the reducing agent were introduced. Saturated Rochelle salt solution was added, and after 1 h of stirring the product was extracted into dichloromethane (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried, and evaporated. The residue was purified by medium pressure liquid chromatography on silica gel (elution with 20% ethyl acetate in petroleum ether) to give **10** as a clear, colorless solid (23 mg, 57.5%), mp >225 °C (from dichloromethane–petroleum ether): IR ($CHCl_3$) cm^{-1} 2970, 2880, 2740, 1700, 1465, 1345, 1235, 1125, 1100, 1070, 1060, 1010, 945, 835; ^1H NMR (300 MHz, $CDCl_3$) δ 8.98 (s, 1 H), 4.55 (t, J = 5.2 Hz, 1 H), 4.40–4.39 (m, 1 H), 3.39–3.35 (m, 1 H), 3.14–2.72 (m, 4 H), 2.23 (br s, 1 H), 1.99 (d, J = 12.6 Hz, 1 H), 1.96 (br s, 1 H), 1.60–1.47 (m, 1 H); ^{13}C NMR ($CDCl_3$) ppm 196.95, 90.52, 80.48, 60.68, 56.49, 53.01, 50.41, 46.85, 40.85, 40.40, 36.80 (quaternary carbon not observed); MS, m/z calcd (M^+) 188.0837, obsd 188.0830.

Octahydro-2,4,5-methenocyclopropa[3,4]pentaleno[1,6-*bc*]pyran-4a(4*H*)-carboxylic Acid (11). When the benzene-d_6 solution of **10** was allowed to stand open to the air, quantitative oxidation to the carboxylic acid occurred, mp 208–209 °C (from ethyl acetate–petroleum ether): IR ($CHCl_3$) cm^{-1} 3300–2900, 2980, 1735, 1695, 1345, 1250, 1100, 1075, 1060, 1005, 995, 900; ^1H NMR (300 MHz, $CDCl_3$) δ 10.1–9.6 (br, 1 H), 4.63–4.57 (m, 1 H), 4.57–4.44 (m, 1 H), 3.47–3.37 (m, 1 H), 3.09–3.05 (m, 1 H), 2.88–2.61 (m, 4 H), 2.45–2.40 (m, 1 H), 2.06 (dd, J = 8.46, 12.5 Hz, 1 H), 1.56 (dt, J = 12.5, 3.9 Hz, 1 H); ^{13}C NMR ($CDCl_3$) ppm 177.27, 90.71, 80.68, 60.75, 56.47, 53.72, 53.08, 48.17, 41.59, 41.33, 36.92, 29.77; MS, m/z calcd (M^+) 204.0798, obsd 204.0792.

Anal. Calcd for $C_{12}H_{12}O_3$: C, 70.58; H, 5.92. Found: C, 70.69; H, 5.88.

Decahydro-7-iodo-2,4,6-methanocyclopropa[3,4]pentaleno[1,6-*bc*]pyran-5-carbonitrile (12). A. Elevated Temperature. A mixture of hexamethyldisilane (300 mg, 2.0 mmol) and iodine (500 mg, 2.0 mmol) was warmed at 65 °C until homogeneous and then heated at reflux for 1.5 h. To the cooled black mixture was added 5 mL of carbon tetrachloride. Cyano ether **8** (55.7 mg, 0.30 mmol) was next introduced and this solution was heated at the reflux temperature for 17 h. Water was added and the product was extracted into dichloromethane (3 × 30 mL). The combined organic layers were washed once each with saturated sodium thiosulfate and sodium chloride solutions, dried, and evaporated. The residue was purified by silica gel chromatography (dichloromethane elution) to give 38.3 mg (40.8%) of **12** as a pale yellow oil: IR ($CHCl_3$) cm^{-1} 2995, 2960, 2870, 2250, 1450, 1365, 1340, 1330, 1270, 1230, 1160, 1110, 1070, 1020, 820; ^1H NMR (300 MHz, $CDCl_3$) δ 5.33 (t, J = 4.28 Hz, 1 H), 4.72 (s, 1 H), 4.65–4.62 (m, 1 H), 3.11–3.10 (m, 2 H), 2.99–2.91 (m, 2 H), 2.60 (br s, 1 H), 2.51–2.50 (m, 1 H), 2.31 (br s, 1 H), 1.95 (d, J = 12.5 Hz, 1 H), 1.46 (dt, J = 12.5, 3.9 Hz, 1 H); ^{13}C NMR ($CDCl_3$) ppm 118.92, 86.96, 79.55, 53.59, 50.40, 50.08, 49.19, 46.96, 41.36, 40.88, 39.43, 28.66; MS, m/z calcd (M^+) 312.9965, obsd 312.9964.

