alumina (chloroform elution) to give 30 mg (12%) of **13** as a colorless crystalline solid: mg 92.5–93 °C (from hexane); ^{1}H NMR ($CDCl_3$) δ 4.84 (s, 2 H), 3.66 (s, 6 H), 1.39 (dd, J = 7.3 and 4.9 Hz, 4 H), 0.77 (dd, J = 7.3 and 4.9 Hz, 4 H); ^{13}C NMR ($CDCl_3$) ppm 166.80, 136.97, 129.94, 51.88, 21.15, 17.83; MS, m/z 248 (M^+, 16.6) 233 (100), 201 (95.7) 185 (55.9), 184 (45.1) 157 (39.9), 156 (29.0), 130 (49.8), 128 (52.1) 115 (36.4), 102 (22.7), 91 (15.4), 77 (23.5).

Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.87; H, 6.63.

C. With Tetracyanoethylene. A solution of **1** (105 mg, 0.99 mmol) in carbon tetrachloride (2 mL) was added under argon to a solution of freshly sublimed tetracyanoethylene (127 mg, 0.99 mmol) in the same solvent (2 mL) at room temperature. Initially, a bright mauve color was observed. After being stirred for 2 days, the reaction mixture was filtered through a bed of Celite, which was rinsed with carbon tetrachloride. Evaporation of the filtrate provided 63 mg (27%) of **14** as a white crystalline solid. Following recrystallization from chloroform and sublimation (90 °C and 0.05 torr), the adduct exhibited a decomposition point of 182 °C: ^{1}H NMR ($CDCl_3$) δ 5.30 (s, 2 H), 1.62 (m, 4 H), 1.25 (m, 4 H); ^{13}C NMR ($CDCl_3$) ppm 128.17, 109.88, 46.55, 22.70, 14.14; MS, m/z 234 (M^+, 63.3), 219 (30.1), 206 (69.9), 192 (100), 181 (35.7), 179 (47.9), 167 (99.5).

Anal. Calcd for $C_{14}H_{10}N_4$: C, 71.78; H, 4.30. Found: C, 71.52; H, 4.47.

D. With *N*-Phenylmaleimide. A solution of **1** (94.1 mg 0.89 mmol) and *N*-phenylmaleimide (154 mg, 0.89 mmol) in benzene (3.5 mL) was deoxygenated by bubbling argon through and heated at reflux under an argon atmosphere overnight. The solvent was evaporated under reduced pressure and the pale yellow crystalline residue (246 mg) was purified by MPLC on silica gel (elution with 21% ethyl acetate in petroleum ether). There was isolated 185 mg (74%) of **15** as fine white needles which were sublimed at 102 °C and 0.05 torr: mp 102–104 °C; ^{1}H NMr ($CDCl_3$) δ 7.50–7.23 (m, 5 H), 5.32 (s, 2 H), 2.81 (s, 2 H), 1.40 (m, 2 H), 0.88 (m, 4 H), 0.67 (m, 2 H); ^{13}C NMR ($CDCl_3$) ppm 176.69, 133.45, 129.05, 128.39, 126.48, 48.40, 17.54, 14.69, 12.45; MS, m/z 279 (M^+, 44.7) 132 (100), 117 (59.7).

Anal. Calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13. Found: C, 77.37; H, 6.26.

Acknowledgment. This research has been supported in large part by a grant from the National Science Foundation.

Registry No. **1**, 36336-98-8; **4**, 81236-83-1; **5**, 91606-47-2; **6**, 91606-48-3; **7**, 91606-49-4; **8**, 91606-50-7; **9**, 91606-51-8; **10**, 91606-52-9; **11**, 91606-53-0; **12**, 91606-54-1; **13**, 91606-55-2; **14**, 91606-56-3; **15**, 91606-57-4; *N*-methyltriazolinedione, 13274-43-6; dimethyl acetylenedicarboxylate, 762-42-5; tetracyanoethylene, 670-54-2; *N*-phenylmaleimide, 941-69-5.

