alkoxy-substituted benzaldehydes were not involved in the discussion of the transition state. Based on the obtained results, it is likely that these alcohols were formed via the same mechanism.

Experimental Section

General Remarks. Reactions with diethylzinc were performed in a dry nitrogen atmosphere by using Schlenk-type glassware. Diethylzinc is commercially available. For the experiments a standard toluene solution, containing 0.3 g/mL diethylzinc was prepared. The aldehydes are all commercially available. They were distilled just before use.

General Procedure for the Addition of Diethylzinc to Aldehydes. In a carefully dried Schlenk vessel was placed a 15-mL toluene solution containing 7 mmol of aldehyde. To this magnetically stirred solution was added 50 mg of the catalyst, followed by slow injection (10 min) of 4 mL of a toluene solution containing 0.3 g/mL diethylzinc. This mixture was stirred overnight. The mixture was cooled to 0 °C by an ice–salt bath and 10 mL of 1 N HCl was slowly added (10 min) with continuous stirring. The toluene layer was separated and subsequently washed with 2 × 8 mL of 1 N HCl. After drying over Na_2SO_4 and filtration, the toluene was removed under reduced pressure. The crude alcohol was purified by bulb-to-bulb distillation under reduced (oil pump) pressure. The isolated yield was between 70 and 90%. The products were characterized by ^1H NMR spectroscopy and derivatized with PCl_3 and Mosher reagent for enantiomeric excess determination when necessary.

1-Phenyl-1-propanol: $[\alpha]_D^{20}$ +27.2° (*c* 1, toluene), ee 68%; ^1H NMR δ 0.9 (t, 6 Hz, 3 H), 1.4–2.0 (m, 2 H), 2.1 (br s, 1 H), 4.5 (t, 7 Hz, 1 H), 7.1–7.4 (m, 5 H).

1-(*o*-Methoxyphenyl)-1-propanol: $[\alpha]_D^{20}$ +47.0° (*c* 1.2, toluene), ee 87%; ^1H NMR δ 0.8 (t, 7 Hz, 3 H), 1.4–2.0 (m, 2 H), 2.6 (br s, 1 H), 3.6 (s, 3 H), 4.7 (t, 7 Hz, 1 H), 6.6–7.3 (m, 4 H).

1-(*p*-Methoxyphenyl)-1-propanol: $[\alpha]_D^{20}$ +20.4° (*c* 1.2, toluene), ee 61%; ^1H NMR δ 0.9 (t, 8 Hz, 3 H), 1.5–2.0 (m, 2 H), 2.1 (s, 1 H), 3.7 (s, 3 H), 4.4 (t, 7 Hz, 1 H), 6.6–7.3 (m, 4 H).

1-(2,5-Dimethoxyphenyl)-1-propanol: $[\alpha]_D^{20}$ +27.7° (*c* 1.2, toluene), ee 84%; ^1H NMR δ 0.9 (t, 7 Hz, 3 H), 1.4–2.0 (m, 2 H), 2.7 (br s, 1 H), 3.7 (s, 6 H), 4.7 (t, 7 Hz, 1 H), 6.6–6.9 (m, 3 H).

1-(*o*-Ethoxyphenyl)-1-propanol: $[\alpha]_D^{20}$ +46.3° (*c* 1.2, toluene), ee 92%; ^1H NMR δ 0.9 (t, 7 Hz, 3 H), 1.4 (t, 7 Hz, 3 H), 1.6 (m, 2 H), 2.8 (br s, 1 H), 4.0 (q, 7 Hz, 2 H), 4.7 (t, 7 Hz, 1 H), 6.4–7.3 (m, 4 H).

1-Phenyl-3-pentanol: $[\alpha]_D^{20}$ −10.6° (*c* 1, EtOH), ee 40%; ^1H NMR δ 0.9 (t, 7 Hz, 3 H), 1.3–2.0 (m, 5 H), 2.4–2.9 (m, 2 H), 3.3–3.7 (m, 1 H), 7.0–7.3 (m, 5 H).

Synthesis of the Enantiomeric K-Region Arene 5,6-Oxides Derived from Chrysene, 7,12-Dimethylbenz[*a*]anthracene, and Benzo[*c*]phenanthrene

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K-region arene 5,6-oxides of chrysene, benzo[*c*]phenanthrene [B[*c*]Ph], and 7,12-dimethylbenz[*a*]anthracene (DMBA) have been synthesized from resolved *cis*-5,6-dihydrodiols by the ortho ester route as well as from separated bromo(menthyloxy)acetate precursors in the cases of chrysene and B[*c*]Ph. Absolute configurations of the 5,6-oxides and their precursors from chrysene and DMBA have been determined by nucleophilic trans addition of methanol to the oxirane ring and correlation by circular dichroism of the adducts with trans dihydrodiols of known configurations. Confirmation of the configurational assignments to the enantiomeric chrysene *cis*-5,6-dihydrodiols was achieved by reduction to *cis*-5,6-dihydroxy-1,2,3,4,5,6-hexahydrochrysene and determination of the skew sense of the resulting biphenyl chromophore through CD measurements. B[*c*]Ph 5,6-oxide enantiomers were assigned by direct comparison with a sample of known configuration on a chiral column.

Optically active arene oxides of known absolute configuration are of substantial interest in that they can be used to define the stereoselectivity of the hepatic cytochromes P450 which form such metabolites and to establish the mechanism and enantioselectivity of nonoxidative drug metabolizing enzymes such as microsomal epoxide hydrolase and glutathione *S*-transferases which utilize arene oxides as substrates. K-region arene oxides are particularly useful in this regard since they are stable and have very low solvolytic reactivity[1] and since they are not subject to spontaneous racemization.[2] Thus, cytochrome P450c either in liver microsomes from 3-methylcholanthrene-treated rats or in homogeneous preparations has been shown to display high stereoselectivity in the formation of enantiomeric K-region arene oxides from benzo[*a*]pyrene, benz[*a*]anthracene, and 7,12-dimethylbenz[*a*]anthracene.[3] A steric model for the catalytic binding site of this enzyme has been proposed,[4] and the metabolism of chiral K-region arene oxides has been examined with microsomal epoxide hydrolase[5] and gluta-

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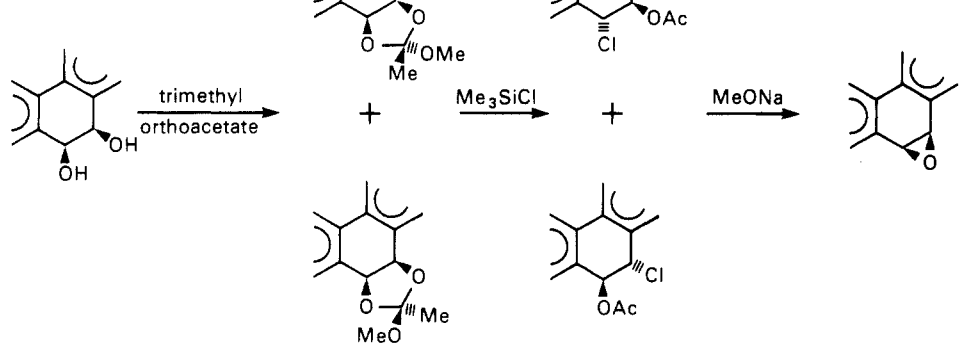
Table I. Rotations (THF) (in Degrees) of K-Region Cis Dihydrodiols, Diastereomeric Diesters with Resolving Agent, and Derived Arene Oxides

