of iodide obscures the species undergoing protonolysis. Note $k_H/k_D \approx 1$.[31]
(44) (a) W. A. Nugent, F. Bertini, and J. K. Kochi, *J. Am. Chem. Soc.*, **96**, 4945 (1974). The electrochemical oxidation potentials are $\eta_{.06}$ values from ref 39b which were unavailable when this work was published. (b) H. C. Gardner and J. K. Kochi, *J. Am. Chem. Soc.*, **98**, 558 (1976); (c) **97**, 1855 (1975). (d) The electrophile E^+ in eq 14 is not intended to apply to only positively charged species since electrophiles as structurally diverse as olefins (TCNE), peroxides (di-*tert*-butyl peroxide) and anions [hexachloroiridate(IV)] have been employed with substitution-labile (Grignard reagents) and substitution-stable (alkylmercury and lead) organometals.
(45) (a) H. Taube, "Electon Transfer Reactions of Complex Ions in Solution", Academic Press, New York, N.Y., 1970. (b) W. L. Reynolds and R. W. Lumry, "Mechanisms of Electron Transfer", Ronald Press, New York, N.Y., 1966. (c) R. A. Marcus and N. Sutin, *Inorg. Chem.*, **14**, 213 (1975), and earlier papers.
(46) (a) J. O. Edwards and R. G. Pearson, *J. Am. Chem. Soc.*, **84**, 16 (1962). (b) See also R. E. Davis and A. Cohen, *Ibid.*, **86**, 440 (1964).
(47) G. Klopman in ref 7, p 57 ff.
(48) The relationship of the covalent terms to the ionization potential is described by M. Arbelot, J. Metzger, M. Chanon, C. Gulmon, and G. Pfister-Guillouzo, *J. Am. Chem. Soc.*, **96**, 6217 (1974).
(49) R. G. Pearson, *Science*, **151**, 172 (1966); *J. Am. Chem. Soc.*, **85**, 3533 (1963).
(50) L. G. Makarova in "Methods of Elemento-Organic Chemistry", Vol. 4, A. N. Nesmeyanov and K. A. Kocheshkov, Ed., North-Holland Publishing Co., Amsterdam, 1967.
(51) Further evidence for a large steric contribution to **C** (and not to **L**) is supplied by preliminary studies on the effect of β-methyl substitution. Thus the value of **L** along the series: Et, *n*-Pr, *i*-Bu, neopentyl remains essentially constant, but **C** drops off sharply. A quantitative study of steric and polar contributions to **C** is in progress.

Dihydrodiols from Anthracene and Phenanthrene

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Abstract: Relative stereochemistry has been assigned to the vicinal dihydrodiols produced from anthracene and phenanthrene by bacterial and mammalian enzymes. The mutant strains, *Beijerinckia* strain B-836 and *Pseudomonas putida* strain 119, which are deficient in dihydrodiol dehydrogenase activity, accumulate (+)-*cis*-1,2-dihydroxy-1,2-dihydroanthracene and (+)-*cis*-3,4-dihydroxy-3,4-dihydrophenanthrene in the culture medium when incubated with anthracene and phenanthrene, respectively. *cis*-1,2-Dihydroxy-1,2-dihydrophenanthrene was also detected as a minor product from phenanthrene. Trans dihydrodiols at the 1,2, 3,4, and 9,10 positions of phenanthrene were synthesized by reduction of *o*-quinones, and the kinetics of their formation by epoxide hydrase from the corresponding arene oxides were determined. The 9,10-oxide proved to be one of the best known substrates for epoxide hydrase. Rates of dehydration and the ratio of phenols produced from the dihydrodiols were highly dependent on both configuration and conformation. Coupling constants for vicinal, trans hydrogens in cyclic systems tend to be much larger than those for the corresponding cis isomers. In contrast to this, *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene was found to have a larger coupling constant ($J_{3,4}$ = 5.5 Hz) than the corresponding trans isomer ($J_{3,4}$ = 2.0 Hz) due to unusual conformational effects for these diols near the hindered bay position of phenanthrene. Coupling constants are reported for cis and trans, non-K-region dihydrodiols in each of the four possible conformations.

Systematic studies of the stereochemistry of the mammalian and bacterial dihydrodiols from anthracene and phenanthrene have not been reported. Relative stereochemistry of vicinal dihydrodiols of biological origin is of interest as it relates to the fundamental mechanisms utilized by living organisms for the metabolism of aromatic hydrocarbons.[2] Dihydrodiols formed during the mammalian metabolism of aromatic hydrocarbons are generally thought to arise by the trans enzymatic hydration of initially formed arene oxides,[3] whereas dihydrodiols formed from aromatic hydrocarbons by bacteria have cis stereochemistry and are produced by the action of dioxygenases which incorporate both oxygen atoms from the same oxygen molecule into the substrate.[4]

Metabolism of phenanthrene to a dihydrodiol can occur at any of three ring positions with destruction of aromaticity in only one of the three aromatic rings. Mammals form dihydrodiols at each of these positions. The preponderant dihydrodiol has been assigned as *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene.[5,6] The trans relative stereochemistry was firmly assigned, since the corresponding cis isomer was known from the action of osmium tetroxide on the parent hydrocarbon.[7] The 1,2-dihydrodiol[6,8,9] has been assumed to be the trans isomer. Very small amounts of another dihydrodiol, assumed to be *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene, have been isolated.[9] These three dihydrodiols are detectable as in vitro metabolites formed when phenanthrene is incubated with hepatic microsomes.[10]

3,4-Dihydroxy-3,4-dihydrophenanthrene was identified as an intermediate in the degradation of phenanthrene by a *Flavobacterium* species,[11] which led to the suggestion that soil pseudomonas oxidize phenanthrene through *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene to 3,4-dihydroxyphenanthrene.[12] However, the authors pointed out that neither of these reactions had been demonstrated enzymatically, nor were definitive assignments of stereochemistry made.

Mammals oxidize anthracene to *trans*-1,2-dihydroxy-1,2-dihydroanthracene.[13] Interestingly, evidence has been presented for the formation of *trans*-9,10-dihydroxy-9,10-dihydroanthracene.[14] Since the carbinol groups are at remote positions of the central aromatic ring, this dihydrodiol may not originate from an arene oxide. A 1,2-dihydrodiol of unknown stereochemistry has been implicated as a metabolite in the degradation of anthracene by a *Flavobacterium* species.[15]

The results described below establish relative stereochemistry of the dihydrodiols formed from anthracene and phenanthrene by mammals and bacteria on the basis of their proton magnetic resonance spectra. A prior study of the cis and trans 1,2-dihydrodiols of naphthalene[2] established the applicability of the Karplus equation[16] to dihydrodiols of polycyclic aromatic hydrocarbons. These four spin systems are particularly suited to conformational analysis due to the angular dependence of the magnitudes of the coupling constants. Although trans isomers generally have much larger coupling constants[17] than the corresponding cis isomers, unequivocal assignments

of relative stereochemistry are best made when both isomers are available for analysis. The cis and trans dihydrodiols formed at the 1,2 position of anthracene and at the 1,2, 3,4, and 9,10 positions of phenanthrene have now been examined and are compared with the previously reported data for the cis and trans isomers from naphthalene.[2] Kinetic parameters for the action of epoxide hydrase (EC 4.2.1.63) on the three arene oxides of phenanthrene and the specificity of bacterial dioxygenases toward anthracene and phenanthrene are reported.

Discussion

Synthesis of Dihydrodiols. The reduction of *o*-quinones with lithium aluminum hydride has been employed[18] to synthesize trans dihydrodiols at the 1,2 position of anthracene (non-K-region) and the 9,10 position of phenanthrene (K-region). Yields of dihydrodiols from K-region *o*-quinones are moderate to excellent, while yields from non-K-region quinones, such as naphthalene and anthracene 1,2-quinone are markedly lower. Although this procedure generally shows very high stereospecificity in generating trans dihydrodiols, the reduction of 7,12-dimethylbenzo[*a*]anthracene 5,6-quinone[19] produced nearly equal amounts of the cis and trans isomers. The present examination of the reduction of anthracene 1,2-quinone with lithium aluminum hydride established that the principal product is 1,2-dihydroxy-1,2,3,4-tetrahydroanthracene. Although *p*-quinones such as naphthalene 1,4-quinone are known to overreduce in this fashion,[20] this is the first example in which an *o*-quinone has been overreduced. Two minor products (~5%) were identified as the desired cis and trans 1,2-dihydrodiols. The cis isomer, which has a lower R_f on boric acid treated TLC plates, was identical with the metabolite formed from anthracene by the bacterium, *Beijerinckia* B-836, with the exception of optical activity. Reduction of phenanthrene 9,10-quinone with lithium aluminum hydride produces the desired trans dihydrodiol in 84% yield.[18] Unfortunately, application of this procedure to the 1,2- and 3,4-quinones of phenanthrene provided only 4 and 1%, respectively, of the desired trans dihydrodiols. No evidence was obtained for the formation of cis stereoisomers in either of these reductions. Although the yields of non-K-region dihydrodiols were unsatisfactory, adequate quantities were obtained to pursue the present studies.