B. Room Temperature. A mixture of hexamethyldisilane (300 mg, 2.0 mmol) and iodine (500 mg, 2.0 mmol) was warmed at 65 °C until homogeneous and then heated at reflux for 1.5 h. To the cooled black mixture was added 5 mL of carbon tetrachloride and 1.1 g of potassium carbonate. Cyano ether **8** (20 mg, 0.108 mmol) was added and stirring was maintained at room temperature for 36 h. Water was added and the product was extracted into dichloromethane (3 × 30 mL). The combined organic layers were washed with sodium thiosulfate (30 mL) and sodium chloride solutions (30 mL), dried, and evaporated. The resulting yellow oil was purified by silica gel chromatography (silica gel elution) to give 29.4 mg (87%) of **12**, identical with the material described in part A.

Recyclization of 12. Potassium hydride in mineral oil (5 mL of 24.6%, 0.8 mmol) was washed 3 times with petroleum ether, and dry tetrahydrofuran (5 mL) was added. A solution of **12** (50 mg) in the same solvent (5 mL) was added, and the reaction mixture was heated at reflux for 2 h and stirred at room temperature for 36 h. Saturated ammonium chloride solution was carefully added and the product was taken up in dichloromethane (3 × 25 mL). The combined organic layers were washed with brine, dried, and evaporated to leave a colorless solid shown to be **8** by IR and ^1H NMR spectroscopy.

Acknowledgment. We thank the National Institutes of Health (Grant AI-11490) and the Science Research Council (U.K.) for financial support, Prof. K. N. Houk for MM2 calculations, and Dr. Kurt Loening (Chemical Abstracts Service) for assistance with nomenclature.

Registry No. **3**, 65181-92-2; (*E*)-**4**, 100019-63-4; (*Z*)-**4**, 99966-06-0; **5**, 99965-98-7; **6**, 99965-99-8; **7a**, 99966-00-4; **7b**, 100019-62-3; **8**, 99966-01-5; **9**, 99966-02-6; **10**, 99966-03-7; **11**, 99966-04-8; **12**, 99966-05-9; diethyl cyanomethylphosphonate, 2537-48-6.

Absolute Configuration of Benzo[*c*]phenanthrene 5,6-Oxide and Other K-Region Derivatives

Jane M. Sayer, Peter J. van Bladeren, Herman J. C. Yeh, and Donald M. Jerina*

Laboratory of Bioorganic Chemistry and Laboratory of Analytical Chemistry, NIADDK, The National Institutes of Health, Bethesda, Maryland 20892

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Synthesis of enantiomerically pure benzo[*c*]phenanthrene (+)-(5*S*,6*R*)- and (−)-(5*R*,6*S*)-oxides is described from diastereomerically pure (−)-(5*R*,6*R*)-*trans*-5-bromo-6-[(menthyloxy)acetyl]- and (+)-(5*S*,6*S*)-*trans*-5-bromo-6-[(menthyloxy)acetyl]-5,6-dihydrobenzo[*c*]phenanthrene derived from (−)-(menthyloxy)acetic acid. Configurational assignment of the enantiomeric arene oxides is based on correlation of the CD spectra of their *trans*-*N*-acetyl-L-cysteine adducts as methyl esters with the bis((−)-α-methoxy(trifluoromethyl)phenylacetate) of (+)-(5*R*,6*R*)-*trans*-5,6-dihydroxy-5,6-dihydrobenzo[*c*]phenanthrene of known absolute configuration. Separable major and minor S adducts were obtained from each arene oxide enantiomer. Structures of the major (attack at C-6) and minor (attack at C-5) adducts were established through the use of 5-deuterated arene oxide. Predominant attack (3:1) of the thiolate at C-6 of the arene oxide is consistent with PMO calculations.

The polycyclic aromatic hydrocarbon benzo[*c*]-phenanthrene (**1**) is a weakly carcinogenic environmental contaminant.[1,2] Although the tumorigenicity of the hydrocarbon can be accounted for by formation of bay-region[3] diol epoxides on the benzo ring,[4] most of the in vitro

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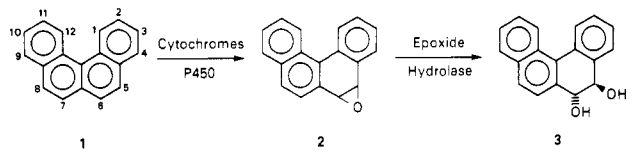
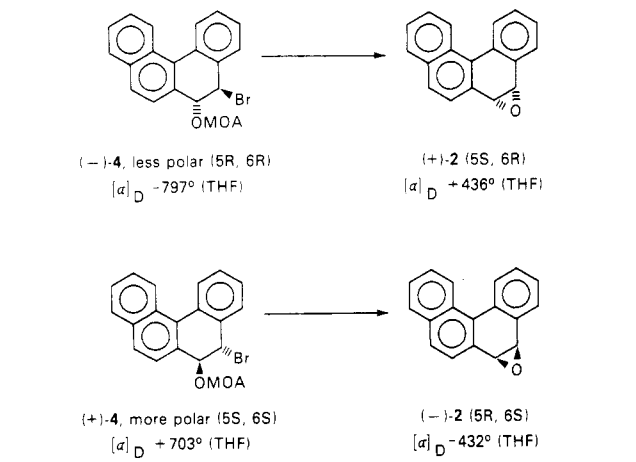
Scheme I