parent hydrocarbon (resolving agent)	diastereomeric diesters[a]	cis dihydrodiols	arene oxides	
			configuration	chiral HPLC[b]
chrysene (−)-MOA	less polar: +167	+290	−9 (5*S*,6*R*)	early
	more polar: −322	−282	+9 (5*R*,6*S*)	late
7,12-dimethylbenz[*a*]anthracene (−)-MTPA	less polar: −31	−97	+117 (5*S*,6*R*)	early
	more polar: −44	+104	−117 (5*R*,6*S*)	late
benzo[*c*]phenanthrene (−)-MTPA	less polar: −190	−159	+436 (5*S*,6*R*)[e]	early
	more polar: +58	+164	−432 (5*R*,6*S*)[e]	late
benz[*a*]anthracene[c] (−)-MTPA	less polar: −126	−107	−119 (5*R*,6*S*)	early
	more polar: +41	+109	+120 (5*S*,6*R*)	late
benzo[*a*]pyrene[c] (+)-MTPA	less polar: +115	+48[d]	+123 (4*S*,5*R*)	late
	more polar: −77	−48[d]	−123 (4*R*,5*S*)	early

[a] Less polar and more polar refer to order of elution on silica gel HPLC of the (menthyloxy)acetyl (MOA) and α-methoxy(trifluoromethyl)phenylacetyl (MTPA) esters. [b] Elution order on dinitrobenzoyl-(*R*)-phenylglycine bonded HPLC column. Data for benz[*a*]anthracene and benzo[*a*]pyrene oxides are from Yang and Chiu.[3e] [c] Diesters separated on DuPont Zorbax SIL columns eluted with 50% methylene chloride in hexane. Values of *k'* were 3.63 and 4.05 for the benz[*a*]anthracene and 1.90 and 2.30 for the benzo[*a*]pyrene derivatives.[5,7a] The (+) enantiomer of MTPA was used in the benzo[*a*]pyrene case. [d] Rotations determined on the diacetates. [e] Rotations from Sayer et al.[8a]

Scheme I. Ortho Ester Route to K-Region Arene Oxides with Optically Active Cis Dihydrodiols. Chirality of the Oxygen Substituents Is Preserved in the Product Arene Oxide without Separation of the Regioisomeric Chlorohydrin Acetates

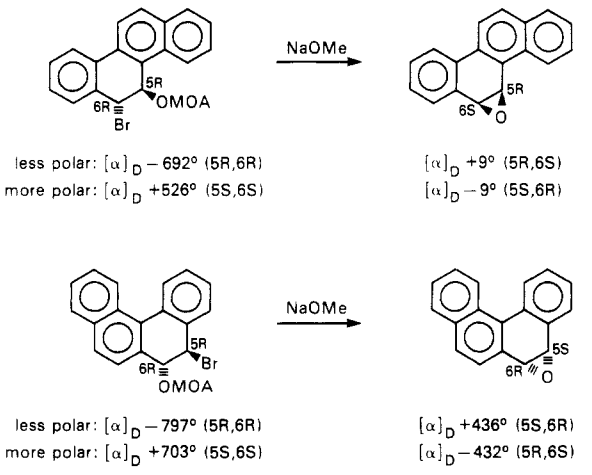


thione *S*-transferases.[6] At present two routes are available for the preparation of K-region arene oxide enantiomers; cyclization of resolved, K-region cis dihydrodiols via the ortho ester method[5,7] and cyclization of resolved K-region bromohydrins with base.[8] Racemic cis dihydrodiols are available via oxidation of the hydrocarbons with osmium tetraoxide,[9] and racemic bromohydrins are obtained by reaction of the hydrocarbons with *N*-bromoacetamide in acetic acid.[8b] The present report describes the applications of these approaches to the hydrocarbons chrysene, 7,12-dimethylbenz[*a*]anthracene, and benzo[*c*]phenanthrene.

Results and Discussion

Chiral Arene Oxide Precursors. Both the present and previous studies have found the resolving agent (−)-α-methoxy(trifluoromethyl)phenylacetic acid (MTPA)[5,7a] to be effective for the preparation of enantiomerically pure, K-region cis dihydrodiols. Although separation of the diastereomeric di-MTPA esters by HPLC is generally the method of choice, enrichment by fractional crystallization has been achieved in the cases of benzo[*a*]pyrene,[6] benz[*a*]anthracene,[5] and 7,12-dimethylbenz[*a*]anthracene (present study). Results of these separations are summarized in Table I. In one case, that of chrysene *cis*-5,6-dihydrodiol, a diester with (−)-(menthyloxy)acetic acid (MOA) was utilized since repeated attempts to prepare the di-MTPA ester of the dihydrodiol resulted in

Scheme II. Formation of Optically Active Chrysene (Top) and Benzo[*c*]phenanthrene (Bottom) 5,6-Oxides from Chiral Bromohydrin Esters



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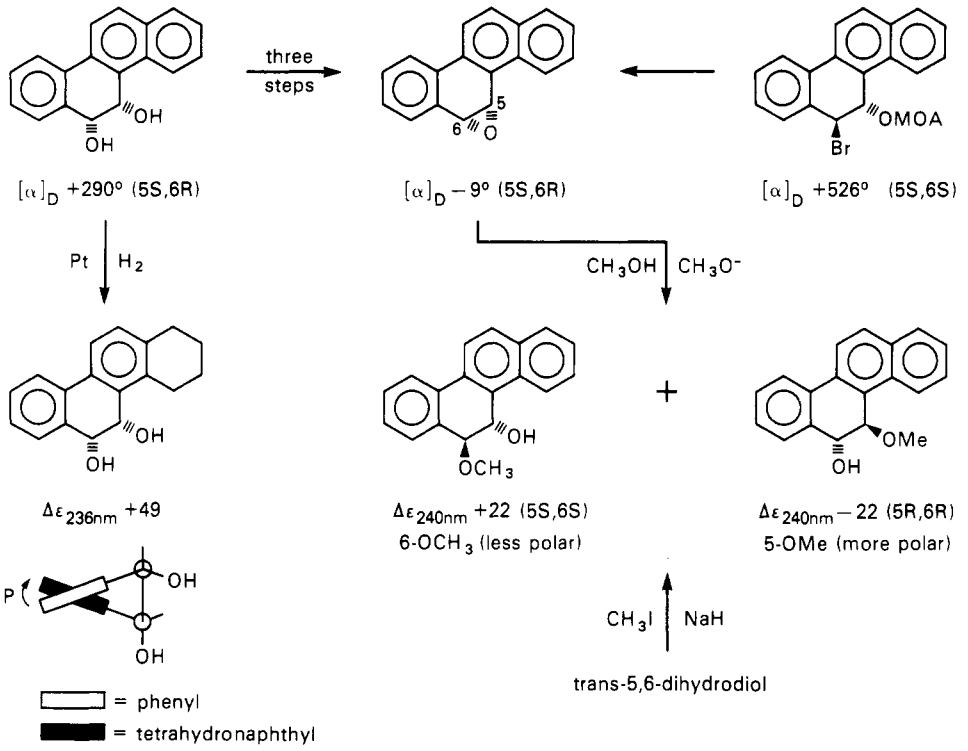
Scheme III. Interrelationship of Chiral Chrysene Derivatives



formation of the di-MTPA ester of the catechol. K-region dihydrodiols have also been resolved[3e,10] through the use of chiral HPLC columns.[11] The method is useful analytically, but limited by the low capacity of such columns for preparative work. With the ortho ester route (Scheme I), the absolute configuration of the precursor cis dihydrodiols is preserved in the product arene oxides.