While alternate reducing agents such as diisobutyl aluminum hydride have shown some promise for the preparation of dihydrodiols from quinones,[21] the present study indicates that reduction of non-K-region quinones will be of limited success in providing the dihydrodiols necessary to study the metabolism of larger polycyclic hydrocarbons. An alternate approach in which the final double bond was introduced into cis or trans diesters of 7,8,9,10-tetrahydrobenzo[*a*]pyrene has shown excellent promise for the synthesis of dihydrodiols at non-K-region positions of polycyclic hydrocarbons.[22]

Bacterial Dihydrodiols. *Pseudomonas putida*, strain 119, and a *Beijerinckia* species, strain B-836, are mutant strains that oxidize naphthalene and biphenyl to cis dihydrodiols.[2,23] When grown in the presence of succinate as a carbon source and an aromatic hydrocarbon which induces the dioxygenase system, these bacteria accumulate dihydrodiols in the culture medium, since the mutants are deficient in dihydrodiol dehydrogenase. Metabolism by the wild strains proceeds to ring-cleaved products without significant accumulation of intermediates. Both organisms, when grown on succinate in the presence of anthracene or phenanthrene were found to accumulate dihydrodiols in the culture medium. The presence of two vinyl hydrogens, two carbinol hydrogens, and the patterns of coupling constants in the ^{1}H NMR spectra of these compounds (Table I) requires dihydrodiol structures for each in the terminal ring of the hydrocarbons. The bacterial product from anthracene is identical with the low R_f synthetic dihydrodiol on boric acid treated TLC plates, readily forms an acetonide, and has a ^{1}H NMR spectrum which is remarkably similar to the cis dihydrodiol from naphthalene[2] (Table I). Taken together, these data allow assignment as (+)-*cis*-1,2-dihydroxy-1,2-dihydroanthracene. In a separate study,[24] this diol was shown to be the optically pure 1(*R*),2(*S*) enantiomer.

With phenanthrene as the substrate for the bacteria, two dihydrodiol metabolites were detected. The major product (>90%) readily dehydrates to 3-phenanthrol (Tables II and III), forms an acetonide, and has an uv (but not ^{1}H NMR) spectrum almost identical with the synthetic trans 3,4-dihydrodiol. Chemical shifts of the benzylic carbinol hydrogen and benzylic vinyl hydrogen (Table I) are instrumental in assigning dihydrodiols. Edge deshielding of hydrogens in bay positions of polycyclic hydrocarbons, such as H(4) and H(5) in phenanthrene, is well recognized.[25] The bacterial diol and the synthetic trans 3,4-dihydrodiol have their benzylic carbinol hydrogens shifted downfield by ~0.6 ppm from the average position (δ 4.81 ± 0.16) observed for all the compounds of this (Table I) and the previous study,[2] indicating these hydrogens are in bay positions. Thus, the major dihydrodiol from phenanthrene has been assigned as (+)-*cis*-3,4-dihydroxy-3,4-dihydrophenanthrene. The diol has 4(*R*),3(*S*) absolute stereochemistry.[26]

The minor (<10%) bacterial dihydrodiol from phenanthrene as well as synthetic *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene have their benzylic vinyl hydrogens shifted downfield by ~0.7 ppm from the average position (δ 6.59 ± 0.19) observed in the ^{1}H NMR spectra for the compounds in this series (Table I and ref 2), indicating these vinyl hydrogens are in bay positions. The bacterial metabolite was assigned as *cis*-1,2-dihydroxy-1,2-dihydrophenanthrene. Analysis of the crude dihydrodiol metabolite fraction from phenanthrene by high pressure liquid chromatography gave no indication of the presence of other diols such as *cis*-9,10-dihydroxy-9,10-dihydrophenanthrene (<0.5%).

The above assignments of cis relative stereochemistry to the bacterial diols have been based mainly on the ease with which these compounds form acetonides. Although it is possible to construct acetonides of trans dihydrodiols with Dreiding stereomodels, attempts to synthesize acetonides of the trans dihydrodiols at the 1,2 position of naphthalene[2] and the 9,10 position of phenanthrene[6] have been unsuccessful. The validity of these assignments is confirmed by the discussion of the ^{1}H NMR spectra in Table I which appears later.

Dehydration Rates and Product Ratios. Theoretical calculations of charge density and the index of free valence have been employed to predict the direction in which unsymmetrical dihydrodiols eliminate water to form phenols.[27] The calculations require the hydroxyl group which is retained to reside on the carbon atom of highest electron density in the parent hydrocarbon. Relative stereochemistry of the hydroxyl groups was suggested to be unimportant in establishing the direction in which water is eliminated. While these correlations have proved substantially correct for the compounds studied, the method fails to take into account the fact that both kinetic and thermodynamic control are capable of dictating product formation.

Rates of dehydration in 3.12 N HCl at 25 °C (Table III) and product distribution from dehydration in 5 N HCl at 100 °C (Table II) were determined for the dihydrodiols used in this study. Dehydration of the cis and trans dihydrodiols from anthracene yielded results similar to those observed on dehydration of cis and trans naphthalene dihydrodiols where 1-naphthol predominates (>95%).[2] Thus, 1-anthrol (86–90%) is the predominant product. The results obtained for both naphthalene and anthracene dihydrodiols are easily rationalized in terms of carbonium-ion stability as indicated below.

Table I. Analysis of Proton Magentic Resonance Spectra Given by Anthracene and Phenanthrene Dihydrodiols and Their Derivatives[a]

Compd	Carbinol protons		Vinyl protons		Aromatic protons
	Benzylic	Other	Benzylic	Other	
cis-1,2-Dihydroxy-1,2-dihydroanthracene (I)	H(1), 4.82	H(2), 4.42 ($J_{1,2}$ = 4.6, $J_{2,3}$ = 4.3, $J_{3,4}$ = 9.8 Hz)	H(4), 6.69	H(3), 6.10	7.30–8.00
Acetonide of I	H(1), 5.18	H(2), 4.96 ($J_{1,2}$ = 6.4, $J_{2,3}$ = 3.0, $J_{2,4}$ = 1.5, $J_{3,4}$ = 10.0 Hz) singlets for methyl groups at δ 1.37 and 1.47	H(4), 6.61	H(3), 5.93	7.30–7.90
Diacetate of I	H(1), 6.27	H(2), 5.75 ($J_{1,2}$ = 4.3, $J_{2,3}$ = 4.0, $J_{2,4}$ = 1.2, $J_{3,4}$ = 9.7 Hz) singlets for acetates at δ 2.02 and 2.13	H(4), 6.81	H(3), 6.03	7.20–7.90
trans-1,2-Dihydroxy-1,2-dihydroanthracene (II)	H(1), 4.89	H(2), 4.50 ($J_{1,2}$ = 10.5, $J_{2,3}$ = 1.8, $J_{2,4}$ = 2.2, $J_{3,4}$ = 10.0 Hz)	H(4), 6.57	H(3), 6.03	7.30–8.10
Diacetate of II	H(1), 6.31	H(2), 5.63 ($J_{1,2}$ = 5.6, $J_{2,3}$ = 4.15, $J_{2,4}$ = 1.0, $J_{3,4}$ = 9.8 Hz) singlets for acetates at δ 2.02 and 2.12	H(4), 6.81	H(3), 6.07	7.30–7.90
trans-1,2-Dihydroxy-1,2-dihydrophenanthrene(III)[b]	H(1), 4.97	H(2), 4.59 ($J_{1,2}$ = 11.75, $J_{2,3}$ = 2.25, $J_{2,4}$ = 2.5, $J_{3,4}$ = 10.0 Hz)	H(4), 7.22	H(3), 6.26	7.34–8.16
Diacetate of III	H(1), 6.33	H(2), 6.56 ($J_{1,2}$ = 6.0, $J_{2,3}$ = 4.3, $J_{2,4}$ = 1.3, $J_{3,4}$ = 10.0 Hz) singlets for acetates at δ 2.04 and 2.11	H(4), ~7.45	H(3), 6.24	7.35–8.23
cis-1,2-Diacetoxy-1,2-dihydrophenanthrene	H(1), 6.23	H(2), 5.79 ($J_{1,2}$ = 4.5, $J_{2,3}$ = 3.8, $J_{2,4}$ = 1.6, $J_{3,4}$ = 9.6 Hz) singlets for acetates at δ 2.06 and 2.11	H(4), 7.44	H(3), 6.18	7.25–8.20
cis-3,4-Dihydroxy-3,4-dihydrophenanthrene (IV)[b,c]	H(4), 5.35	H(3), 4.74 ($J_{3,4}$ = 5.5, $J_{2,4}$ = 1.3, $J_{2,3}$ = 12.1, $J_{1,3}$ = 2.75, $J_{1,2}$ = 9.75 Hz)	H(1), 6.55	H(2), 6.03	7.23–8.23
Acetonide of IV	H(4), 5.75	H(3), 5.02 ($J_{3,4}$ = 8.0, $J_{2,3}$ = 3.0, $J_{1,3}$ = 1.5, $J_{1,2}$ = 9.95 Hz) singlets for methyl groups at δ 1.47 and 1.53	H(1), 6.45	H(2), 5.98	7.10–8.30
Diacetate of IV[d]	H(4), 6.91	H(3), 5.88 or 6.01 ($J_{3,4}$ = 5.05, $J_{2,4}$ = 1.35, $J_{1,3}$ = 3.3, $J_{1,2}$ = 10.2 Hz) singlets for acetates at δ 1.99 and 2.15	H(1), 6.65	H(3), 5.88 or 6.01	7.22–8.08
trans-3,4-Dihydroxy-3,4-dihydrophenanthrene[b]	H(4), 5.46	H(3), 4.48 ($J_{3,4}$ = 1.5–2.0, $J_{2,3}$ = 5.5, $J_{1,2}$ = 9.5 Hz)	H(1), 6.78	H(2), 6.31	7.23–8.30
cis-9-Acetoxy-10-hydroxy-9,10-dihydrophenanthrene[e]		H(9), 6.04, H(10), 4.93 $J_{9,10}$ = 3.8; singlet for acetate at δ 1.92			7.14–7.86
trans-9-Acetoxy-10-hydroxy-9,10-dihydrophenanthrene[d]		H(9), 6.06, H(10), 4.83 $J_{9,10}$ = 8.2; singlet for acetate at δ 2.10			7.20–7.90