Scheme II

MOA = Menthyloxyacetyl, absolute configurations as shown



microsomal metabolism of the hydrocarbon proceeds via the 5,6-oxide (**2**) at the K region.[5] With liver microsomes from either control or induced rats, from 77% to 89% of the metabolites consists of the *trans*-5,6-dihydrodiol (**3**) which is optically active.[6] Study of the stereospecificity of the cytochromes P450 and epoxide hydrolase involved in this transformation (Scheme I; absolute configuration not implied for **3**) has necessitated the preparation of optically active benzo[*c*]phenanthrene 5,6-oxides (**2**) of known absolute configuration. The present report describes the synthesis of these enantiomers and assigns their configurations.

We have recently described a convenient, high yield synthesis of *trans*-5-bromo-6-hydroxy-5,6-dihydrobenzo[*c*]phenanthrene and its resolution via chromatographic separation of the diastereomeric esters formed on esterification with (−)-(menthyloxy)acetic acid.[7] The early eluting, less polar diastereomer was tentatively assigned 5*R*,6*R* absolute configuration on the basis of two NMR correlation techniques.[7,8] However, since these methods had not been applied to K-region derivatives, the assignment could not be considered definitive. Both diastereomers have now been converted to benzo[*c*]phenanthrene 5,6-oxide (**2**) by treatment with dry sodium methoxide in tetrahydrofuran (Scheme II). Configurational assignments in Scheme II are based on CD experiments which relate the arene oxide to dihydrodiol **3** of known absolute configuration.[9] This necessitated preparation of nucleophile adducts of (+)-**2** and (−)-**2** whose structures and solution conformations were known with certainty such that their CD spectra could be compared to that of optically active dihydrodiol **3** or an appropriate derivative.

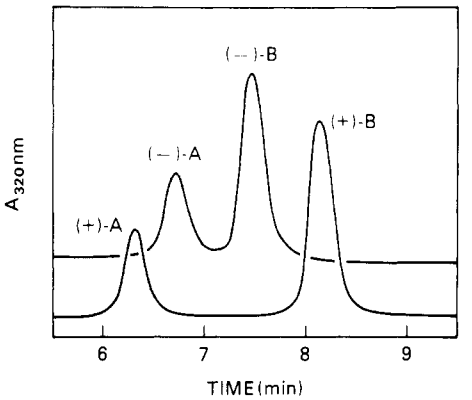
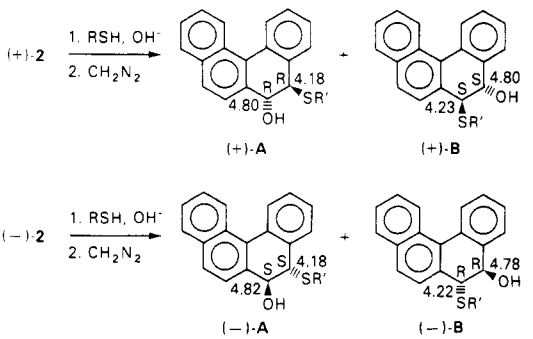


Figure 1. Analytical HPLC separation of the *N*-acetyl-L-cysteine adducts *as the free acids* from optically active benzo[*c*]phenanthrene 5,6-oxide (**2**) on an Applied Sciences Adsorbosphere ODS column (9.4 × 100 mm) eluted isocratically (5 mL/min) with 46% methanol in 0.05 M Tris acetate buffer (pH 7). UV spectra were monitored continuously throughout the chromatogram with a Hewlett-Packard 1040A diode array detector. The four peaks had practically identical spectra very similar to that of the 5,6-dihydrodiol **3**.[10] The designations (+) and (−) specify the sign ($[\alpha]_D$) of the starting arene oxide whereas A and B indicate minor and major adducts, respectively. Thus, (+)-A is the minor adduct from the (+)-(5*S*,6*R*)-arene oxide (+)-**2**. For structures of the adducts, see Scheme III.