The second procedure examined for the preparation of chiral, K-region arene oxides consisted of resolution of trans bromohydrins as their esters with (−)-(menthyloxy)acetic acid (MOA). Although this procedure proved satisfactory for the preparation of chrysene and benzo[c]phenanthene 5,6-oxides (Scheme II), it is limited by the fact that certain hydrocarbons do not form K-region trans bromohydrin acetates with NBA in acetic acid but instead undergo ring bromination (i.e., benzo[a]pyrene) or bromination at methyl substituents (i.e., 7,12-dimethylbenz[a]anthracene).[8b]

Chrysene 5,6-Oxide. Both enantiomers of chrysene 5,6-oxide have been prepared by two independent routes and assigned absolute configuration as shown in Scheme III. 5,6-Dihydrochrysenes may be thought of as substituted biphenyl chromophores. Given the fact that a 5-substituent in the hindered bay region of such molecules must be pseudoaxial[12] and that the absolute configuration of the 5-substituent will then determine the skew-sense of the biphenyl, we had initially reasoned that comparison of the CD spectra of the *trans*-5,6-dihydrodiol of known absolute configuration[3c] with the resolved *cis*-5,6-dihydrodiols would allow their assignment. Although the major CD extrema should derive from the skew-biphenyl chromophore in these molecules, the shapes of the CD spectra of the resolved *cis*- vs. *trans*-5,6-dihydrodiols either

Table II. NMR Spectral Data (300 MHz, $CDCl_3$) of Chrysene 5,6-Dihydrodiol Derivatives after Exchange with CD_3OD as Applicable

dihydrodiol derivative	$J_{5,6}$	H_5	H_6	acetyl or methyl ether
trans diacetate	2.7	6.88	6.11	1.87, 1.97
trans dihydrodiol	2.9	5.57	4.90	
trans-5,6-dimethyl ether	2.8	5.35	4.56	3.30, 3.42
trans-5-methyl ether	2.9	5.36	5.03	3.44
trans-6-methyl ether[a]	2.7	5.66	4.52	3.32
cis-6-methyl ether[b]	3.9	5.72	4.61	3.83
cis dihydrodiol	4.0	5.46	4.95	
cis-6-acetate	3.7	5.64	6.25	2.40
cis-5-acetate	4.1	6.94	5.15	1.92
cis-diacetate	4.2	7.11	6.32	1.94, 2.30
chrysene 5,6-oxide	4.3	5.32	4.67	

[a] Identical products were obtained either by methylation of the *trans*-5,6-dihydrodiol with methyl iodide or by addition of methoxide to chrysene 5,6-oxide. When $^{13}CH_3I$ was utilized for methylation of the *trans*-5,6-dihydrodiol, the signals for the methoxyl groups appeared as doublets with J = 142 Hz, the signal for H_5 in the 5-methyl ether had a 3.5 Hz coupling to ^{13}C, and the signal for H_6 in the 6-methyl ether had a 3.3 Hz coupling to ^{13}C. [b] When $^{13}CH_3I$ was utilized for methylation of the *cis*-5,6-dihydrodiol, the signal for the methoxyl group appeared as a doublet with J = 140 Hz and an additional coupling of 4.4 Hz was observed at H_6 due to the presence of ^{13}C.

free (spectra not shown) or as their diacetates (Figure 1A) were not identical. The results were suggestive, however, that the (+)-*cis*-5,6-dihydrodiol had 5*S*,6*R* absolute configuration but could not be considered definitive.

As a second approach to assigning absolute configuration to the (+)-*cis*-5,6-dihydrodiol and derived (−)-5,6-oxide, the oxide (Scheme III) was allowed to react with sodium methoxide saturated methanol at 40 °C overnight, at which time complete conversion to a pair of methanol adducts had occurred in a ratio of 3:2 (less polar/more polar based on HPLC elution order). The adducts were thought to arise by nucleophilic, trans-opening of the arene oxide since a control experiment in methanol alone resulted in no reaction of the arene oxide. Notably, NMR is not particularly helpful in distinguishing a cis adduct from a

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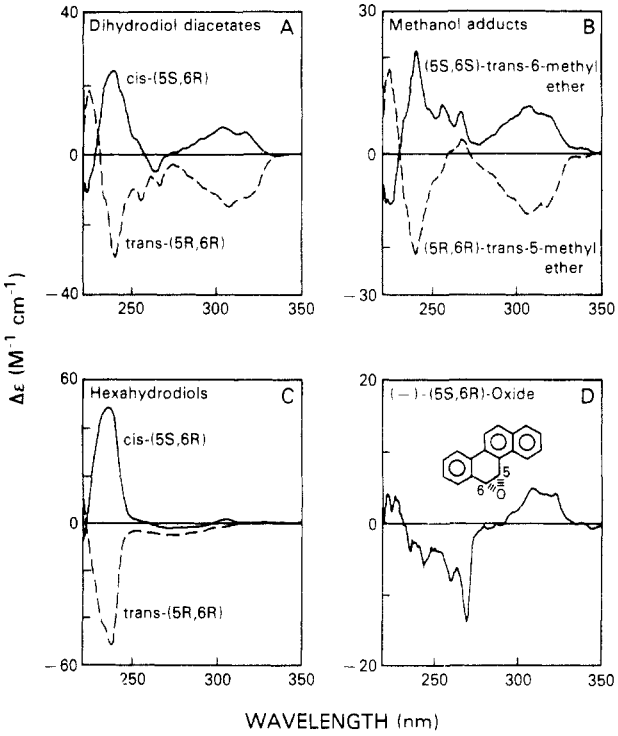

Figure 1. Circular dichroism spectra (THF in 10-mm cylindrical cells) of selected chrysene derivatives. For ease of comparison, all compounds with 5*R* substituents are shown with broken lines: (A) Comparison of the (+)-*cis*-5(*S*),6(*R*)-dihydrodiol derived from the less polar (early eluting) di-MOA diastereomer and the known (−)-*trans*-5(*R*),6(*R*)-dihydrodiol[3c] as their acetates. Opposite configurations are shown at carbon-5 for clarity. If compounds with the same configuration at carbon-5 were shown, the spectra would be similar but not quite superimposable. (B) Methyl ethers derived by addition of methoxide to (−)-chrysene 5(*S*),6(*R*)-oxide ($\epsilon_{266\,nm}$ 71 000 for acetates and methyl ethers). Although the shape of the CD spectrum of the 5-*O*-methyl ether adduct more resembles that of the cis dihydrodiol, the adduct must be trans since the same CD spectrum was obtained on resolution (early eluting peak on the chiral column) of the 5-*O*-methyl ether formed by methylation of racemic *trans*-5,6-dihydrodiol. (C) Comparison of the hexahydrodiols derived from the (+)-*cis*-5(*S*),6(*R*)- and the known (−)-*trans*-5(*R*),6(*R*)-dihydrodiols.[3c] Skew sense of the biphenyl chromophores is determined by their configuration at carbon-5, and nearly mirror-image spectra are expected. (D) The spectrum of (−)-chrysene 5(*S*),6(*R*)-oxide ($\epsilon_{270\,nm}$ 64 150) is markedly shifted and less intense than the related 5,6-dihydro derivatives.