[a] Spectra were measured in $CDCl_3$ at 100 or 220 MHz. Where necessary 10–20% acetone-d_6 was added to improve solubility. This amount of acetone is thought to have no effect on the conformational equilibrium, since no significant changes in coupling constants were observed when *trans*-1,2-dihydroxy-1,2-dihydroanthracene and phenanthrene were recorded in acetone as solvent. Previously, the spectra of the cis and trans dihydrodiols of naphthalene remained essentially unchanged with $CDCl_3$, acetone, or Me_2SO as the solvent. Line positions are reported in δ (ppm) relative to internal tetramethylsilane. Coupling constants (J) are given in Hertz (Hz). All spectra were recorded after replacement of exchangeable (hydroxyl) protons with deuterium. Decoupling was required for assignments in some spectra. Attempts to observe individual conformers for diols at low temperature (cf. ref 2) were impractical due to poor solubility. [b] Spectra recorded at 220 MHz. [c] Produced by *Beijerinckia* B8-36. [d] Chemical shifts for H(2) and H(3) are so close that these hydrogens cannot be assigned. Furthermore, $J_{2,3}$ could not be measured, since this portion of the spectrum is so far from first order at 100 MHz. [e] The cis and trans monoacetates were also examined in CD_3OD at 100 MHz and showed comparable coupling constants for $J_{9,10}$ (J_{cis} = 3.7, J_{trans} = 7.7 Hz).

Table II. Ratios of Phenols Formed after Acid-Catalyzed Dehydration of Anthracene and Phenanthrene Dihydrodiols[a]

Dihydrodiol	Phenols (%)
trans-1,2-Dihydroxy-1,2-dihydrophenanthrene	1-Phenanthrol (66); 2-phenanthrol (34)
trans-3,4-Dihydroxy-3,4-dihydrophenanthrene	3-Phenanthrol (59); 4-phenanthrol (41)
cis-3,4-Dihydroxy-3,4-dihydrophenanthrene	3-Phenanthrol (>98); 4-phenanthrol (<2)
trans-1,2-Dihydroxy-1,2-dihydroanthracene	1-Anthrol (86); 2-anthrol (14)
cis-1,2-Dihydroxy-1,2-dihydroanthracene	1-Anthrol (90); 2-anthrol (10)

[a] Conditions of dehydration and quantitation of phenols are described in Experimental Section.

Support for this concept is found in the spontaneous isomerization of arene oxides to phenols via carbonium ions. Naphthalene 1,2-oxide, phenanthrene 1,2-oxide, and phenanthrene 3,4-oxide rearrange predominantly to 1-naphthol, 1-phenanthrol, and 4-phenanthrol, respectively.[28] Thus, 1- and 4-phenanthrol could be expected as the principal products formed on dehydration of the 1,2- and 3,4-dihydrodiols of phenanthrene, respectively. Such is not the case (Table II). The most extreme example is the cis 3,4-dihydrodiol, which produces practically none of the expected 4-phenanthrol. In contrast, the corresponding trans isomer produces 41% of 4-phenanthrol.

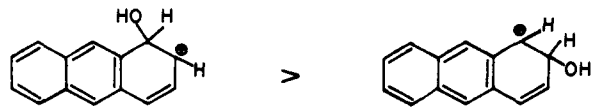


Table III. Rates of Dehydration of Phenanthrene and Anthracene Dihydrodiols in 3.12 M HCl at 25 °C[a]

Dihydrodiol	$10^6 \times k$, s^{-1}
cis-9,10-Dihydroxy-9,10-dihydrophenanthrene	95.6
trans-9,10-Dihydroxy-9,10-dihydrophenanthrene	0.85
cis-3,4-Dihydroxy-3,4-dihydrophenanthrene	122000 (estd)[b]
trans-3,4-Dihydroxy-3,4-dihydrophenanthrene	1460
trans-1,2-Dihydroxy-1,2-dihydrophenanthrene	156
cis-1,2-Dihydroxy-1,2-dihydroanthracene	4700
trans-1,2-Dihydroxy-1,2-dihydroanthracene	131

[a] Conditions as described in Experimental Section. [b] The observed rate constant for *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene at 2.0% of the above acid concentration and the same ionic strength was 2.43 $\times 10^{-3}$ s^{-1}.

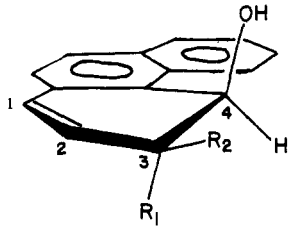
The observed dehydration ratios establish with certainty that relative stereochemistry plays a significant role in determining the ratio of dehydration products. The effect of conformation on product ratios will be discussed below.

Comparison of dehydration rates for the dihydrodiols (Table III) consistently showed the cis isomers to dehydrate substantially faster than the corresponding trans isomers. Previously, the cis 1,2-dihydrodiol of naphthalene was noted to dehydrate 45 times faster than the corresponding trans isomer.[2] For the three pairs of compounds studied, the cis stereoisomers dehydrated 36–113 times faster than the trans stereoisomers. An earlier study of the relative dehydration rates for the cis and trans dihydrodiols at the 9,10 position of phenanthrene showed the cis isomer to dehydrate 14 times faster than the trans isomer[6] compared to >100 times faster in the present study. Since cis dihydrodiols uniformly dehydrate much faster than the corresponding trans isomers, relative rates of dehydration appear to be a reliable method for assigning stereochemistry to these systems. Rates of glycol cleavage are occasionally unreliable.[6,29]

Analysis of ^{1}H NMR Spectra. In general, dihydrodiols of aromatic hydrocarbons can be considered as rapidly interconverting pairs of conformers at room temperature. Coupling constants represent the weighted average of the couplings in these conformers provided interconversion is fast on the ^{1}H NMR time scale. Factors such as a proximate bulky group or an intramolecular bridge might be anticipated to restrict or inhibit the population of one of these states. Intramolecular hydrogen bonding can also be a significant factor in determining the population of the two conformers, particularly for a trans dihydrodiol, where the conformer in which the hydroxyl groups are quasi-equatorial would be stabilized. For vicinal, non-K-region[30] dihydrodiols, the conformers of the trans isomer have the hydroxyl groups either quasi-axial or quasi-equatorial. For the cis isomer, the conformers have the benzylic and allylic hydroxyl groups quasi-axial and quasi-equatorial, respectively, or vice versa. Due to distortion in these systems, the dihedral angle between the adjacent carbinol hydrogens is somewhat less than 180° and greater than 60° in the two conformers of trans dihydrodiols and is less than 60° for both conformers of cis dihydrodiols. Values of $^3J_{1,2}$ (H(1)-C-C-H(2)), $^3J_{2,3}$ (H(2)-C-C-H(3)), and $^4J_{2,4}$ (H(2)-C-C=C-H(4)) were calculated from the bond angles observed in Dreiding stereomodels of the four conformers of *trans*- and *cis*-1,2-dihydroxy-1,2-dihydronaphthalenes[2] with the aid of the three appropriate Karplus curves as described by Becker.[31] The calculations predict $J_{1,2}$ = 5.1 Hz for both of the conformers of the cis isomer and $J_{1,2}$ = 4.1 Hz when the hydroxyl groups are quasi-axial vs. $J_{1,2}$ = 12.7 Hz when the hydroxyl groups are quasi-equatorial in the conformers of the trans isomer. Values of $J_{2,3}$ = 2.7 and $J_{2,4}$ = −2.3 Hz were calculated for the cis isomer with the benzylic hydroxyl group quasi-axial and the trans isomer with both hydroxyl groups quasi-equatorial, since the dihedral angles are the same for these conformers. For the cis isomer with the benzylic hydroxyl group quasi-equatorial and the trans isomer with both hydroxyl groups quasi-axial, values of $J_{2,3}$ = 5.5 and $J_{2,4}$ = 0.3 Hz were calculated. Coupling constants of $J_{1,2}$ = 12.0, $J_{2,3}$ = 1.6, and $J_{2,4}$ = −2.3 were observed at −70 °C for the trans isomer in the conformation in which the hydroxyl groups are quasi-equatorial. This conformer also greatly predominates at room temperature. Correction for the reduction in coupling constants due to the electronegative hydroxyl groups was unnecessary in this case, since these effects are maximal only when the dihedral angle between the hydrogen in question and the electronegative substituent is either 180° (antiperiplanar) or 0° (eclipsed) (cf ref 17 for a discussion). The coupling constants

for the cis isomer ($J_{1,2}$ = 5.1, $J_{2,3}$ = 3.8, $J_{2,4}$ < 0.5 Hz) indicate that the conformer with the benzylic hydroxyl group quasi-equatorial (as with the trans isomer) is greatly favored at room temperature. The conformational preference of 1- and 2-substituents in related 1,2-dihydronaphthalenes appears to be markedly dependent on the nature of the substituent.[57-60] The extent to which $J_{1,2}$ is reduced by the effect of the electronegative oxygen substituent at C(2) in this conformer would be expected to be rather small, since the Dreiding stereomodel suggests a deviation of at least 30° from the optimal antiperiplanar geometry. Formation of an acetonide increases $J_{1,2}$ to 6.9, since the dihedral angle for both conformers of the acetonide are decreased compared to the diol. Acetylation of the trans isomer greatly decreases $J_{1,2}$ to 6.0 Hz, while $J_{2,3}$ and $J_{2,4}$ approach the values in the cis isomer, as would be expected for extensive population of the conformer with both oxygen substituents quasi-axial due to steric repulsion between the acetates and the loss of intramolecular hydrogen bonding.