Scheme III

Chemical Shifts for H_5 and H_6 of Methyl Esters in $CDCl_3$
(Absolute configurations as shown)

RSH = N-acetyl-L-cysteine
R'SH = N-acetyl-L-cysteine methyl ester



N-Acetyl-L-cysteine reacts rapidly and quantitatively with **2** in alkaline, aqueous dioxane to form minor (A) and major (B) positional adducts (ratio 1:3) from each enantiomer. The adducts were readily separable by reverse-phase HPLC (Figure 1). Based on the knowledge that *trans*-5,6-dibromo-5,6-dihydrobenzo[*c*]phenanthrene eliminates HBr to form 5-bromobenzo[*c*]phenanthrene,[5] that arene oxides generally react with nucleophiles more extensively at the oxirane carbon atom which can form the more stable carbocation,[11] and that Dewar PMO (ΔE_{deloc}) calculations[12] indicate protonated **2** should open to form

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Table I. Chemical Shifts and Coupling Constants for K-Region Derivatives Derived from [5-^{2}H]Benzo[*c*]phenanthrene[a]

benzo[c]phenanthrene derivative	H_5 (d), δ	H_6 (appt t), δ	J, Hz
benzo[c]phenanthrene (1)	7.90	7.83	8.5
trans-5-bromo-6-acetoxy-5,6-dihydrobenzo[c]phenanthrene	5.38	6.20	2.9
benzo[c]phenanthrene 5,6-oxide (2)	4.85	4.72	4.0
(5*R*,6*R*)-dihydrodiol (3)[b]	4.69	4.64	10.9
diacetate of 3[c]	6.10	6.19	6.5
bis((menthyloxy)acetate) of (+)-3[d,e]	6.14	6.24	5.6
bis((−)-α-methoxy(trifluoromethyl)-phenylacetate) of (+)-3[d,f]	6.14	6.28	3.5

[a] Spectra were recorded at 300 MHz in $CDCl_3$. Assignments of H_5 and H_6 are based on partial deuteration at H_5 in selected compounds. [b] The dihydrodiol prefers the conformation in which the hydroxyl groups are pseudoequatorial in $CDCl_3$ as well as in CD_3OD (J = 10.0 Hz) and tetrahydrofuran-d_8 (J = 11.0 Hz). [c] Little conformational change was observed in CD_3OD (J = 6.2 Hz) or in tetrahydrofuran-d_8 (J = 6.7 Hz). [d] Sample not deuterated. Positional assignments assumed from the diacetate of 3. [e] The value of $J_{5,6}$ = 5.8 Hz in CD_3CN and 6.4 Hz in tetrahydrofuran-d_8. [f] The value of $J_{5,6}$ = 3.4 Hz in tetrahydrofuran-d_8.

a more stable carbocation at C-6 (0.658β) relative to C-5 (0.606β), it was anticipated that the major adduct (B) from each enantiomer of **2** was formed by nucleophilic trans addition of the thiolate at C-6 (Scheme III).

Confirmation of the position of attack in the major and minor adducts was achieved through analysis of the NMR spectra of their methyl esters. Unequivocal assignment of relative stereochemistry (position of nucleophile attack) to the four adducts was achieved through the use of specifically deuterated **2**. Although the proton NMR spectra of **1** and 5-bromobenzo[*c*]phenanthrene had previously been examined at 360 MHz,[5] a more detailed investigation of these compounds was required as the stereochemical assignments would hinge on their accuracy. Through the application of 2D-NMR, all proton and ^{13}C resonances for these compounds have been assigned.[13] These studies confirmed the structure of 5-bromobenzo[*c*]phenanthrene and removed an ambiguity concerning the chemical shifts of the K-region protons H_5 and H_6 in **1** (H_6 δ 7.83 and H_5 δ 7.90 relative to Me_4Si with J = 8.5 Hz in $CDCl_3$). Hydrogenolysis of 5-bromobenzo[*c*]phenanthrene with deuterium gas provided [5-^{2}H]benzo[*c*]phenanthrene. The signal for H_5 remained as a doublet but of reduced intensity (half deuterated due to identity of H_5 and H_8 resulting from the plane of symmetry for **1**) while H_6 appeared as a triplet consisting of the original doublet (from molecules in which H_5 is hydrogen) with a singlet (uncoupled when H_5 is deuterium) about midway between the lines of the doublet. This effective partial deuteration at H_5 has allowed assignment of chemical shifts for a number of K-region derivatives of benzo[*c*]phenanthrene (Table I).