diaxial trans adduct since values of $J_{5,6}$ must be small and similar in either case. Monomethylation of the *trans*-5,6-dihydrodiol (CH_3I, NaH in THF) resulted in the same pair of products based both on HPLC retention time (again in the ratio of 3:2 for less polar/more polar) and NMR analysis (Table II). Since methylation of a hydroxyl group results in an upfield shift of the carbinol hydrogen relative to the starting dihydrodiol (discussed in ref 8a), the major adduct (early eluting) of the arene oxide was assigned as the 6-*O*-methyl ether and the minor adduct (late eluting) as the 5-*O*-methyl ether of the *trans*-5,6-dihydrodiol (see Experimental Section for further details). The CD spectrum of the major adduct (6-*O*-methyl ether) from the (−)-oxide was identical in shape (Figure 1B) to that of the (+)-*trans*-5(*S*),6(*S*)-dihydrodiol as its diacetate (Figure 1A, enantiomer shown), thus again requiring 5*S*,6*R* absolute configuration for the (+)-*cis*-5,6-dihydrodiol and derived (−)-5,6-oxide. The minor adduct (5-*O*-methyl ether) had opposite signs for its major CD bands (Figure 1B) as expected, but the shape of the CD resembled more that of the cis rather than that of the trans diacetate (Figure 1A). CD evidence that this adduct is indeed trans in relative configuration was obtained by resolution, on a chiral HPLC column, of the 5-*O*-methyl ether formed on methylation of the racemic *trans*-5,6-dihydrodiol. Mirror image spectra, *identical in shape* with that of the minor (late eluting) methanol adduct of the (−)-arene oxide, were obtained. Thus, the late-eluting methanol adduct (5-*O*-methyl ether) of the arene oxide is clearly trans.

To avoid any possible ambiguity in assignment of absolute configuration to the (−)-5,6-oxide of chrysene, its precursor (+)-*cis*-5,6-dihydrodiol (Scheme III) was reduced (3 atm H_2, Pt, 3 days) to *cis*-5,6-dihydroxy-1,2,3,4,5,6-hexahydrochrysene such that the CD spectrum of the skew-biphenyl chromophore could be examined. The presence of a strong positive CD band (Figure 1C, $\Delta\epsilon_{236\,nm}$ +49) requires 5*S*,6*R* absolute configuration (discussed in ref 3c) for the (+)-*cis*-5,6-dihydrodiol and resulting (−)-5,6-oxide. The CD spectrum of the oxide (Figure 1D) is relatively weak possibly due to diminished contribution by the substituted, skew-biphenyl chromophore.

Chrysene (−)-5(*S*),6(*R*)-oxide has also been obtained by cyclization of the more polar (menthyloxy)acetate of the *trans*-5,6-bromohydrin of chrysene (Schemes II and III). This diastereomer therefore has 5*S*,6*S* absolute configuration. The degree of magnetic nonequivalence of H_A and H_B judged by the NMR signals for the $-OCH_AH_BCO_2-$ portion of (menthyloxy)acetates of tetrahydro benzo ring trans bromohydrins[13] and benzo ring dihydro and tetrahydro trans diols,[14] as well as K-region trans dihydrodiols[3c] has been a useful tool in predicting absolute configuration. Generally this methylene group appears as a singlet (100 MHz, benzene-d_6) when the esterified carbinol has *R* absolute configuration and as a pair of doublets (H_A and H_B nonequivalent, $J \sim 16$ Hz) when the carbinol is *S* in configuration. This correlation held true for the diastereomeric (menthyloxy)acetates of *trans*-5-bromo-6-hydroxy-5,6-dihydrobenzo[*c*]phenanthrene[8a] and also holds true for the present chrysene K-region derivatives (i.e., $-CH_2-$ as a singlet in the less polar (−)-5*R*,6*R* isomer and as a pair of doublets in the more polar (+)-5*S*,6*S* isomer, Scheme II).

Benzo[*c*]phenanthrene 5,6-Oxide. The enantiomers of benzo[*c*]phenanthrene 5,6-oxide had previously been synthesized by the bromohydrin route (Scheme II) and assigned absolute configuration.[8a] Although the method was satisfactory, difficulty in resolving the bromohydrin precursor prompted our present evaluation of the ortho ester route (Scheme I). The di-MTPA esters of the racemic *cis*-5,6-dihydrodiol (Table I) with an $\alpha = 1.09$ also proved difficult to separate, but excellent peak shape on HPLC prompted pursuing the ortho ester method. Once resolved, the *cis*-5,6-dihydrodiols were readily converted into ortho esters as described.[7b] However, treatment with trimethylsilyl chloride under a variety of conditions gave the desired chlorohydrin acetate in a maximum yield of only 10% (Scheme I). Most of the product consisted of the phenol(s) and phenolic acetate(s). Use of trimethylsilyl bromide, or BF_3–etherate in acetonitrile containing KBr did not improve the yield of the desired halohydrin acetate. The low yield for formation of the chlorohydrin acetates had not been anticipated since this reaction proceeded quite well with 7,12-dimethylbenz[*a*]anthracene,[7b] another nonplanar hydrocarbon with a sterically congested bay region. Configurational assignments for the benzo[*c*]-

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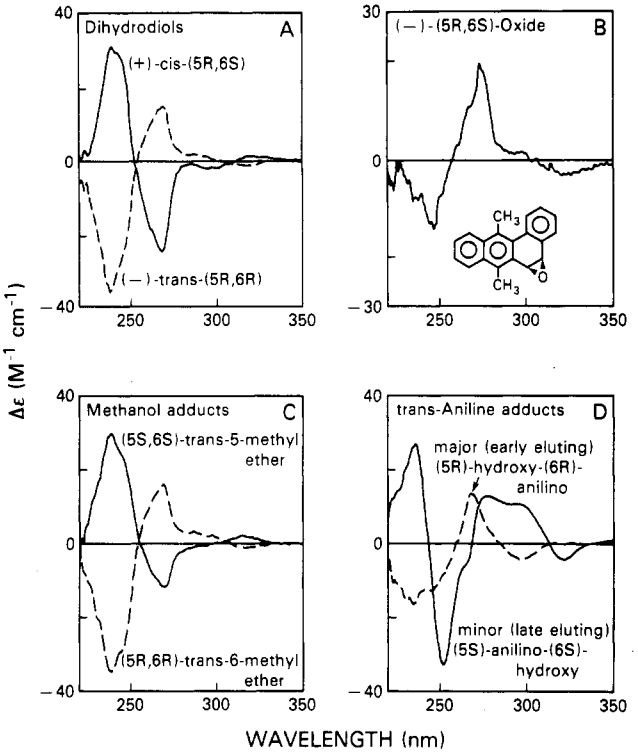
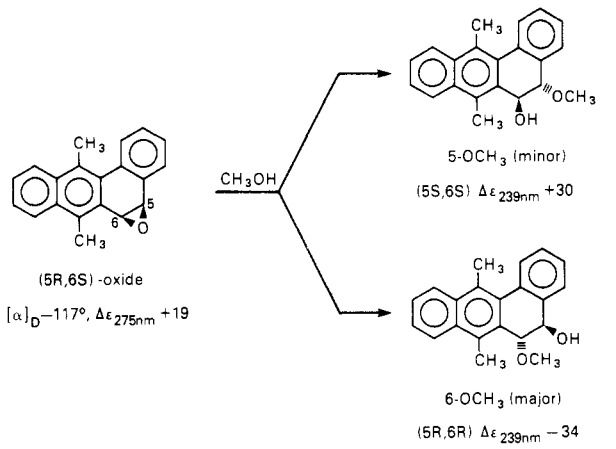