Comparison of the coupling constants (Table I) for the free cis diols, their diacetates, and their acetonides for the 1,2-dihydrodiols from anthracene and phenanthrene with those for *cis*-1,2-dihydroxy-1,2-dihydronaphthalene[2] show a striking similarity, i.e., mainly the conformer with the benzylic hydroxyl group quasi-equatorial. A similar correlation holds for the trans isomers at the 1,2 positions of anthracene and phenanthrene when compared to *trans*-1,2-dihydroxy-1,2-dihydronaphthalene, where the hydroxyl groups are almost entirely quasi-equatorial. Striking exceptions to the above correlations are the dihydrodiols at the 3,4 position of phenanthrene. The pattern of coupling constants for the vinyl hydrogens of *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene looks similar to the previous trans isomers and the pattern for *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene resembles that of the previous cis isomers. These observations are entirely consistent with the previous data, provided these two dihydrodiols exist predominantly as the conformers in which the benzylic hydroxyl groups are pseudo-axial as shown below.



trans R_1 = OH, R_2 = H

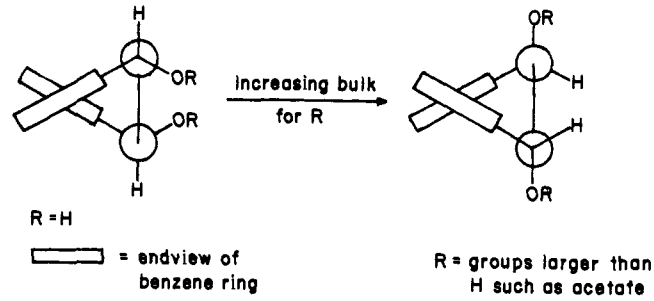
cis R_1 = H, R_2 = OH

This change in conformation is reasonable and, to some extent, predictable due to the highly hindered bay region of the hydrocarbon. *cis*-3,4-Dihydroxy-3,4-dihydrophenanthrene has

an interesting long range "W" coupling of $^4J_{2,4}$ = 1.3 Hz (cf. ref 17 and 31) which causes the nonbenzylic vinyl hydrogen (H(2)) to appear as a doublet of triplets. Since the trans diols not in bay regions show this coupling only as slight line broadening, this "W" coupling may be diagnostic for cis dihydrodiols in hindered bay regions. A similar "W" coupling of 1.3 Hz has been observed in related chlorinated dihydronaphthalenes,[60] but could not be confirmed, since one of the hydrogens was obscured by other components in the sample. The value of $J_{3,4}$ = 1.5–2.0 Hz for *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene is somewhat lower than predicted.[2] Steric hindrance between the carbinol H(4) and the aryl H(5) in the bay region may cause an expansion of the dihedral angle between H(3) and H(4) such that this angle approaches 90°, the minimum in the Karplus curve[31] at which J = 1.9 Hz. At the same time, the hydroxyl groups and the adjacent carbinol hydrogens would be approaching eclipsed conformations, a maximum for reduction of vicinal coupling constants due to electronegativity effects.[17] The somewhat low value for $J_{3,4}$, thus, may be due to a slight distortion of the conformer with both hydroxyl groups in quasi-axial environments.

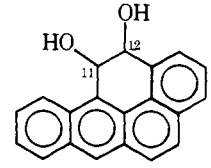
The present study thus provides examples of cis and trans non-K-region dihydrodiols which exist either entirely or preponderantly in all four possible conformations. The cis diols show vicinal coupling constants between the carbinol hydrogens of 4.6–5.5 Hz, which decrease 0.3–0.4 Hz on acetylation and increase 1.8–2.5 Hz on formation of acetonides. Long-range coupling (^4J) and particularly edge deshielding induced changes in chemical shift are diagnostic for positional isomers in hindered bay regions. Typically, trans isomers show coupling constants of 10–12 Hz between the carbinol hydrogens, which is reduced 4–6 Hz on acetylation. When the trans diol is near a bay region, the vicinal coupling is reduced to ~2 Hz. For cis and trans non-K-region dihydrodiols, the benzylic hydroxyl group prefers a quasi-equatorial position, except when hindered by a bay region.

The monoacetates of *cis*- and *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene ($J_{9,10}$ = 3.8 and 8.2 Hz, respectively) were examined as examples of K-region[29] dihydrodiols (Table I). The monoacetates were required due to symmetry in the free diols. For cis dihydrodiols at K-regions, inspection of Dreiding stereomodels suggests that the dihedral angles between the carbinol hydrogens in both conformers expands relative to the non-K-region dihydrodiols and almost reaches 60° (^3J = 4.2 Hz).[31] In each of these conformers, one of the hydroxyl groups and the adjacent carbinol hydrogen are antiperiplanar, the optimal geometry for reduction of the coupling constant due to the electronegative hydroxyl group.[17] Thus, coupling constants of ≤4 Hz are to be expected. *cis*-9-Acetoxy-10-chloro-9,10-dihydrophenanthrene has a value of $J_{9,10}$ = 4.0 Hz.[60] The dihedral angles between the carbinol hydrogens in the two conformers of trans dihydrodiols at K-regions approximate 60 (^3J = 4.2 Hz) or 180° (^3J = 13 Hz). These coupling constants would be only marginally reduced due to electronegativity effects in either conformer. In contrast to the cis isomers, the observed coupling constant for trans isomers should be highly dependent on conformation. By analogy to the trans 1,2-dihydrodiols of naphthalene, anthracene, and phenanthrene, *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene (K-region) should exist mainly as the conformer in which both hydroxyl groups are quasi-equatorial due to intramolecular hydrogen bonding. This coupling cannot be directly observed due to symmetry. Mono- or diacetylation should cause increased population of the conformer in which the substituents are quasi-axial due to adverse steric interactions and decrease in intramolecular hydrogen bonding as shown below. The coupling constant of 8.2 Hz in the monoacetate (Table I) is of the magnitude expected for an equal mixture of both conformers. Introduction of a second acetate



should reduce the coupling constant further. Such a decrease has been observed on acetylation of *trans*-9-hydroxy-10-methoxy- and *trans*-9-hydroxy-10-tertiarybutylamino-9,10-dihydrophenanthrene, where $J_{9,10}$ decreases from ~9 to ~4 Hz.[32] Burton et al.[60] have calculated that *trans*-9-acetoxy-10-chloro-9,10-dihydrophenanthrene, with $J_{9,10}$ = 3.4 Hz, has the substituents quasi-axial 74% of the time. Miura et al.[33] have concluded that optically active *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene exists mainly as the conformer in which the oxygen substituents are quasi-axial and that the diacetate has the substituents mainly quasi-equatorial based on circular dichroism spectra. These assignments are inconsistent with the present study, and are probably in error when compared with the circular dichroism studies of Joshua et al.[34] on a closely related structure.