Racemic, deuterated **2**, obtained by reaction of [5-^{2}H]-benzo[*c*]phenanthrene with NBA in acetic acid and cyclization of the resulting bromohydrin acetate with dry sodium methoxide in THF,[7] was used in the preparation of the adducts shown in Scheme III. After separation of the adducts by preparative HPLC and conversion to their methyl esters with diazomethane, their NMR spectra (300 MHz, $CDCl_3$) were recorded. For the four adducts (as the methyl esters) NMR signals for the *N*-acetyl-L-cysteine methyl ester moiety fell in the following range: δ 1.95–1.98 (Ac), 2.76–3.25 (CH_2), 3.70–3.73 (OCH_3), and 4.76–4.87 (CH), corresponding to δ 1.95 (Ac), 2.90 (CH_2), 3.73 (OCH_3), and 4.81 (CH) for the model compound *S*-benzyl-*N*-acetyl-DL-cysteine methyl ester. Chemical shifts ($CDCl_3$) of the key signals at the 5- and 6-positions in the esters are tabulated on Scheme III. Assignments of chemical shifts to protons on either carbon-5 (doublet) or carbon-6 (overlapping doublet and singlet) is easily made on the basis of the presence or absence of deuterium. Assignment of the nature of the substituent, either hydroxyl or thioether, is based on the NMR spectra of thiolate and alkoxide adducts of benzene oxide[14] and phenanthrene 9,10-oxide[15] (K region) for which the proton on the carbon atom bearing the hydroxyl substituent is always at lower field (0.5 to 0.7 ppm higher chemical shift) relative to the proton on the carbon atom bearing the ether or thioether substituent. Thus, for the present adducts, chemical shifts of 4.78 to 4.82 ppm correspond to protons attached to carbon atoms substituted by hydroxyl groups and chemical shifts of 4.18 to 4.23 ppm correspond to positions bearing the thioether residues of the *N*-acetyl-L-cysteine methyl esters. This assignment is confirmed by the 1.2-ppm downfield shift of the doublet corresponding to H_5 (hydroxyl substitution) upon acetylation of one of the major adducts, (+)-B, whereas the apparent triplet (H_6) is shifted only 0.2 ppm downfield. Thus, the positional pattern of substitution in the adducts is established; i.e., the primary site of attack by the thiolate on **2** is at carbon-6 as anticipated (B type adducts). Values of $J_{5,6}$ in $CDCl_3$ for the four adducts indicate a preference for the pseudodiaxial conformation of the substituents: $J_{5,6}$ (+)-A, 5.3–5.4; (−)-A, 4.4–4.5; (+)-B, 4.6–4.7; (−)-B, 4.9–5.0 Hz. In acetonitrile or tetrahydrofuran, a stronger preference for the pseudoaxial orientation is observed: $J_{5,6}$ (CD_3CN) (+)-A, 2.6–2.9; (+)-B, 2.7–2.8 Hz; $J_{5,6}$ (THF-d_8) (−)-A, 3.7; (−)-B, ~2.7 Hz. Acetylation of the free hydroxyl group in (+)-B also changed $J_{5,6}$ ($CDCl_3$) from ~4.7 to ~2.6 Hz, as did addition of methanol-d_4 to $CDCl_3$ solutions of the adducts. These observations confirm the trans stereochemistry of the adducts since the value of $J_{5,6}$ for cis adducts would be expected to be insensitive to solvent changes or acetylation.

CD spectra of the adducts (measured on a JASCO J500A spectropolarimeter) are shown in Figure 2. As anticipated, the spectra of (+)-A and (−)-A are essentially mirror images of each other as is the case for (+)-B and (−)-B. Contribution of the *N*-acetyl-L-cysteine methyl ester substituent to the overall spectrum in the region of 220–400 nm was expected to be small.[16] Tetrahydrofuran was selected as the solvent for the CD spectra of the adducts since they show a strong preference in this solvent for the conformation in which the K-region substituents (hydroxyl and -*S*-(*N*-acetylcysteine methyl ester)) prefer the axial environment. Under these conditions the benzofused, bridged biphenyl chromophore has maximal helicity and should show a pronounced CD band related to this helicity. Strong, short-wavelength CD (or ORD) bands in the region of ~225–235 nm have been used to assign the helix sense to the preferred conformation of substituted bridged bi-

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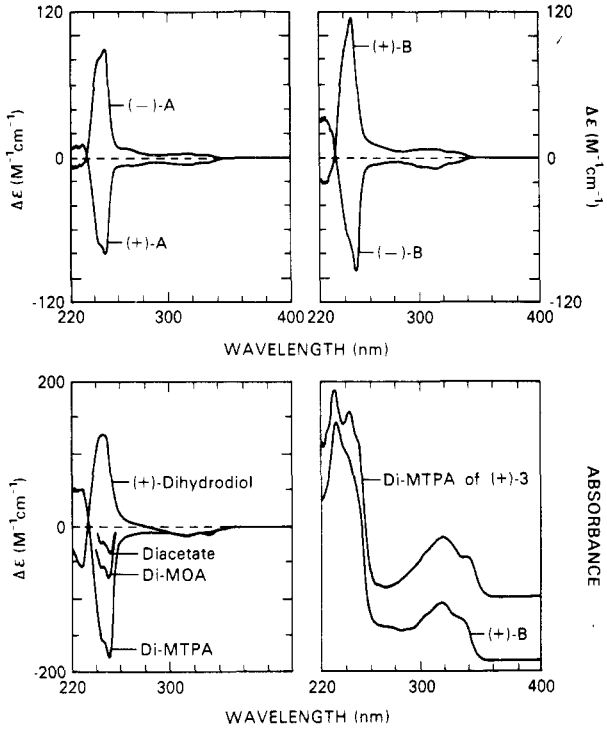