Synthesis and Absolute Configuration of the Bacterial *cis*-1,2-, *cis*-8,9-, and *cis*-10,11-Dihydrodiol Metabolites of Benz[*a*]anthracene Formed by a Strain of *Beijerinckia*

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Received January 10, 1984

Metabolism of the environmental contaminant benz[*a*]anthracene has been examined with the bacterium *Beijerinckia* B8/36. This organism is a mutant strain of the wild type, which lacks the ability to oxidize further initially formed *cis*-dihydrodiol metabolites of aromatic hydrocarbons. The main isolated metabolites of benz[*a*]anthracene consist of the *cis*-1,2-, *cis*-8,9-, and *cis*-10,11-dihydrodiols in a ratio of 73:15:12, respectively. Synthesis of the dihydrodiols in optically pure form from precursors whose configurations were previously known or have been assigned in the present study has established that the metabolites are of very high enantiomeric purity and have 1*R*,2*S*, 8*R*,9*S*, and 10*S*,11*R* absolute configurations. In the course of these assignments, the (+)-isomer of 1,2-epoxy-1,2,3,4-tetrahydrobenz[*a*]anthracene has been established to have 1*R*,2*S* absolute configuration, and a prior assignment of (−)-*trans*-(1*R*,2*R*)-1,2-dihydroxy-1,2-dihydrobenz[*a*]anthracene has been confirmed. The chemical interrelationships of absolute configuration have been done in such a manner that the *cis*-1,2-, *cis*-8,9-, and *cis*-10,11-dihydrodiols formed by the bacterium are tied directly to structures which have been used to assign the corresponding *trans*-1,2-, *trans*-8,9-, and *trans*-10,11-dihydrodiols formed from benz[*a*]anthracene in mammalian liver.

Introduction

Polycyclic aromatic hydrocarbons such as benz[*a*]anthracene represent a widespread class of environmental contaminants. Their identification in soils and ancient marine sediments[2] has led to the suggestion that they have been present in the environment during geological periods of time.[3] It is therefore not surprising that microbial flora have developed enzyme systems capable of their metabolism. Sisler and Zobell,[4] for example, have reported the release of carbon dioxide during the oxidation of benz[*a*]anthracene. Although conventional enrichment culture techniques in mineral salts media failed to identify organisms capable of growth on polycyclic aromatic hydrocarbons, a *Beijerinckia* species was isolated based on its

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(2) Blumer, M. *Science (Washington, D.C.)* **1961**, *134*, 474–475. Kern, W. *Helv. Chim. Acta* **1947**, *30*, 1595–1599.

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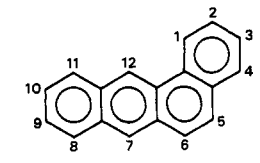
0022-3263/84/1949-3621$01.50/0

Table I. NMR Spectra (220 MHz, Acetone-d_6) of the Bacterial *cis*-1,2- (1a), *cis*-8,9- (1b), and *cis*-10,11-Dihydrodiols (1c) after Deuterium Exchange of the Hydroxyl Groups[a]

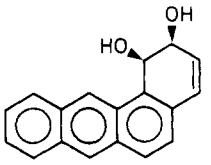
	carbinol		olefinic		aromatic hydrogens			
compd	benzylic	nonbenzylic	nonbenzylic	benzylic	H_7	H_{12}	$H_{5,6}$	benzo ring
1a	H_1 5.46	H_2 4.72	H_3 6.05	H_4 6.55	8.51	8.88	7.35, 8.04	$H_{8,11}$ 8.05, 8.17, $H_{9,10}$ 7.50
	($J_{1,2}$ = 5.4 Hz, $J_{2,3}$ ~ 2 Hz, $J_{2,4}$ = 2.8 Hz, $J_{3,4}$ = 9.9 Hz)							
1b	H_8 4.82	H_9 4.40	H_{10} 6.16	H_{11} 6.86	8.05	8.55	7.81	H_1 8.79, H_4 7.95, $H_{3,4}$ 7.64
	($J_{8,9}$ = 4.8 Hz, $J_{9,10}$ = 4.3 Hz, $J_{10,11}$ = 9.6 Hz)							
1c	H_{11} 4.87	H_{10} 4.40	H_9 6.15	H_8 6.74	7.68	8.88	7.79	H_1 8.79, H_4 7.95, $H_{2,3}$ 7.63
	($J_{10,11}$ = 4.7 Hz, $J_{9,10}$ = 4.4 Hz, $J_{8,9}$ = 9.6 Hz)							

[a] Chemical shifts are in ppm relative to Me_4Si.