The coupling constant of 3.8 Hz for the monoacetate of *cis*-9,10-dihydroxy-9,10-dihydrophenanthrene (Table I) is as expected. Similar values should be observed for free cis dihydrodiols and their diacetates at K-regions of other hydrocarbons. The cis and trans dihydrodiols at the 11,12 position (K-region) of benzo[*a*]pyrene provides an interesting case for comparison, since the dihydrodiols are near a bay region and can be considered as analogues of the 3,4-dihydrodiols of phenanthrene; i.e., the predominant conformers should have the 11-hydroxy group quasi-axial. In the ^{1}H NMR spectra of



the diacetates[35] the benzylic hydrogens appear as doublets at δ 7.52 and 6.64 for the cis isomer and at δ 7.27 and 6.49 for the trans isomer. The lower field signal in each case is due to H(11), which is edge deshielded by the bay region. The coupling constants for the cis ($J_{11,12}$ = 4.4 Hz) and trans ($J_{11,12}$ = 2.6 Hz) isomers are as predicted from the 3,4-dihydrodiols in the phenanthrene series; i.e., $^3J_{11,12}$ is again smaller for the trans isomer. Notably, the cis isomer has acetate signals at δ 1.89 and 2.36, whereas the acetates for the trans isomer appear at δ 1.85 and 1.93. Presumably, the acetate at C(12) of the cis isomer is edge deshielded and appears at lower field since it is quasi-equatorial. A similar effect has been observed for the *cis*- and *trans*-9-acetoxy-10-chloro-9,10-dihydrophenanthrenes.[59] Finally, since *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene shows measurable coupling between H(3) (allylic) and H(1) (benzylic vinyl) as the dihedral angle for these hydrogens approaches 90°, coupling between H(12) and nearby aryl hydrogens can be expected for *cis*-11,12-diacetoxy-11,12-dihydrobenzo[*a*]pyrene and is observed. The signal for H(12) appears as a doublet ($J_{11,12}$) of apparent triplets due to weak coupling ($2J_{12,Ar} < 1$ Hz) to proximate aryl hydrogens. Such long-range couplings between quasi-axial hydrogens in K-region, cis dihydrodiols ($J_{H_{ax},H_{Ar}}$) for which conformational interconversion is severly limited (bay region or steric interaction with bulky groups) can be expected for other hydrocarbons such as chrysene or 7,12-dimethylbenzo[*a*]anthra-

Table IV. Kinetic Parameters for the Hydration of Phenanthrene Oxides by Epoxide Hydrase[a]

Phenanthrene oxide	K_m, mM	V_{max}[b]
Phenanthrene 1,2-oxide	2.9	26.7
Phenanthrene 3,4-oxide	2.0	4.6
Phenanthrene 9,10-oxide	0.2	38.0

[a] Details of these experiments are given in Experimental Section. [b] Dihydrodiol (nmol)/min/mg of protein.

cene. Clearly, assignment of relative stereochemistry for two electronegative substituents at a dihydro K-region of a polycyclic hydrocarbon is complicated by not knowing the population of the two conformers for the trans isomers. Values of $J_{K\text{-region}}$ for trans isomers vary from as little as 2.6 Hz for the above trans diacetate from benzo[*a*]pyrene and 3.0–3.5 Hz for trans halohydrin acetates of several polycyclic hydrocarbons[36,60] to as high as 9.4 Hz for the trans adduct between *tert*-butylamine and phenanthrene 9,10-oxide.[32] In a series of cis and trans dihydrodiol diacetates at K-regions, values of $J_{K\text{-region}}$ were found to vary in the narrow range of 3–5 Hz.[37]

Epoxide Hydrase. Metabolism of phenanthrene by mammals has been examined in vivo and with hepatic preparations. With liver microsomes,[10] about 90% of the metabolism proceeds to the three dihydrodiols via the action of epoxide hydrase[3] on the intermediate arene oxides. The kinetics for hydration of these oxides have been examined with a solubilized preparation of epoxide hydrase from rat liver. Preparative incubations of phenanthrene 1,2- and 9,10-oxides, the two best substrates, indicated that only trans dihydrodiols were formed based on chromatography and ^{1}H NMR. All three oxides show substrate inhibition above 4 mM substrate concentration (Figure 2) with inhibition by the 1,2-oxide being most severe. Inhibition of epoxide hydrase by high concentrations of naphthalene oxide can be eliminated by ethanol.[38] Apparent values of K_m and V_{max} for the three phenanthrene oxides under the indicated incubation conditions are given in Table IV. The most stable of the oxides toward isomerization to a phenol is the 9,10-oxide at the K-region.[28,39] This oxide also has the lowest apparent K_m and highest apparent V_{max}. The very high reactivity of epoxide hydrase toward phenanthrene 9,10-oxide may partially account for the fact that practically no 9-phenanthrol can be isolated from in vitro[10] or in vivo[40] studies on phenanthrene. Very low spontaneous isomerization and solvolysis rates[28] coupled with reasonable epoxide hydrase activity could partially explain the inability of many investigations to detect phenols and their conjugates as metabolites at the K-region of polycyclic aromatic hydrocarbons. The low specificity of epoxide hydrase toward phenanthrene 3,4-oxide only partially explains the fact that the 3,4-dihydrodiol is a very minor metabolite from phenanthrene both in vivo and in vitro. A more complete understanding of the metabolite profile from aromatic hydrocarbons with the microsomal system from liver will be possible when the specificities of the hepatic oxygenases which form the arene oxides are also known.

Concluding Remarks

Reduction of *o*-quinones to dihydrodiols with metal hydrides has proved to be generally useful only when the quinones are at K-regions. In contrast, reduction of non-K-region quinones produces substantial amounts of tetrahydrodiols and/or catechols. In addition, both cis and trans dihydrodiols were observed from anthracene 1,2-quinone. The unpredictable nature of the reductions of non-K-region quinones suggests that alternate methods[22] may prove more useful.

A unique aspect of the present study lies in the identification of non-K-region dihydrodiols which exist either entirely or preponderantly as single conformers. In the absence of any unusual steric factors, non-K-region dihydrodiols greatly prefer the conformer in which the benzylic hydroxyl group is quasi-equatorial, regardless of whether the diol is cis or trans. When a cis or trans K-region or non-K-region dihydrodiol is part of a sterically hindered bay region of the hydrocarbon (e.g., phenanthrene 3,4 or benzo[*a*]pyrene 11,12 positions), the hydroxyl group or derived acetate which is part of the bay region greatly prefers the conformation in which the electronegative substituent is quasi-axial. In this latter case, the somewhat unusual situation pertains in which the coupling constant (J_{diol}) for the cis isomer (4.4–5.5 Hz) greatly exceeds that of the trans isomer (1.5–2.6 Hz). Thus, the four possible conformers of cis and trans dihydrodiols at non-K-region positions have been observed.

The actual mechanism by which dihydrodiols dehydrate in aqueous acid has yet to be explored. Carbonium ion stability adequately explains the 1-phenol as the primary product from the 1,2-dihydrodiols of anthracene and phenanthrene, but breaks down at the 3,4 position of phenanthrene, where the cis isomer produces almost all 3-phenthrol. This highly directed elimination (loss of the quasi-axial hydroxyl group at C(4)) may result from better charge stabilization at the transition state when the protonated hydroxyl group leaves from a quasi-axial position. Both factors could operate for the 1,2-dihydrodiols described here; the cis isomers all have the 2-hydroxyl group mainly quasi-axial at the position of the most stable carbonium ion, whereas the trans isomers have a minor amount of this conformer in the equilibrium. Thus, the cis 1,2-diols would dehydrate faster if both hydroxyl groups were equally basic, and both isomers should favor the 1-phenol as the product. This, however, is not the entire explanation, since *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene has a quasi-axial hydroxyl group at C(3), the position of the more stable carbonium ion, yet dehydrates 80 times slower than the cis isomer. The data are suggestive of a dehydration mechanism for the cis isomer in which loss of a protonated quasi-axial hydroxyl group is at least partially concerted with loss of the antiperiplanar proton at the transition state in a trans diaxial elimination. Relief of steric strain on reaching the transition state may also be a factor responsible for the higher dehydration rates observed for the cis isomers. Selective dehydration of hindered cis dihydrodiols has had practical value in the synthesis of 12-hydroxybenzo[*a*]pyrene[41] and 5-hydroxy-7,12-dimethylbenzo[*a*]anthracene.[42]

For metabolism of polycyclic aromatic hydrocarbons by mammals, both the monoxygenase system which forms arene oxides and epoxide hydrase play a role in establishing the relative amounts of various dihydrodiols. The present study represents the first comparison of epoxide hydrase activity for all of the metabolically reasonable arene oxides from a given polycyclic hydrocarbon. For phenanthrene, the K-region arene oxide (9,10-position) not only is the best substrate within this set, but also appears to be one of the best substrates known for the enzyme. Although phenanthrene 3,4-oxide is the least effective substrate in this set, it may be premature to assume that a hindered bay region arene oxide is necessarily a poor epoxide hydrase substrate, since benzo[*a*]pyrene 9,10-oxide appears to be a good substrate.[43] A major reason for our interest in dihydrodiols stems from the possibility that diol epoxides derived from non-K-region dihydrodiols may be ultimate carcinogens.[44,45]

Experimental Section

Ultraviolet spectra and reaction rates were recorded on a Cary Model 14 spectrophotometer. Reaction rates were also measured with a Perkin-Elmer MPF-3L fluorescence spectrophotometer. Mass spectra were measured with a Hitachi RMU-7 spectrometer. Only selected major fragments are reported. Proton magnetic resonance

spectra were recorded on Varian HA-100 and 220 MHz spectrometers. Unless noted otherwise $CDCl_3$ was used as solvent. Coupling constants (J) are recorded in Hertz and chemical shifts in parts per million (δ) with tetramethylsilane as internal standard. High pressure liquid chromatography was performed with a Du Pont 830 instrument equipped with a 254-nm photometer.

Preparation of Phenanthrols[46] and Anthrols. 1- and 4-Phenanthrol were synthesized by dehydrogenation of the corresponding tetrahydro ketones.[47] 2-Phenanthrol was prepared from the 2-sulfonate as described by Fieser.[48] Synthesis of 1- and 2-anthrol was by sulfonation of anthracene to yield the 1- and 2-sulfonates.[49] Each sulfonate was fused with KOH to yield the appropriate anthrol.