Figure 2. CD spectra in freshly distilled tetrahydrofuran. Extinction coefficients for the adducts (as the methyl esters) were assumed on the basis of a value of 1.3×10^4 M^{-1} cm^{-1} (316 nm), as measured for the diacetate of the *trans*-5,6-dihydrodiol **3**. Notably, the designations for the adducts (see also Figure 1 and Scheme III) are meant to imply their origin and not their rotation or configuration. For purposes of comparison with the adduct spectra (two upper panels), CD spectra of the (+)-(5*R*,6*R*)-dihydrodiol ((+)-**3**), its diacetate, and its diesters with (2)-(menthyloxy)acetic acid (MOA) and (−)-α-methoxy(trifluoromethyl)phenylacetic acid (MTPA) (all extinction coefficients based on the diacetate) are shown in the lower left panel. The CD spectrum of the diester of (−)-**3** with (−)-α-methoxy(trifluoromethyl)phenylacetic acid was the mirror image of the corresponding derivative of (+)-**3** but was about 8% less intense. UV spectra (tetrahydrofuran) of the MTPA ester of (+)-**3** and methyl ester of adduct (+)-B are compared in the lower right panel.

phenyls.[16-18] This CD band, negative for conformations with a left-handed *M* helicity and positive for conformations with a right-handed *P* helicity (Figure 3), appears at 249–250 nm in the present adducts. Looking down the substituted, bridged biphenyl axis of adduct (+)-B (axial sulfur at C-6) indicates *P* (plus) helicity, and a positive CD band at 250 nm is observed (Figures 2 and 3). Similarly a negative band is observed for (−)-B indicative of *M* (minus) helicity, etc. (Figure 3). Thus, the antipodal relationship expected between (+)-A and (−)-A as well as between (+)-B and (−)-B required by Scheme III is observed. Both the shape and the magnitude of the spectra (Figure 3) are relatively insensitive to the position at which sulfur is attached (A adducts vs. B adducts) unlike the positional isomers of glutathione adducts of benzo[*a*]pyrene 4,5-oxide and benz[*a*]anthracene 5,6-oxide.[16,19] Although the CD arguments in combination with the

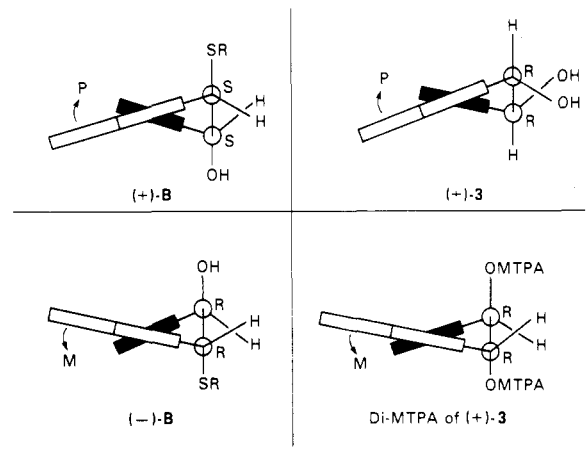

Figure 3. Conformational representations of adducts (+)-B and (−)-B which illustrate their *P* and *M* helicities, respectively (left), as well as the conformational inversion of the (+)-(5*R*,6*R*)-dihydrodiol (+)-**3** (*P* helicity) on conversion to its diester with (−)-α-methoxy(trifluoromethyl)phenylacetic acid (*M* helicity) shown on the right. Notably the pairs (+)-B and (+)-**3** as well as (−)-B and the diester of (+)-**3** with MTPA have practically identical CD spectra (Figure 2).

known positional substitution and preferred conformations of the adducts led to the assignments in Scheme III, additional evidence in support of the CD argument was sought.