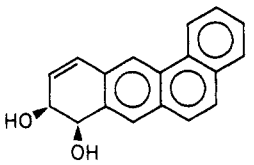
ability to grow on biphenyl as its sole source of carbon. A mutant strain of this bacterium,[5] *Beijerinckia* B8/36, has now been demonstrated to accumulate vicinal *cis*-dihydrodiols from a number of aromatic hydrocarbons and related compounds.[6] Such bacterial *cis*-dihydrodiols are generally believed to be formed by dioxygenase enzymes which incorporate both atoms of a single oxygen molecule into the hydrocarbon substrate.[7] The major metabolite from benz[*a*]anthracene has been identified as *cis*-1,2-dihydroxy-1,2-dihydrobenz[*a*]anthracene.[8] The present study utilizes a combination of chemical and spectral techniques to characterize these *cis*-dihydrodiol metabolites formed from benz[*a*]anthracene by *Beijerinckia* B8/36. Their structures are shown below. Major reasons



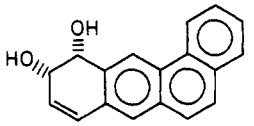
benz[a]anthracene



(1R, 2S) - 1a



(8R, 9S) - 1b



(10S, 11R) - 1c

for our interest in the bacterial metabolism of the weak carcinogen benz[*a*]anthracene[9] stem from the possibility that a 3,4-dihydrodiol possibly could act as a proximate carcinogen in mammals[10] and that a knowledge of the absolute configuration of the *cis*-dihydrodiols could provide some insight into the nature of the dioxygenase enzyme binding site. The potentially carcinogenic *cis*-3,4-dihydrodiol was not detected as a bacterial metabolite.

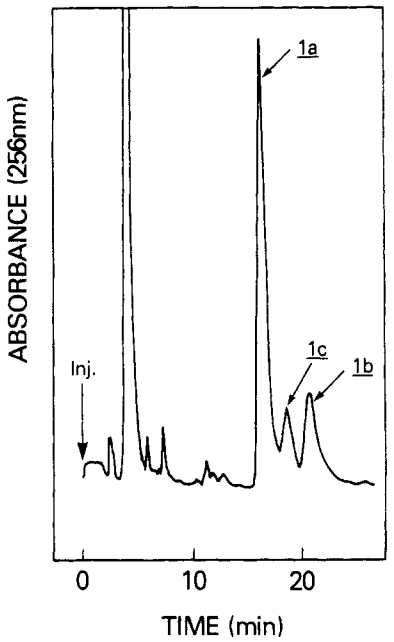


Figure 1. HPLC separation of the bacterially formed *cis*-1,2 (**1a**), 8,9 (**1b**), and 10,11 (**1c**) dihydrodiols of benz[*a*]anthracene. The three dihydrodiols have about the same extinction coefficient at 256 nm. A trace amount of the *cis*-5,6-dihydrodiol, which elutes just prior to **1a**, was also detected. HPLC was run on a Du Pont Zorbax SIL column (0.62 × 25 cm) eluted with 2% dioxane and 0.5% ethanol in hexane at a flow rate of 1.2 mL/min.

Results and Discussion

Isolation and Characterization of Dihydrodiols. Benz[*a*]anthracene was incubated with *Beijerinckia* B8/36 which had been grown in the presence of succinate and biphenyl. A combination of open column chromatography and TLC provided a dihydrodiol fraction of metabolites which was subsequently separated into individual components by HPLC (Figure 1). Three major dihydrodiol isomers were isolated and a very minor isomer was identified. Initial assignment of the position of the diol groups was based on their UV spectra since the spectra of the five possible vicinal *trans*-dihydrodiols of benz[*a*]anthracene