Preparation of Phenanthrene Oxides. Phenanthrene 9,10-oxide was prepared from *cis*-9,10-dihydroxy-9,10-dihydrophenanthrene[7] via the dioxolane route.[36] Phenanthrene 1,2- and 3,4-oxides were prepared from the corresponding tetrahydro ketones (see above) via the halohydrin acetate route.[50]

Preparation of Phenanthrene Dihydrodiols. *cis*-9-Acetoxy-10-hydroxy-9,10-dihydrophenanthrene was obtained by partial hydrolysis of the corresponding 2-methyl-2-methoxy-1,3-dioxolane in aqueous acetone.[36]

Reduction of *o*-quinones with lithium aluminum hydride in ether[18] allowed the synthesis of the trans dihydrodiols used in this study. Hydride reductions were terminated with alkali.[51] *trans*-9,10-Dihydroxy-9,10-dihydrophenanthrene, prepared by reduction of the 9,10-quinone, was converted to *trans*-9-acetoxy-10-hydroxy-9,10-dihydrophenanthrene by dissolving the diol (53 mg, 0.25 mmol) and acetic anhydride (0.37 mmol) in 1.0 ml of pyridine. Conventional procedures led to the isolation of 30 mg (0.12 mmol) of the monoacetate. Unreacted diol and its diacetate accounted for the balance of the material. Reduction of 1.4 g of phenanthrene 1,2-quinone[52] by the above procedure provided 68 mg (4% yield) of pure *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene after workup and repeated crystallization from cyclohexane: $\lambda_{max}^{CH_3OH}$ 237 (ϵ 40 264) and 316 nm (ϵ 7235); M^+ 212 (32), 194 (38), 166 (100), and 165 (80). Reduction of 1.5 g of phenanthrene 3,4-quinone[53] was even less satisfactory due, in part, to the instability of the diol. After workup, the crude product was subjected to preparative TLC (1.00 mm silica gel-GF, Analtech, benzene/chloroform/ethyl acetate/triethylamine (30:30:30:1)). The *trans*-3,4-dihydroxy-3,4-dihydrophenanthrene (R_f 0.2) was further purified by high pressure liquid chromatography on a Du Pont preparative ETH column (⅜ in. × 1.0 m) with 3% ethanol in hexane as the mobile phase: 12.0 mg, 0.8% yield; λ_{max}^{MeOH} 242 (ϵ 20 000), 250 (ϵ 36 800), and 259 nm (ϵ 48 000); M^+ 212 (11), 166 (3), 165 (33), and 57 (100). The synthetic trans 1,2- and 3,4-dihydrodiols appear to be identical, with the exception of optical activity, with the same dihydrodiols isolated as metabolites from phenanthrene during the mammalian oxidation of phenanthrene.[9] The proton magnetic resonance spectra of the synthetic trans dihydrodiols and the monoacetate derivatives are presented in Table I.

Preparation of Anthracene Dihydrodiols. Anthracene 1,2-quinone (0.6 g) was reduced as previously described.[18] The authors indicated that the dihydrodiol was difficult to isolate in pure form. The crude product (0.4 g) was crystallized from ethyl acetate to yield 120 mg of 1,2-dihydroxy-1,2,3,4-tetrahydroanthracene, which was identified by the absence of vinyl protons in its ^1H NMR spectrum and by the presence of a molecular ion at m/e 214. The mother liquor from above was subjected to preparative TLC on boric acid treated silica gel plates (benzene/chloroform/ethyl acetate (1:1:1)). Two products (R_f 0.58 and 0.30) were isolated. The compound with R_f 0.58 (30 mg) was identified as *trans*-1,2-dihydroxy-1,2-dihydroanthracene: M^+ 212; ^1H NMR spectrum, Table I; λ_{Max}^{MeOH} 244 (ϵ 38 600), 276 (ϵ 9400), 286 (ϵ 12 200), and 298 nm (ϵ 12 000). The other compound (R_f 0.30, 30 mg) was identified as *cis*-1,2-dihydroxy-1,2-dihydroanthracene (see below).

Microbiological Oxidation of Phenanthrene. *Beijerinckia* B-836 was grown in 10 l. of mineral salts medium which contained (per liter): sodium succinate, 1.0 g; yeast extract, 1.0 g; and phenanthrene, 0.4 g. The culture was grown in a New Brunswick Model M14 Microferm fermentor. After 24 h the culture was filtered through glass wool to remove the remaining phenanthrene and then extracted with 6 l. of ethyl acetate. The ethyl acetate extract was dried (Na_2SO_4) and the solvent removed to yield 1.80 g of a brown oil. The oil (200 mg) was dissolved in benzene and applied to a column (2 × 20 cm) of deactivated silica gel (20% H_2O). The column was washed with benzene (100 ml), chloroform (100 ml), and finally with 0.25% methanol in chloroform. The last solvent eluted a chromatographically pure product (R_f 0.20, chloroform/acetone, 80:20): yield 170 mg; mp 123 °C from benzene (opaque 97–98 °C); λ_{max}^{MeOH} 214 (ϵ 23 800), 243 sh (ϵ 26 400), 252 (ϵ 38 300), 260 (ϵ 43 000), and 306 nm (ϵ 5300); mass spectrum, calcd for $C_{14}H_{12}O_2$, 212.0837; found 212.0832; $[\alpha]^{25}D$ = +58° (c = 0.034, MeOH). The bacterial metabolite was identified as *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene by acid-catalyzed dehydration to yield 3-hydroxyphenanthrene (Figure 1 and TLC) and by analysis of its ^1H NMR spectrum (Table I). In an analogous experiment *Pseudomonas putida* 119 produced 600 mg of *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene. There were no detectable differences between the dihydrodiol produced by *Beijerinckia* B-836 and *Pseudomonas putida* 119. The ultraviolet spectra of these cis diols are nearly identical with the corresponding trans diol. The dihydrodiol (100 mg) formed by *Beijerinckia* B-836 was converted to an acetonide by reaction with 2,2-dimethoxypropane (10 ml). The solution was cooled in an ice bath and reaction was initiated by addition of 1.0 mg of *p*-toluenesulfonic acid. After 25 min 1.0 g of Na_2CO_3 was added and the mixture was stirred for 10 min. Benzene (15 ml) was added and solids were removed by filtration. The filtrate was evaporated to dryness at room temperature, the residue dissolved in chloroform, and the solution was applied to a column (17 × 1.0 cm) of basic alumina. Elution with chloroform and removal of the solvent gave 56 mg of a viscous oil: mp 76–77 °C (lyophilized from benzene); $[\alpha]^{25}D$ +186° (c = 0.046, MeOH); λ_{max}^{MeOH} 216 (ϵ 29 000), 242 (ϵ 29,000), 250 (ϵ 53 000), 258 (ϵ 64 700), and 310 nm (ϵ 5800); mass spectrum, calculated for $C_{17}H_{16}O_2$, 252.1150; found 252.1146; ^1H NMR spectrum, Table I.

A crude sample of diol fraction (206 mg) obtained by column chromatography as above to remove phenanthrene was examined by high pressure liquid chromatography: two coupled 2.1 mm × 0.25 m Zorbax-5μ analytical Du Pont SIL columns eluted with 3% ethanol and 6% dioxane in petroleum ether (0.8 ml/min, 2300 psi). The sample was free of *cis*-9,10-dihydroxy-9,10-dihydrophenanthrene (<0.3%), but contained a minor, unresolved component (>5%) with longer retention time. The sample was acetylated, purified by TLC (R_f 0.2, benzene) on silica gel, and crystallized from cyclopentane to yield 87 mg of the cis 3,4-diacetate ($[\alpha]D$ −204°). The pmr ^1H NMR of the components in the mother liquor (122 mg) indicated that a minor diacetate was present (~20%). Further TLC (lower R_f component, 15% ether in cyclohexane) on silica gel allowed isolation of 24 mg (~10% of crude diacetate fraction) of *cis*-1,2-diacetoxy-1,2-dihydrophenanthrene: m/e M^+ 296 (100%), 236 (15%), 194 (100%), and 178 (95%). Although the sample exhibited a very weak, negative $[\alpha]D$, further studies have indicated that it is as highly optically active as other derivatives.[26]

Microbiological Oxidation of Anthracene. *Beijerinckia* B-836 was grown as described above with anthracene (0.2 g/l.) replacing phenanthrene. Extraction of the culture with ethyl acetate followed by removal of the solvent gave 600 mg of a solid residue: mp 125–126 °C (benzene/petroleum ether); $[\alpha]^{25}D$ +246° (c = 0.056, MeOH); λ_{max}^{MeOH} 244 (ϵ 55 600), 276 (ϵ 13 600), 287 (ϵ 17 000), and 298 nm (ϵ 16 300); mass spectrum calculated for $C_{14}H_{12}O_2$, 212.0837; found 212.0831. This product was identified as *cis*-1,2-dihydroxyl-1,2-dihydroanthracene by dehydration to 1- and 2-anthrol (Figure 1 and Table III) and by its ^1H NMR spectrum (Table I). An acetonide derivative of the cis anthracene dihydrodiol was prepared as described above for phenanthrene dihydrodiol: mp 140 °C (vacuum sublimation); $[\alpha]^{25}D$ +284° (c = 0.034, MeOH); mass spectrum, calculated for $C_{17}H_{16}O_2$, 252.1157, found, 252.1150; ^1H NMR spectrum, Table I.