The (+)-(5*R*,6*R*)-dihydrodiol ($[\alpha]_D$ +409°, THF)[9] prefers the conformation in which its hydroxyl groups are equatorial ($J_{5,6}$ = 11.0 Hz, THF). This requires *P* helicity (Figure 3) as observed (Figure 2). Upon acetylation ($J_{5,6}$ = 6.7 Hz), formation of a diester (di-MOA) with (−)-(menthyloxy)acetic acid ($[\alpha]_D$ −326°, $J_{5,6}$ = 6.4 Hz),[9] and formation of a diester (di-MTPA) with (−)-α-methoxy(trifluoromethyl)phenylacetic acid ($J_{5,6}$ = 3.4 Hz), the CD band ~250 nm becomes increasingly negative (CD and NMR spectra in THF, Table I and Figure 2), indicative that (i) the short-wavelength CD band ~250 nm is associated with the helicity of the benzofused, bridged biphenyl chromophore and that (ii) *M* helicity required by axial 5*R*,6*R* substitution corresponds to a negative sign of this CD band as in the bridged biphenyl system. As in the case of $\Delta\epsilon_{228}$ for (9*R*,10*R*)-substituted 9,10-dihydrophenanthrenes[16] there is a good linear correlation (slope 41, intercept −324, correlation coefficient 0.998) between $J_{5,6}$ and $\Delta\epsilon$ at the 246–251-nm extremum for (+)-**3** and three O-substituted 5*R*,6*R* derivatives (cf. Figure 2, lower left) with values of $\Delta\epsilon$ ranging from −183 to +127 M^{-1} cm^{-1}. Thus it may be concluded that the *N*-acetyl-L-cysteine adducts have the absolute configurations shown in Scheme III and hence that the enantiomers of arene oxide **2** (Scheme II) and their precursors[7] have been assigned correctly.

Optically active arene oxides are particularly useful in the study of the mechanism of action of the cytochromes P450 which form them[19] and of epoxide hydrolase[20] which converts them to *trans*-dihydrodiols. These enzymes are major contributors to the in vivo metabolism of a wide variety of nonpolar xenobiotic substances to reactive metabolites which can display toxic and carcinogenic activity.[21] Currently, the metabolism of **1** to the enantiomers of **2** is being utilized to probe the catalytic binding site of

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cytochrome P450c,[6] the isozyme which is strongly induced in rat liver by prior treatment of the rats with 3-methylcholanthrene.

Experimental Section

[5-^{2}H]Benzo[*c*]phenanthrene. The availability of 1,2-dihydro-4(3*H*)-benzo[*c*]phenanthrenone,[22] obtained as described previously,[23] prompted its use in the synthesis of 1.[24] The ketone was reduced to the corresponding alcohol ($NaBH_4$ in methanol/THF, 4:1), the alcohol was dehydrated to 1,2-dihydrobenzo[*c*]phenanthrene (toluenesulfonic acid in refluxing benzene, 5 min) which was purified by filtration of the benzene solution through a bed of silica gel, and the dihydro compound was oxidized to benzo[*c*]phenanthrene by treatment with a 10% excess of DDQ in dioxane at room temperature. Although oxidation appeared to be complete within a few minutes, the dark mixture was stored overnight before workup. The reaction mixture was filtered to remove most of the insoluble hydroquinone product, then diluted with benzene and extracted several times with 1 M sodium carbonate solution. After drying and concentration of the organic layer, the residue was dissolved in benzene and filtered through a bed of silica gel. The oxidation is essentially quantitative. Benzo[*c*]phenanthrene and its 1,2-dihydro precursor are separable by HPLC on a Perkin-Elmer HS-3 C_{18} column (4.6 × 100 mm) eluted with acetonitrile at a flow rate of 1 mL/min: retention times 1.71 and 1.80 min, respectively.

Benzo[*c*]phenanthrene (1.14 g, 5 mmol) and bromine (10 mmol) were stored in benzene (50 mL) at room temperature for 40 min at which time >98% conversion to the *trans*-5,6-dibromide had occurred. Evaporation of the solvent under vacuum removed excess bromine. The resulting oil was dissolved in 50 mL of benzene and refluxed for 10 min to eliminate HBr. In addition to 5-bromobenzo[*c*]phenanthrene (90% yield after purification by HPLC), traces of 1 along with three minor late eluting impurities were present: HPLC on a Du Pont Zorbax ODS column (21.2 × 250 mm) eluted with acetonitrile at 22 mL/min gave retention times of 2.5 min for benzene (void volume), 3.0 min for the *trans*-5,6-dibromide, 4.4 min for 1, and 6.5 min for 5-bromobenzo[*c*]phenanthrene. The present reaction conditions represent a significant improvement over those previously described.[5]

A mixture of 5-bromobenzo[*c*]phenanthrene (0.6 g) and 10% palladium on carbon (100 mg) was stirred under 1 atm of deuterium gas in 15 mL of ethyl acetate containing 1 mL of triethylamine for 50 min. The catalyst and triethylamine hydrobromide were removed by filtration and the solvents removed in vacuum. Analysis by HPLC as above indicated quantitative hydrogenolysis to [5-^{2}H]-1. Mass spectral analysis indicated >98% incorporation of one deuterium. The NMR spectrum is described in the text and Table I.