Figure 2. Circular dichroism spectra (THF) of K-region derivatives of 7,12-dimethylbenz[a]anthracene. (A) Comparison of the (−)-*trans*-5(*R*),6(*R*)-dihydrodiol[18] ($\epsilon_{266\,nm}$ 54 000 for dihydrodiols and their methyl ethers) with the (+)-*cis*-5,6-dihydrodiol derived from the more polar (late eluting) di-MTPA diastereomer. The nearly mirror image spectra require 5*R*,6*S* absolute configuration for the (+)-cis enantiomer. (B) Spectrum of (−)-7,12-dimethylbenz[a]anthracene 5(*R*),6(*S*)-oxide ($\epsilon_{276\,nm}$ 55 100) derived from the (+)-*cis*-5(*R*),6(*S*)-dihydrodiol. This is the late eluting enantiomer of the arene oxide on the chiral HPLC column (Table I). (C) Spectra of the trans methanol adducts derived from the (−)-5(*R*),6(*S*)-oxide. Near identity of the late eluting 6-methyl ether (major methanol adduct) with that of the known[18] (−)-*trans*-5(*R*),6(*R*)-dihydrodiol confirms the assignment of the arene oxide. (D) Spectra of the trans aniline adducts (ϵ 54 000 assumed) derived from the (−)-5(*R*),6(*S*)-oxide. The 5(*S*)-anilino-6(*S*)-hydroxy (minor) adduct has a CD spectrum similar to that of the *trans*-5(*S*),6(*S*)-dihydrodiol (enantiomer shown in panel A).

phenanthrene *cis*-5,6-dihydrodiols given in Table I are based (Scheme I) on their conversion to benzo[c]-phenanthrene 5,6-oxide of known absolute configuration.

7,12-Dimethylbenz[a]anthracene 5,6-Oxide. By analogy with chrysene, 7,12-dimethyl-5,6-dihydrobenz-[a]anthracene may also be thought of as a substituted biphenyl chromophore whose skew sense is determined in this case by the absolute configuration of the 6-carbon bearing an axial substituent. Such 6-substituents prefer the axial conformation in order to avoid adverse steric interactions with the 7-methyl group. CD spectra of the (+)-*cis*-5,6-dihydrodiol (Figure 2A) obtained from the more polar (late eluting) di-MTPA ester (Table I) and the (−)-*trans*-5(*R*),6(*R*)-dihydrodiol[3c,15] are nearly mirror images: cis isomer with $\Delta\epsilon_{240\,nm}$ +32 and $\Delta\epsilon_{269\,nm}$ −25, trans isomer with $\Delta\epsilon_{239\,nm}$ −36 and $\Delta\epsilon_{271\,nm}$ +15. This allows assignment of 5*R*,6*S* absolute configuration to the (+)-*cis*-5,6-dihydrodiol. Yang and Weems[16] had correctly ascribed 5*R*,6*S* absolute configuration to the early eluting peak of the *cis*-5,6-dihydrodiol on a chiral HPLC column identical with that used in the present study. Assignment of absolute configuration to the enantiomeric 7,12-dimethylbenz[a]anthracene 5,6-oxides (Table I, Figure 2B) is based on the configuration of their precursor *cis*-5,6-dihydrodiols which were converted to arene oxide (Scheme I) by the route previously described for racemic material.[7b] The CD spectrum of the arene oxide (Figure 2B) bears no relationship to the CD spectrum of its precursor cis dihydrodiol.

Scheme IV. Methanolysis of Optically Active 7,12-Dimethylbenz[a]anthracene 5,6-Oxide



7,12-Dimethylbenz[a]anthracene 5,6-oxide is known to undergo solvolysis in methanol to form major and minor adducts with methanol.[17] The minor, early eluting adduct on HPLC results from trans addition of methanol at position-5 and the major, late eluting adduct from trans addition of methanol at position-6.[18] These same adducts were prepared by allowing (−)-(5*R*,6*S*)-7,12-dimethylbenz[a]anthracene 5,6-oxide to react with methanol (Scheme IV). Since the CD spectrum (Figure 2C) of the minor, 5-*O*-methyl ether is virtually identical with that of the known (+)-*trans*-5(*S*),6(*S*)-dihydrodiol[3c,15,18] (Figure 2A) and the CD spectrum of the major, 6-*O*-methyl ether is identical with that of the (−)-*trans*-5(*R*),6(*R*)-dihydrodiol (Figure 2A, enantiomer shown), assignment of 5*R*,6*S* absolute configuration to 7,12-dimethylbenz[a]anthracene (−)-5,6-oxide is certain. It should be noted that Mushtaq et al.[3d] had misassigned absolute configuration[18] to these K-region arene oxides.

Aniline has been suggested as a model compound for the reaction of nucleic acid bases with the 5,6-oxide of 7,12-dimethylbenz[a]anthracene.[19] For this reason, we have prepared the major and minor, trans aniline adducts of (−)-(5*R*,6*S*)-7,12-dimethylbenz[a]anthracene 5,6-oxide as described for the racemic arene oxide. Although the CD spectrum of the minor, late-eluting 5(*S*)-anilino-6(*S*)-hydroxy adduct (Figure 2D) is quite similar to that of the (+)-*trans*-5(*S*),6(*S*)-dihydrodiol, the symmetric pair of extrema ($\Delta\epsilon_{237\,nm}$ +27, $\Delta\epsilon_{254\,nm}$ −32) are highly suggestive of an exciton interaction[20] between the aniline and 7,12-dimethyl-5,6-dihydrobenz[a]anthracene chromophores. The major, early eluting 5(*R*)-hydroxy-6(*R*)-anilino adduct had a rather weak CD spectrum compared with the minor isomer (Figure 2D), suggestive that any exciton interaction might be negated by the skew-biphenyl present. We anticipate that optically active 7,12-dimethylbenz[a]-anthracene 5,6-oxide will prove to be extremely useful in further defining the structures of nucleic acid adducts

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prepared from racemic material.[21] For example, Jeffrey et al.[21d] have allowed racemic 7,12-dimethylbenz[a]anthracene 5,6-oxide to react with polyguanylic acid. Four major adducts (compounds I–IV) were separated by HPLC. Structural analysis had indicated that they resulted from trans addition of the exocyclic N^2-amino group of the guanine at C-5 and at C-6 of both enantiomers of the 5,6-oxide. On the basis of their CD spectra that result from an exciton coupling between the hydrocarbon and the base, it was tentatively concluded that compounds I (C-6 substitution) and III (C-5 substitution) were derived from the 5(*R*),6(*S*)-oxide. We have repeated this study with the (+)-5(*S*),6(*R*)-oxide and have found, on the basis of HPLC and CD comparison with the four adducts formed from racemic 5,6-oxide, that compounds II and IV were produced. Thus the CD assignment from the initial study is indeed correct.