Dehydration of Dihydrodiols. Dihydrodiols were dehydrated under nitrogen with 5 N HCl at 100 °C for 5 min. Phenols were extracted into ether after the solutions were saturated with sodium chloride and analyzed by TLC and high pressure liquid chromatography. Since losses during this procedure are small, the ratios of phenols (Table II) are not corrected for recoveries.

A. Kinetic Measurements. Rates of dehydration for each dihydrodiol were measured in 3.12 N HCl at 25 ± 0.1 °C. Formation of phenols from *cis*- and *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene and *trans*-1,2-dihydroxy-1,2-dihydrophenanthrene were monitored by the increase in absorbance at 252 nm. The similarity of the ultraviolet spectra given by the cis and trans dihydroduols at the 3,4 position of phenanthrene with the 3- and 4-phenanthrols precluded rate measurements by this procedure. Rates for these dihydrodiols were determined by excitation at 254 nm and measurement of the increase

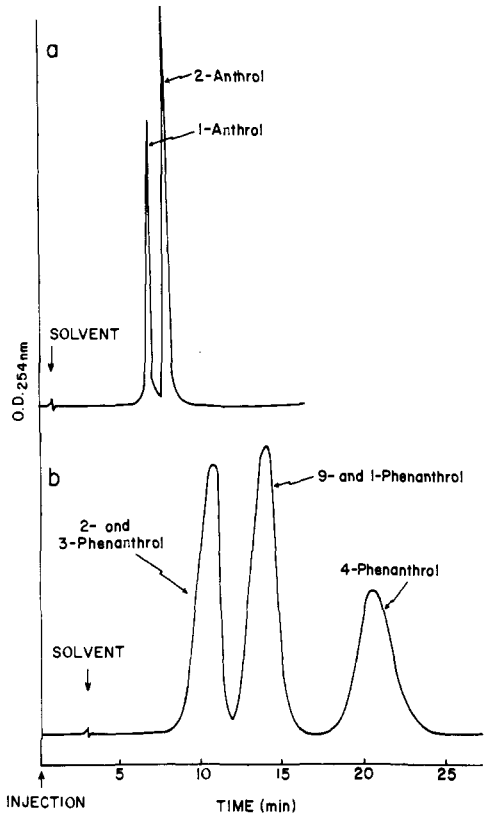


Figure 1. Separation of phenanthrols and anthrols by high pressure liquid chromatography. Zero time is the point at which the sample was injected. Breakthrough of the carrier solvents is shown. Anthrols (a) were separated on a Du Pont Zorbax-Sil column (25 cm) with 0.5% ethanol in hexane as the mobile phase. The flow rate was 0.9 ml/min. Phenanthrols (b) were separated on a Du Pont analytical ODS column (1 m) with 40% methanol in water as the mobile phase. The flow rate was 0.6 ml/min. Under these conditions, separation of 2- and 3-phenanthrol and 1- and 9-phenanthrol does not occur. For quantitative studies the photometer response was calibrated with known amounts of each phenol.

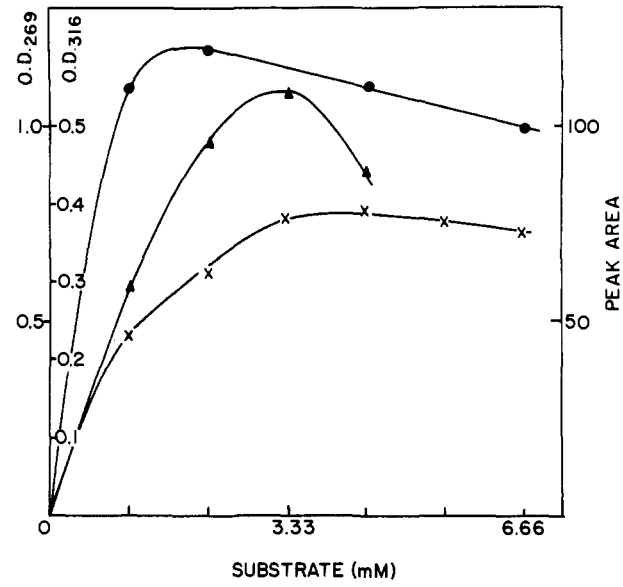


Figure 2. Hydration of phenanthrene oxides by epoxide hydrase. Details of incubation conditions and assay of products are given in the Experimental Section: ●, diol from phenanthrene 9,10-oxide (measured at 269 nm); ▲, diol from phenanthrene 1,2-oxide (measured at 316 nm); ×, diol from phenanthrene 3,4-oxide (measured by high pressure liquid chromatography).

in fluorescence at 373 nm. The formation of anthrols from *cis*- and *trans*-1,2-dihydroxy-1,2-dihydroanthracene was determined by measuring the increase in absorbance at 254 nm. All reactions were initiated by the addition of 5–10 μl of each dihydrodiol (in methanol) to 3.0 ml of aqueous acid. Reactions were monitored for 3–5 half-lives with the exception of *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene. Dehydration of this compound was so slow that data were collected for only 1 half-life (approximately 4 days). In contrast, the dehydration of *cis*-3,4-dihydroxy-3,4-dihydrophenanthrene was so rapid in 3.12 N HCl that accurate measurements could not be obtained. Thus, the acid was diluted by a factor of 50 and returned to the original ionic strength with lithium chloride. First-order rate constants were derived from plots of log $(A_\infty - A)$ vs. time (s). Lines were constructed from data points by application of least-squares analysis with the aid of a computer (Table III).

B. Identification and Quantitation of Phenols. A procedure for the complete separation of the five possible phenanthrols in unavailable. Thus, the pair 1- and 9-phenanthrol and the pair 2- and 3-phenanthrol have very similar chromatographic properties on high pressure liquid chromatography (Figure 1). However, a combination of mobility on TLC and color reaction with 2,6-dichloroquinone-4-chloroimide (Gibb's reagent) does allow qualitative identification of these isomers. Furthermore, the pairs of phenanthrols of interest in this study are readily separated by high pressure liquid chromatography. Details for the separations are given in the legend to Figure 1.

Epoxide Hydrase. Microsomes from phenobarbital-induced, immature (50–55 g), male, Long-Evans rats were solubilized with deoxycholate. The microsomal preparation was fractionated on a DEAE-cellulose column in the presence of deoxycholate.[54,55] Epoxide hydrase activity[56] eluted from the column with 0.2–0.3 M potassium chloride. All fractions containing epoxide hydrase activity were pooled and concentrated by membrane ultrafiltration with a PM-30 membrane. The concentrated solution was centrifuged at 160 000*g* for 90 min and the supernatant used as a source of epoxide hydrase in the following studies.

Reaction mixtures contained in a final volume of 0.45 ml: Tris–HCl buffer, pH 9.0, 50 μmol; Tween 80, 100 μg; lipid fraction, 100 μl;[55] and epoxide hydrase preparation (amounts indicated in the text). After preincubation at 30 °C for 1 min, reaction was initiated by the addition of substrate in 50 μl of acetonitrile. After incubation at 30 °C for 10 min, each reaction mixture was cooled to 0 °C and extracted with 2 × 2 ml of ethyl acetate. Extracts were dried (Na_2SO_4), concentrated to approximately 50 μl in vacuo, and applied to 5 × 20 cm thin-layer plates (250 μm, silica gel, Analtech) which had been prewashed with the developing solvent (benzene/chloroform/ethyl acetate/triethylamine (30:30:30:1)). The 1,2- and 9,10-dihydrodiols of phenanthrene were measured spectrophotometrically at 316 (ε 7235) and 269 nm (ε 15 142), respectively, after the dihydrodiols were extracted from the gel with 3.0 ml of methanol. The silica gel containing the 3,4-dihydrodiol was transferred to a small column and the reaction product eluted with 1% triethylamine in ethyl acetate. The eluate was concentrated to dryness in a stream of nitrogen, dissolved in 75 μl of ethanol, and analyzed by high pressure liquid chromatography with an ETH column as previously described. Results are corrected for control experiments in which equivalent amounts of bovine serum albumin was substituted for the epoxide hydrase preparation and recoveries of the 1,2-dihydrodiol (81 ± 2%), 3,4-dihydrodiol (71 ± 5%), and 9,10-dihydrodiol (87 ± 2%).

The amount of each oxide required to saturate the enzyme (0.328 mg protein) was determined after 10 min of incubation. The rate of the reaction is proportional to the protein concentration in this region. Substrates were used over the range 1–6 mM (Figure 2). Apparent Michaelis constants (K_m) were obtained from double reciprocal plots of initial reaction rates. Dihydrodiol formation was measured at 2-min intervals. Hydration of the 1,2-oxide was linear for 10 min, while hydration of the 9,10-oxide was linear for up to 8 min. In contrast, severe deviation from linearity was found for the 3,4-oxide. The K_m and V_{max} values given in Table IV were calculated from initial rates observed after 3 min of incubation. The relatively low sensitivity of these spectroscopic assays required the use of high protein concentrations. The kinetic data, especially the K_m, are anticipated to change when more sensitive radiometric assays are utilized at low protein concentrations.