Benzo[*c*]phenanthrene 5,6-Oxide (2). We have previously reported that the treatment of K-region, *trans*-halohydrin acetates (~50–100 mg) with dry sodium methoxide (~200–400 mg) in freshly distilled THF (~20–30 mL) at room temperature for 24 h results in near quantitative formation of K-region arene oxides.[7,25] For the present study, the enantiomers of *trans*-5-bromo-6-hydroxy-5,6-dihydrobenzo[*c*]phenanthrene were separated by HPLC as their (–)-(menthyloxy)acetates and were converted into arene oxides (–)-**2** and (+)-**2**, as shown in Scheme II. Similarly, [5-^{2}H]-1 was converted to racemic **2** which is partially deuterated at positions 5 and 8. In this latter case, the precursor, *trans*-5-bromo-6-acetoxy-5,6-dihydrobenzo[*c*]phenanthrene (Table I), was purified by HPLC on a Du Pont Zorbax SIL column (21.2 × 250 mm) eluted with 15% ethyl acetate in hexane at a flow rate of 25 mL/min; retention time 8 min. Mass spectral analysis indicated >99% incorporation of one deuterium. NMR data are given in Table I.

Liver Microsomal Hydration of 2. Approximately 2.5 mg of partially deuterated **2** in 0.5 mL of acetonitrile was added in five equal portions over a period of 1 h at 37 °C to 60 mL of 0.1 M potassium phosphate buffer (pH 7.4) containing a suspension of liver microsomes (81.2 mg protein; ~2.4 mg epoxide hydrolase) from 3-methylcholanthrene-treated rats (immature Long-Evans, male).

The product was extracted with ethyl acetate and after solvent evaporation was purified by chromatography on a Du Pont Zorbax SIL column (9.4 × 250 mm) eluted with 2.5% methanol and 15% ethyl acetate in cyclohexane at a flow rate of 5.5 mL/min; $k' = 1.3$. Partial purification of the ultraviolet-absorbing material, **3** (detected at 317 nm), from an early shoulder that was detectable only by refractive index was effected by concentration of the sample and rechromatography under the same conditions with two or three recycles. Relevant features of the NMR spectrum of **3** in $CDCl_3$ are given in Table I.

Diol **3** was acetylated with excess acetic anhydride in pyridine for 16 h at room temperature. The reaction mixture was taken up in benzene, evaporated, and dried in vacuo, and the diacetate was purified by chromatography under the conditions described above for the diol; $k' = 0.65$.

Reaction of 2 with *N*-Acetyl-L-cysteine. Deuterated oxide **2** (50 mg, 204 μmol) dissolved in 10 mL of freshly distilled dioxane was added in three equal portions at 37 °C over a period of 30 min to 140 mL of an aqueous solution containing 12 mmol of potassium phosphate buffer, pH 7.4, 2 mmol of *N*-acetyl-L-cysteine, 20 mL of freshly distilled dioxane, and sufficient sodium hydroxide to adjust the solution to pH 11. After ~45 min the basic reaction mixture was extracted twice with ether; the aqueous layer was then cooled in an ice bath and acidified to pH ~2.8 with concentrated phosphoric acid. The acidified solution was extracted with three portions of ethyl acetate, and the extracts were combined, dried with magnesium sulfate, and evaporated to dryness. The four adducts were separated by chromatography on a Du Pont Zorbax ODS column, 21.2 × 250 mm, eluted with methanol/0.05 M Tris phosphate buffer (pH 7.0) 46:54, at a flow rate of 18 mL/min. The elution order of the products is the same as that in the analytical system described in Figure 1. Four fractions were collected, concentrated to remove methanol, and acidified to pH 2.8 in the cold with concentrated phosphoric acid. The desired products were extracted into ethyl acetate. For further purification each fraction was rechromatographed by using the same column and solvent described above.

The purified acids in ethyl acetate solution were treated with excess ethereal diazomethane to give their methyl esters. The methyl esters were freed from traces of diastereomeric contaminants prior to circular dichroism measurements by chromatography on the Applied Sciences Adsorbosphere ODS column (9.4 × 100 mm) eluted with 50% methanol in water at a flow rate of 5 mL/min. Colored impurities were removed by chromatography of the methyl esters on a Du Pont Zorbax SIL column (9.4 × 250 mm), eluted with 5% methanol and 15% ethyl acetate in hexane at a flow rate of 12 mL/min. Retention time for all four compounds was ~11 min. Mass spectra for (+)-B and (–)-B (CI, NH_3) gave m/z 405 (M + 1 – 18). Relevant NMR data for the methyl esters are given in the text and Scheme III, and their circular dichroism spectra are shown in Figure 2. It should be noted that chromatographic mobile phases containing tetrahydrofuran should be avoided with these sulfur-containing adducts; extensive degradation of the adducts was observed when such solvents were used. This degradation may result from oxidation mediated by traces of peroxides that are concentrated upon solvent evaporation.

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