Conclusions

Synthesis of optically active, K-region 5,6-oxides of established absolute configuration are described for chrysene, benzo[*c*]phenanthrene, and 7,12-dimethylbenz[*a*]anthracene. For all three hydrocarbons, the ortho ester route with resolved *cis*-5,6-dihydrodiols was utilized. Only in the case of benzo[*c*]phenanthrene were low overall yields obtained. Resolved K-region bromohydrins were also effective precursors except in the case of 7,12-dimethylbenz[*a*]anthracene where the racemic bromohydrin could not be prepared. On the basis of the present results obtained with the MOA esters of the *trans*-5,6-bromohydrins of chrysene and benzo[*c*]phenanthrene, the splitting pattern for H_A and H_B in the $-OCH_AH_BCO_2-$ portion of these esters may be predictive of absolute configuration: a higher degree of magnetic nonequivalence implies *S* configuration.

Although the degree of magnetic nonequivalence between H_A and H_B in the $-OCH_AH_BCO_2-$ portion of di-MOA esters of K-region trans dihydrodiols allows prediction of their absolute configuration based on NMR spectra,[3c] sufficient examples of resolved and assigned di-MOA and di-MTPA esters of K-region cis dihydrodiols are not available to make predictions of configuration based on such spectra. The NMR spectra of the di-MTPA esters of benzo[*c*]phenanthrene *cis*-5,6-dihydrodiol did, however, prove quite interesting in that each of the diastereomers was found to consist of a pair of slowly interconverting conformational isomers on the NMR timescale based on the presence of two sharp singlets for each OCH_3 group in the di-MTPA ester (Figure 3). Temperature-dependent studies were performed in Me_2SO-d_6 where at 22 °C the NMR spectrum showed two overlapped signals at δ 3.58 and two sharp singlets at δ 2.74 and 2.93 with an intensity ratio of 1:2. Exchange rate constant calculations[22] at a coalesence point of 80 °C gave $k \sim 127$ s^{-1} (less polar 5*S*,6*R* enantiomer) relative to the above methoxyl signals from the two conformers. Free energy of activation, $\Delta G^{\ddagger}$ at 80 °C was calculated to be 17.4 kcal/mol. The benzo[*c*]phenanthrene di-MTPA ester conformers may result from changes in the skew sense of the substituted biphenyl (5 ax, 6 eq $\rightleftharpoons$ 5 eq, 6 ax). This possibility is supported by the fact that both diastereomers show two sets of K-region signals as well. In fact, individual conformers have been separated for the highly hindered K-region dihydrodiols of 4,5-dimethylphenanthrene.[23]

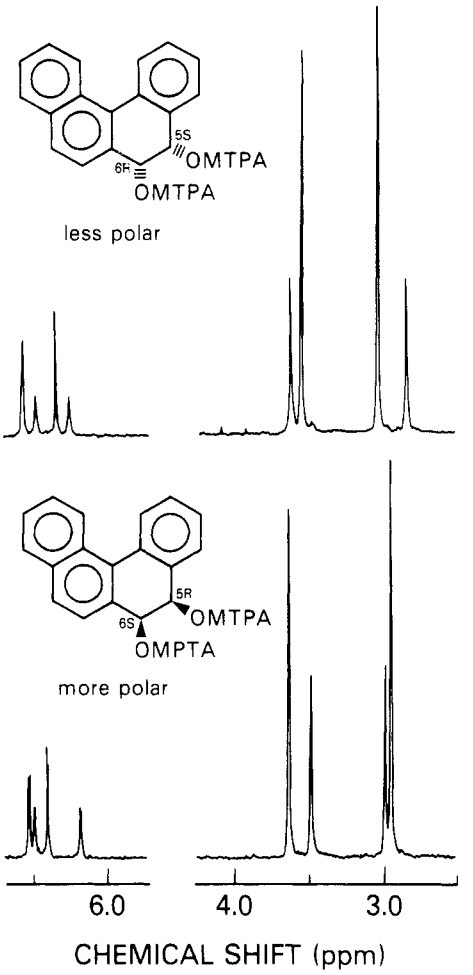

Figure 3. NMR signals (300 MHz, $CDCl_3$, 25 °C) for the methoxyl and the benzylic protons in the diastereomeric di-MTPA esters of the benzo[*c*]phenanthrene *cis*-5,6-dihydrodiol enantiomers.

Weems et al.[24] have described the resolution of nine K-region arene oxides on analytical chiral HPLC columns and have attempted to predict the absolute configuration of seven of these arene oxides based on the elution order for the known enantiomers[5] of benzo[*a*]pyrene 4,5-oxide and benz[*a*]anthracene 5,6-oxide. In a recent study,[18] their previous assignment of absolute configuration to the enantiomers of 7,12-dimethylbenz[*a*]anthracene 5,6-oxide[3d] was shown to be incorrect. Since assignment of absolute configuration to the enantiomers of 12-methylbenz[*a*]anthracene 5,6-oxide was done by comparison of CD spectra with those of 7,12-dimethylbenz[*a*]anthracene 5,6-oxide, the 12-methylbenz[*a*]anthracene oxide assignments may also be incorrect. Thus, a useful model for predicting the elution order of enantiomeric, K-region

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arene oxides on chiral HPLC columns is presently unavailable.

Note: Since submission of this study, Weems et al.[28] have described assignment of the enantiomers of chrysene *cis*-5,6-dihydrodiol on the basis of CD spectra of their bis(*p*-(dimethylamino)benzoates). Their assignment and ours (CD spectrum of a skew-biphenyl chromophore) reach the same conclusion and complement each other. These authors further assigned configuration to the chrysene 5,6-oxides on the basis of the CD spectra of their methoxide adducts with conclusions identical with ours.

Experimental Section

Tetrahydrofuran was freshly distilled from lithium aluminum hydride before use and was the solvent for all determinations of extinction coefficients, CD spectra, and rotations. NMR spectra were recorded at 300 MHz in the solvents specified. All compounds gave the required molecular ions either by EI or by CI-NH_3. Melting points of solid compounds are indicated.