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References and Notes

(1) (a) NIAMDD; (b) University of Texas.
(2) A. M. Jeffrey, H. J. C. Yeh, D. M. Jerina, T. R. Patel, J. F. Davey, and D. T. Gibson, *Biochemistry*, **14**, 575 (1975).
(3) D. M. Jerina and J. W. Daly, *Science*, **185**, 573 (1974).
(4) D. T. Gibson, *Crit. Rev. Microbiol.*, 1, 199 (1971).
(5) L. Young, *Biochem. J.*, **41**, 417 (1947).
(6) E. Boyland and G. Wolf, *Biochem. J.*, **47**, 64 (1950).
(7) R. Criegee, B. Marchand, and H. Wannowius, *Justus Leibigs Ann. Chem.*, **550**, 99 (1942).
(8) E. D. S. Corner and L. Young, *Biochem. J.*, **61**, 132 (1955).
(9) E. Boyland and P. Sims, *Biochem. J.*, **84**, 571 (1962).
(10) P. Sims, *Biochem. Pharmacol.*, **19**, 795 (1970).
(11) C. Colla, C. Biaggi, and V. Treccani, *Rend. Accad. Naz. Lincei*, **23**, 66 (1957).
(12) W. C. Evans, H. N. Fernley, and E. Griffiths, *Biochem. J.*, **95**, 819 (1965).
(13) E. Boyland and A. A. Levi, *Biochem. J.*, **29**, 2679 (1935).
(14) P. Sims, *Biochem. J.*, **92**, 621 (1964).
(15) C. Colla, A. Fiecchi, and V. Treccani, *Ann. Microbiol. Enzimol.*, **9**, 1 (1959).
(16) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).
(17) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2d ed, Pergamon Press, New York, N.Y., 1969, pp 280–304, 334–341.
(18) J. Booth, E. Boyland, and E. E. Turner, *J. Chem. Soc.*, 1188 (1950).
(19) S. H. Goh and R. G. Harvey, *J. Am. Chem. Soc.*, **95**, 242 (1973).
(20) E. Boyland and D. Manson, *J. Chem. Soc.*, 1837 (1951).
(21) A. M. Jeffrey, H. J. C. Yeh, and D. M. Jerina, *J. Org. Chem.*, **39**, 1405 (1974).
(22) D. T. Gibson, V. Mahadevan, D. M. Jerina, H. Yagi, and H. J. C. Yeh, *Science*, **189**, 295 (1975). See also D. J. McCaustland and J. F. Engle, *Tetrahedron Lett.*, 2549 (1975).
(23) D. T. Gibson, R. L. Roberts, H. C. Wells, and V. M. Kobal, *Biochem. Biophys. Res. Commun.*, **50**, 211 (1973).
(24) M. N. Akhtar, N. J. Thompson, D. R. Boyd, M. Koreeda, D. T. Gibson, V. Mahadwan, and D. M. Jerina, *J. Chem. Soc., Perkin Trans. 1*, 2506 (1975).
(25) K. D. Bartle and D. W. Jones, *Adv. Org. Chem.*, **8**, 317–424 (1972).
(26) Manuscript in preparation.
(27) G. M. Badger, *J. Chem. Soc.*, 2497 (1949).
(28) P. Y. Bruice, T. C. Bruice, P. M. Dansette, H. G. Selander, H. Yagi, and D. M. Jerina, *J. Am. Chem. Soc.*, **98**, 2965 (1976).
(29) A. S. Perlin, *Oxidation*, 1, 196 (1969).
(30) K-regions are typified by the 9,10 position of phenanthrene. See W. C. Herndon, *Trans. N. Y. Acad. Sci.*, **36**, 200 (1974) for a discussion.
(31) E. D. Becker, "High Resolution NMR", Academic Press, New York, N.Y., 1969, p 104.
(32) P. Y. Bruice, T. C. Bruice, H. Yagi, and D. M. Jerina, *J. Am. Chem. Soc.*, **98**, 2973 (1976).
(33) R. Miura, S. Honmaru, and M. Nakazaki, *Tetrahedron Lett.*, 5271 (1968).
(34) H. Joshua, R. Gans, and K. Mislow, *J. Am. Chem. Soc.*, **90**, 4884 (1968).
(35) O. Hernandez, P. M. Dansette, H. D. Mah, and D. M. Jerina, manuscript in preparation.
(36) P. Dansette and D. M. Jerina, *J. Am. Chem. Soc.*, **96**, 1224 (1974).
(37) R. G. Harvey, S. H. Goh, and C. Cortez, *J. Am. Chem. Soc.*, **97**, 3468 (1975), have reported chemical shifts and coupling constants for cis and trans dihydrodiol diacetates at K-regions of several polycyclic hydrocarbons. The basis of the assignments given were not provided, and, in some instances, are divergent with the concepts presented here.
(38) P. M. Dansette, H. Yagi, D. M. Jerina, J. W. Daly, W. Levin, A. Y. H. Lu, R. Kuntzman, and A. H. Conney, *Arch. Biochem. Biophys.*, **164**, 517 (1974).
(39) D. M. Jerina, H. Yagi, and J. W. Daly, *Heterocycles*, 1, 267 (1973).
(40) P. Sims, *Biochem. J.*, **84**, 558 (1962).
(41) H. Yagi, G. M. Holder, P. M. Dansette, O. Hernandez, H. J. C. Yeh, R. A. LeMahieu, and D. M. Jerina, *J. Org. Chem.*, **41**, 977 (1976).
(42) M. S. Newman and D. R. Olson, *J. Am. Chem. Soc.*, **96**, 6207 (1974).
(43) G. Holder, H. Yagi, P. Dansette, D. M. Jerina, W. Levin, A. Y. H. Lu, and A. H. Conney, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 4356 (1974).
(44) H. Yagi, O. Hernandez, and D. M. Jerina, *J. Am. Chem. Soc.*, **97**, 6881 (1975).
(45) P. Sims, P. L. Grover, A. Swaisland, K. Pal, and A. Hewer, *Nature (London)*, **252**, 326 (1974).
(46) 9- and 3-phenanthrols were gifts from Dr. E. May, National Institutes of Health.
(47) E. Mosettig and H. M. Duvall, *J. Am. Chem. Soc.*, **59**, 367 (1937).
(48) L. A. Fieser, *J. Am. Chem. Soc.*, 51, 2460 (1929).
(50) H. Yagi and D. M. Jerina, *J. Am. Chem. Soc.*, **95**, 243 (1973).
(51) V. M. Micovic and M. L. Mihailovic, *J. Org. Chem.*, **18**, 1190 (1953).
(52) L. A. Fieser, *J. Am. Chem. Soc.*, 51, 1896 (1929).
(53) L. A. Fieser, *J. Am. Chem. Soc.*, 51, 940 (1929).
(54) A. Y. H. Lu and M. J. Coon, *J. Biol. Chem.*, **243**, 1331 (1968).
(55) A. Y. H. Lu, K. W. Junk, and M. J. Coon, *J. Biol. Chem.*, **244**, 3714 (1969).
(56) F. Oesch, D. M. Jerina, and J. W. Daly, *Biochem. Biophys. Acta*, **227**, 685 (1971).
(57) M. J. Cook, A. R. Katritsky, F. C. Pennington, and B. M. Semple, *J. Chem. Soc. B*, 523 (1969).
(58) M. J. Cook and N. L. Dassanyake, *J. Chem. Soc., Perkin Trans. 2*, 1901 (1972).
(59) P. B. D. de la Mare, A. Singh, E. A. Johnson, R. Koenigsberger, J. S. Lomas, V. Sanchez del Olmo, and A. M. Sexton, *J. Chem. Soc. B*, 717 (1969).
(60) G. W. Burton, M. D. Carr, P. B. D. de la Mare, and M. J. Rosser, *J. Chem. Soc., Perkin Trans. 2*, 710 (1972).

Poly(vinylamine hydrochloride). Synthesis and Utilization for the Preparation of Water-Soluble Polymeric Dyes

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Abstract: A simple and practical route to poly(vinylamine hydrochloride) has been developed. Ethylidene bisacetamide was prepared by condensing (sulfuric acid catalyst) acetaldehyde with 2 equiv of acetamide. After neutralization of the acid with calcium carbonate, the intermediate was directly pyrolyzed (175–195 °C) in the presence of a surface catalyst (Celite) to *N*-vinylacetamide. Polymerization of the unpurified product, followed by acid hydrolysis of the resulting poly(*N*-vinylacetamide), afforded poly(vinylamine hydrochloride) in an overall yield of greater than 80% from acetaldehyde. A series of water-soluble, polymeric azo dyes was prepared from poly(vinylamine hydrochloride) by a sequence of reactions which consisted of: (1) a Schotten–Baumann reaction of the polymer with *p*-acetamidobenzenesulfonyl chloride in aqueous tetrahydrofuran; (2) hydrolysis of the *p*-acetamido function in hydrochloric acid to afford a polymeric sulfanilamide; (3) diazotization; (4) reaction of the resulting diazonium salt with sulfonated coupling agents. Some of the physical properties of these polymeric dyes are briefly discussed.

In the past few years chemists have become increasingly interested in preparing and using functionalized polymers.[1] Materials of this type have been shown to have numerous advantages over their monomeric counterparts. Polymeric reagents,[2] which consist of a reactive functional group attached to an insoluble support, are receiving a growing amount of