]P by liver microsomes[13], is the better substrate for epoxide hydrolase[17], and has 1.5- to 5.5-fold less mutagenic activity when compared to the (-)-enantiomer[18].



$[\alpha]_D$ +48° (THF) $[\alpha]_D$ +123° (THF) (+)-*1* ($[\alpha]_D$ +39°, THF)

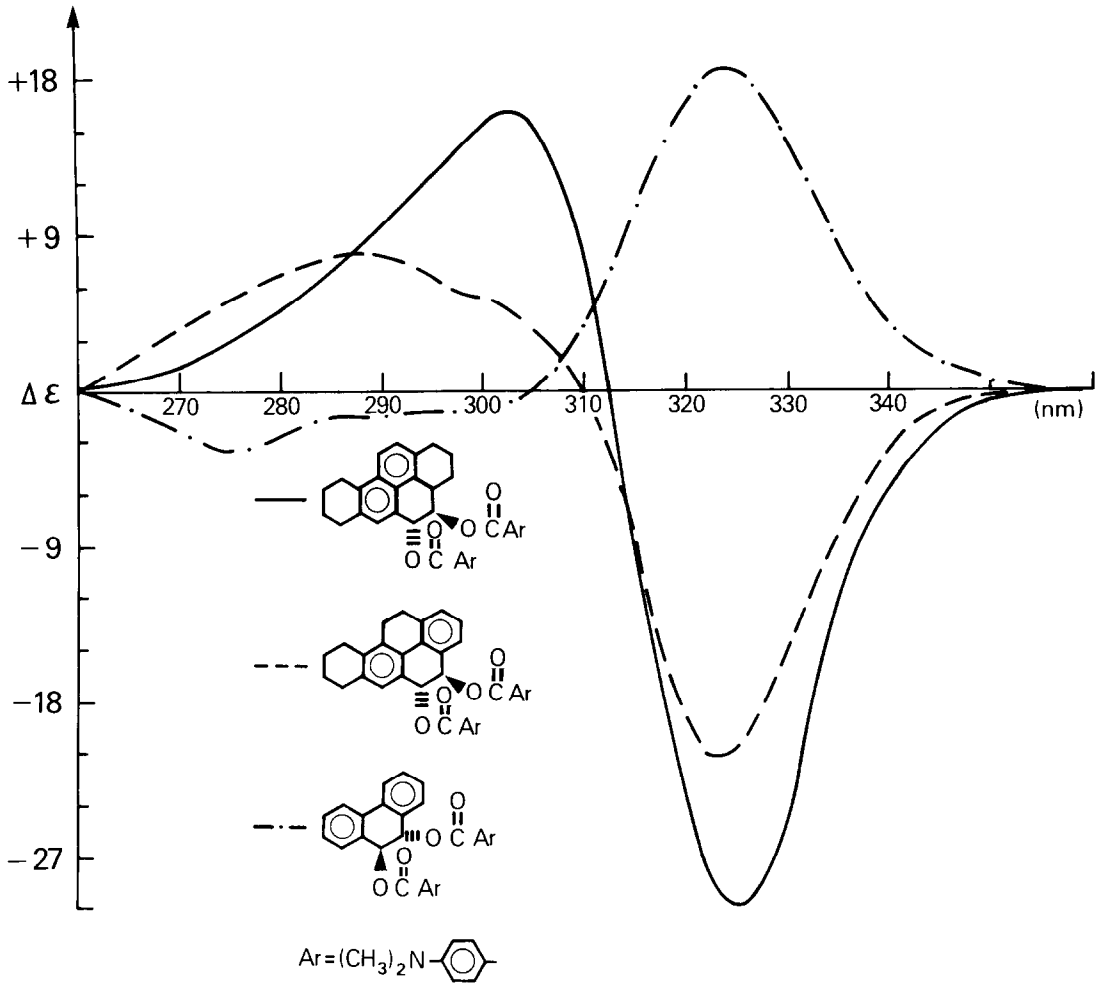


Figure 1. Circular dichroism spectra (CH_3OH) of the bis-(p-N,N-dimethylamino)benzoates of the hexahydro (---, *3*) and octahydro (——, *4*) derivatives of (+)-*1*. The strong negative interact bands (∿320-325 nm) require (4R,5R) absolute configuration. The circular dichroism spectrum of the corresponding diester of (-)-(9S,10S)-dihydroxy-9,10-dihydrophenanthrene (-·-) of opposite configuration is shown for comparison.

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10. The enantiomer described as (+)-*1* in the present study has been designated as the minus enantiomer based on ORD[11] and CD[12] data (CH_3OH) in earlier metabolism studies. The bis-(-)-α-methoxy-α-trifluoromethylphenylacetate of (+)-*1* emerges earlier from Zorbax ODS columns eluted with CH_3OH/H_2O gradients than does the diastereomer formed from (-)-*1*[12,13].
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15. Compound *3* has a biphenyl chromophore (λ_{max} = 285 nm, CH_3OH) very similar to that described by Yagi et al.[3]. Although two such hexahydro derivatives with a biphenyl chromophore are possible, nmr signals (100 MHz, $CDCl_3$) for two benzylic ester hydrogens (H_4 and H_5, 6.01 δ with $J_{4,5} \sim 0$ Hz) and four aromatic hydrogens as two singlets (3H at 7.10 δ and 1H at 7.02 δ) require structure *3*.

    Not counting stereoisomers, three octahydro derivatives with a naphthalene chromophore (wide band at 288 nm with two sharp bands at 318 and 333 nm in CH_3OH) are possible. Structure *4* was selected since it is the only one which has three aromatic hydrogens (H_6 at 6.95 δ as a singlet and H_{11}, H_{12} at 7.24, 7.76 δ with $J_{11,12} \sim 11$ Hz; H_4 at 5.44 δ (m) and H_5 at 6.43 δ (br. d) with $J_{3a,4} \sim J_{4,5} \sim 10$ Hz). Compound *4* is probably a mixture of epimers at carbon 3a.
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