Racemic K-Region Cis Dihydrodiols. The parent hydrocarbons were allowed to react with a 10% excess of osmium tetraoxide in pyridine for 3–5 days with stirring.[9] After decomposition of the resulting osmate esters with sodium bisulfite in aqueous pyridine,[25] crude extracts were applied to open silica gel columns which were eluted first with chloroform to remove impurities and then with 10–15% methanol in chloroform to isolate the dihydrodiols. Yields were >70% in all cases. The *cis*-5,6-dihydrodiol of benzo[c]phenanthrene does not appear to have been described previously: mp 161–162 °C; NMR ($CDCl_3$) δ 4.82 H_5 and δ 4.75 H_6 with $J_{5,6}$ = 3.6 Hz. Assignments are by analogy to the *trans*-5,6-dihydrodiol for which H_5 at δ 4.69 and H_6 at δ 4.64 with $J_{5,6}$ = 10.9 Hz have been unequivocally assigned.[8a]

Diastereomeric Diesters of Cis Dihydrodiols. Acid chlorides of (−)-MTPA and (−)-MOA (Aldrich Chemical Co.) were prepared as described.[26] In a typical experiment, 1 mmol of cis dihydrodiol in 3 mL of pyridine was cooled to 0 °C and 2.2 mmol of acid chloride was added. The mixture was then stored for 1 day at room temperature prior to standard workup. The resulting oily mixtures of diastereomers were dissolved in mobile phase for separation by HPLC on silica gel. In the case of the di-MTPA esters of the 7,12-dimethylbenz[a]anthracene *cis*-5,6-dihydrodiol, the early eluting (less polar) diastereomer readily crystallized from the solvent (8% ether in cyclohexane) in >95% diastereomeric purity. Recrystallization provided pure material with mp 170–171 °C. The diastereomeric 7,12-dimethylbenz[a]anthracene *cis*-5,6-dihydrodiol di-MTPA esters were also separated on a Perkin-Elmer preparative 10-μm silica gel column (2.35 × 25 cm) eluted with 8% ether in cyclohexane: the diastereomers had k' = 0.98 and k' = 1.29 for an α = 1.32. In the case of the di-MOA ester of chrysene *cis*-5,6-dihydrodiol, the diastereomer mixture was first subjected to cleanup by open column chromatography on silica gel (separated from polar contaminants by elution with 20% ether in hexane) prior to HPLC. Diastereomeric chrysene *cis*-5,6-dihydrodiol di-MOA esters were separated on a DuPont Zorbax SIL column (2.12 × 25 cm) eluted with 0.2% dioxane in methylene chloride: less polar diastereomer (early eluting, mp 42 °C) had k' = 1.17 and the more polar (late eluting) had k' = 1.48 for an α = 1.26. Diastereomeric benzo[c]phenanthrene *cis*-5,6-dihydrodiol di-MTPA esters were separated on a DuPont Zorbax SIL column (0.95 × 25 cm) eluted with 35% methylene chloride in hexane: the diastereomers had k' = 5.77 and k' = 6.26 for an α = 1.09.

NMR spectra (300 MHz, $CDCl_3$) of both di-MOA diastereomers of chrysene *cis*-5,6-dihydrodiol showed H_6 at δ 6.45 and H_5 at δ 7.16 with $J \sim 3.9$ Hz. For the less polar (+)-5*S*,6*R* diastereomer, the signals for the $-OCH_2CO_2-$ portion of the molecules appeared as a pair of quartets centered at δ 3.95 and 4.37 due to magnetic nonequivalence. For the more polar (−)-5*R*,6*S* diastereomer, these methylene groups appeared as singlets at δ 3.94 and 4.36. For the di-MTPA diastereomers of 7,12-dimethylbenz[a]anthracene *cis*-5,6-dihydrodiol: less polar, 7-CH_3 δ 2.75, 12-CH_3 δ 2.82, two OCH_3 at δ 2.93 and 3.64, H_5 δ 6.43 ($J_{5,6}$ < 2 Hz); more polar, 7-CH_3 δ 2.72, 12-CH_3 δ 2.81, two OCH_3 at δ 3.02 and 3.56 ($J_{5,6}$ < 2 Hz). For benzo[c]phenanthrene derivatives, see Discussion.

Enantiomerically pure dihydrodiols were regenerated by addition of an excess of solid sodium methoxide to the diesters dissolved in THF/methanol (2/1). MOA esters required 0.5 h for complete reaction, and MTPA esters were allowed to react overnight. Optical properties of the diesters and *cis*-5,6-dihydrodiols are listed in Table I.

Reduction of (+)-*cis*-Chrysene 5(*S*),6(*R*)-Dihydrodiol. A mixture of the (+)-*cis*-5(*S*),6(*R*)-dihydrodiol (28 mg), derived from the less polar (early eluting) (+)-di-MOA diastereomer, and platinum oxide (80 mg) in ethyl acetate (30 mL) was agitated for 3 days under 3 atm of hydrogen at room temperature. The desired *cis*-5(*S*),6(*R*)-dihydroxy-1,2,3,4,5,6-hexahydrochrysene (12 mg, 3.4 min) was separated from the starting dihydrodiol (4.2 min) by HPLC on a DuPont Zorbax SIL column (0.95 × 25 cm) eluted with 2.5% methanol and 15% ethyl acetate in hexane at a flow rate of 12.5 mL/min. For the desired hexahydrodiol: MS (EI) (relative intensity) gave M^+, 266 (3) and $M^+ - H_2O$, 248 (100); NMR (300 MHz, $CDCl_3$) showed $H_{5,6}$ δ 4.80/4.92 with $J_{5,6}$ = 3.9 Hz, $H_{1,1',4,4'}$ δ 2.77–2.96 (m), $H_{2,2',3,3'}$ δ 1.75–1.98 (m); UV (THF), broad band at 275 nm (ϵ 18 300) with shoulders at 266 and 282 nm; mp 182 °C dec. A value of ϵ 18 000 at 277 nm (THF) has been reported for the trans isomer.[3c]

Diastereomeric (−)-(Menthyloxy)acetates of *trans*-5-Hydroxy-6-bromo-5,6-dihydrochrysene. A solution of *trans*-5-acetoxy-6-bromo-5,6-dihydrochrysene[8b] (268 mg, 0.73 mmol) was stored in 5 mL of 1 M diborane in THF overnight. Excess diborane was destroyed by slow addition of 5 mL of 10% water in methanol. Standard workup provided a near quantitative yield of bromohydrin which crystallized from ether–pentane, mp 138 °C: NMR ($CDCl_3$) showed H_5 at δ 5.75 and H_6 at 5.47 with $J_{5,6}$ = 2.6 Hz. The bromohydrin was dissolved in pyridine containing (−)-(menthyloxy)acetyl chloride (0.33 g, 1.4 mmol) and stored at room temperature for 3 h before addition of ice and water. Standard workup provided the diastereomeric bromo MOA esters (Scheme II) as an oil in 95% yield. The diastereomers were separated on a DuPont Zorbax SIL column (2.12 × 25 cm) eluted with 3% ether in cyclohexane. Less polar diastereomer (k' = 1.67): 84 mg; $[\alpha]_D$ −692° (THF); NMR (100 MHz, benzene-d_6) $-OCH_2CO_2-$ δ 3.50 (s), H_6 5.45 with $J_{5,6}$ = 2.5 Hz. More polar diastereomer (k' = 1.95): 41 mg; $[\alpha]_D$ +526° (THF); NMR (100 MHz, benzene-d_6) $-OCH_2CO_2-$ δ 3.42 (d) and 3.63 (d) with J_{gem} = 16 Hz, H_6 δ 5.46 with $J_{5,6}$ = 2.5 Hz.

Chrysene 5,6-Oxide. When the 5,6-oxide was prepared by the halohydrin route, 25 mg of either diastereomeric bromo(menthyloxy)acetate was stirred with 75 mg of dry sodium methoxide in 5 mL of freshly distilled THF for 3 days at room temperature. Water and ether were added, and the ether phase was washed with dilute potassium carbonate. Yields were in excess of 80%. When prepared from *cis*-5,6-dihydrodiol by the ortho ester route (Scheme I), the standard procedure was followed without isolation of intermediates.[7b] Overall yields were ~40%. See Scheme II and Tables I and II for details.

Methylation of Chrysene 5,6-Dihydrodiols. In order to establish trans relative stereochemistry for the two methoxide adducts formed from chrysene 5,6-oxide, several K-region derivatives of 5,6-dihydrochrysene were prepared. Chromatographic separations were done on a DuPont Zorbax SIL column (0.95 × 25 cm) eluted at 12.5 mL/min with 2.5% methanol and 15% ethyl acetate in hexane. NMR spectra of the various products are listed in Table II.

To a solution of the *trans*-5,6-dihydrodiol (26 mg, 0.1 mmol) in 3 mL of freshly distilled THF containing 0.1 mL of methyl iodide was added small portions of sodium hydride (50% suspension in oil) under nitrogen. Although the mixture darkened considerably, HPLC indicated substantial conversion to the dimethyl ether (1.8 min) as well as to the desired 6-methyl ether (3.4 min) and 5-methyl ether (3.8 min). Further additions of methyl iodide and sodium hydride over the next hour led to consumption of the trans dihydrodiol (9.0 min). The methylated products (about half as the dimethyl ether) were isolated in ~50% yield along with substantial quinone, and the early eluting 6-methyl ether was favored over the 5-methyl ether by 3:2. Positions of methylation were deduced by comparison of NMR spectra with

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that of the *trans*-5,6-dihydrodiol, the signal for H_5 in which is shifted downfield 0.7 ppm relative to H_6 due largely to edge-deshielding in the bay region. Methylation of either carbinol results in an expected upfield shift (0.2–0.4 ppm) as confirmed by use of [^{13}C]methyl iodide (see Table II). Signals for the O-methyl groups in the di- and monomethyl ethers clustered tightly in the region of 3.3–3.4 ppm. The 5-methyl ether was readily resolved by analytical, chiral HPLC (5.3% ethanol and 2.7% acetonitrile in hexane at 3.0 mL/min, see arene oxide separations described later) into its early eluting (7.12 min) and late eluting (7.59 min) enantiomers which had mirror-image CD spectra. The spectrum of the early eluting enantiomer on the chiral column was identical in shape and magnitude with that of the more polar (5*R*,6*R*)-*trans*-5-methyl ether on the Zorbax SIL column obtained by addition of methoxide to (-)-chrysene 5(*S*),6(*R*)-oxide (see Figure 1B, Scheme III).

In a further attempt to exclude the possibility that either of the methoxide adducts from chrysene 5,6-oxide had formed by cis addition, chrysene *cis*-5,6-dihydrodiol was methylated as above. In this case, more severe decomposition of the dihydrodiol occurred, and only a small amount (<5% yield) of a single monomethyl ether could be isolated. Comparison of NMR spectra (Table II) with that of the *cis*-5,6-dihydrodiol (4.2 min on HPLC) indicated it was the *cis*-6-methyl ether (2.7 min). It eluted earlier and was well separated from the trans methyl ethers which had retention times identical with the methoxide adducts formed from chrysene 5,6-oxide. The NMR signal (Table II) for the OCH_3 group in the *cis*-6-methyl ether (δ 3.83) is shifted downfield ~0.4 ppm relative to the trans methyl ethers due to edge-deshielding in the bay region. A similar downfield shift for the acetyl group of the *cis*-6-acetate (3.6 min, longer retained isomer due to more polar, axial 5-hydroxyl group) compared to the *cis*-5-acetate (2.7 min) was also observed. Partial acetylation of the *cis*-5,6-dihydrodiol (limited acetic anhydride in pyridine) favored (4:1) esterification of the equatorial 6-hydroxyl group as apparently did methylation. Although the *cis*-5-methyl ether was not isolated and identified among the methylation products, it should elute earlier on HPLC than the *cis*-6-methyl ether which has a more polar, axial hydroxyl group. Thus, the *cis*-5-methyl ether (as well as the *cis*-6-methyl ether) can be excluded as a methoxide adduct of chrysene 5,6-oxide. Of the four possible monomethyl ethers of the *cis*- and *trans*-5,6-dihydrodiols from 7,12-dimethylbenz[*a*]anthracene, the comparable *cis*-6-methyl ether (axial methyl ether and equatorial hydroxyl) was the first to elute under similar HPLC conditions.[18]

Arene Oxide Separations by Chiral HPLC. Analytical separations of enantiomeric arene oxides were achieved on a covalently bonded dinitrobenzoyl-(*R*)-phenylglycine column (0.46 × 25 cm, Regis Chemical Co.). In all cases the elution solvent (3.0 mL/min) was hexane containing 0.33% ethanol and 0.17% acetonitrile: chrysene oxides, (-)-5*S*,6*R* at 11.18 min and (+)-5*R*,6*S* at 11.57 min; benzo[*c*]phenanthrene oxides, (+)-5*S*,6*R* at 7.46 min and (-)-5*R*,6*S* at 7.83 min; 7,12-dimethylbenz[*a*]anthracene oxides, (+)-5*S*,6*R* at 9.87 min and (-)-5*R*,6*S* at 10.20 min. We attribute no particular significance to the fact that the 5*S*,6*R* enantiomers elute first. It should be noted, however, that the order of arene oxide enantiomer elution may reverse when dinitrobenzoyl-(*S*)-leucine columns are utilized.[24]

Synthesis of a Parabactin Photoaffinity Label

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The synthesis of parabactin azide, the first catecholamide siderophore photoaffinity label, is described. Its preparation is predicated on the generation of ethyl 4-azido-2-hydroxybenzimidate. This imidate is coupled with N^1,N^8-bis(2,3-dihydroxybenzoyl)-N^4-threonylspermidine hydrobromide to produce parabactin azide. The photoaffinity label is shown to have the same biological activity as parabactin in stimulating the growth of *Paracoccus denitrificans* when the microorganism is cultured under low iron conditions. Furthermore, parabactin azide is shown to form a gallium(III) complex identical with the parabactin gallium(III) complex as determined by 300-MHz ^{1}H NMR. Finally, ethyl 2-hydroxy-4-nitrobenzimidate hydrochloride, an intermediate in the synthesis of ethyl 4-azido-2-hydroxybenzimidate, is used in the preparation of aminoparabactin which is subsequently attached to an activated sepharose resin to produce a parabactin affinity column.

Introduction

The fact that iron plays a critical role in the growth of microorganisms is certainly well established.[1-3] An "excess" of the metal causes a fulminant growth of many microorganisms while iron deprivation can substantially slow or even halt growth.[4-6] Because of the poor solubility of iron in aqueous solution, (K_{sp} = 10^{-38} M)[7] at the pH which most bacteria grow, microbes have developed a rather sophisticated apparatus for solubilizing and incorporating the metal.[8-10] They produce low-molecular-weight virtually ferric ion specific ligands which tightly chelate iron and assist in its transport into the cell. These ligands are typically either hydroxamates[11] as exemplified by desferrioxamine[12] or catecholamides[13] as exemplified by enterobactin[14] and the linear catecholamide, parabactin.[15] Both desferrioxamine and parabactin form extremely tight complexes with iron, with formation constants, K_f, of 10^{31} M^{-1} and 10^{48} M^{-1} respectively.[16,17] Two

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