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(6) *cis*-Dihydrodiols have been isolated as bacterial metabolites formed from benzene,[7a] toluene (Ziffer, H.; Jerina, D. M.; Gibson, D. T.; Kobal, V. M. *J. Am. Chem. Soc.* **1973**, *95*, 4048–4049), naphthalene,[7b] biphenyl,[5] anthracene (Akhtar, M. N.; Boyd, D. R.; Thompson, N. J.; Koreeda, M.; Gibson, D. T.; Mahadevan, V.; Jerina, D. M. *J. Chem. Soc.*, Perkin *Trans. 1* 1975, 2506–2511), phenanthrene (Koreeda, M.; Akhtar, M. N.; Boyd, D. R.; Neil, J. D.; Gibson, D. T.; Jerina, D. M. *J. Org. Chem.* **1978**, *43*, 1023–1027), dibenzofurane, thiophene, and dioxane (Cerniglia, C. E.; Morgan, J.; Gibson, D. G. *Biochem. J.* **1979**, *180*, 175–185; Laborde, A. L.; Gibson, D. T. *Appl. Environ. Microbiol.* **1977**, *34*, 783–790; Klecka, G. M.; Gibson, D. T. *Biochem. J.* **1979**, *180*, 639–645), benzoic acids (Reiner, A. M.; Hegeman, A. M. *Biochemistry* **1971**, *10*, 2530–2536; Reineke, W.; Otting, W.; Knackmuss, H. J. *Tetrahedron* **1978**, *34*, 1707–1714), and 5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone (Frenne, E.; Eberspacher, J.; Lingens, F. *Eur. J. Biochem.* **1973**, *33*, 357–363).

(7) (a) Gibson, D. T.; Cardini, G. E.; Maseles, F. C.; Kallio, R. E. *Biochemistry* **1970**, *9*, 1631–1635. (b) Jeffery, A. M.; Yeh, H. J. C.; Jerina, D. M.; Patel, T. R.; Davey, J. F.; Gibson, D. T. *Ibid.* **1975**, *14*, 575–584.

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(9) Dipple, A. *ACS Monogr.* **1976**, No. 173, 245–314. Levin, W.; Thakker, D. R.; Wood, A. W.; Chang, R. L.; Lehr, R. E.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1978**, *38*, 1705–1710.

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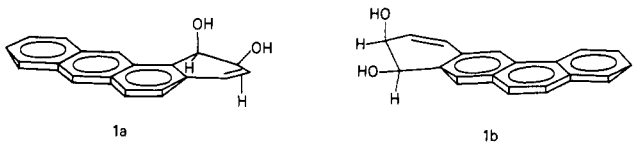
are known and are substantially different from each other.[11] The values of λ_{max} for the bacterial *cis*-dihydrodiols (75% methanol in water) were within 1–2 nm of those for the corresponding synthetic *trans*-dihydrodiols (ethanol). In their order of elution, the bacterial dihydrodiols consisted of the 5,6- (trace), 1,2-(**1a**, 73%), 10,11- (**1c**, 12%), and 8,9-positional isomers (**1b**, 15%) (Figure 1). The values of the coupling constants between the carbinol hydrogens (J_{diol}) of the isolated *cis*-dihydrodiols ranged from 4.7 to 5.4 Hz (Table I) and were markedly different from the trans isomers.[11] For the *trans*-8,9- and *trans*-10,11-dihydrodiols, $J_{diol} \sim 10$ Hz indicates that these dihydrodiols prefer the conformation in which the hydroxyl groups are pseudodiequatorial.[7b,11,12] Steric strain in the bay region causes the *trans*-1,2-dihydrodiol to prefer the pseudodiaxial conformation (J_{diol} = 1.7 Hz). For vicinal, benzo ring dihydrodiols, the magnitude of the W coupling (cf. ref 13) between the allylic carbinol hydrogen and the benzylic vinyl hydrogen is particularly informative. When this coupling is significant (J = 2–3 Hz), the allylic hydroxyl group prefers the pseudoequatorial conformation. When this coupling is not observed ($J \sim 0$ Hz), the allylic hydroxyl group prefers the pseudoaxial conformation.[7b,12] In this latter conformation, the coupling between the allylic carbinol hydrogen and the non-benzylic vinyl hydrogen is larger (4–5 Hz relative to ~2 Hz). The correlation is independent of the relative cis/trans configuration of the dihydrodiol. Based on the magnitude of the above two coupling constants, the *cis*-dihydrodiols in the present study prefer the conformations shown below. The preferred conformations of **1b** and **1c** are identical. In the absence of steric and electrostatic factors which specifically alter the conformation of dihydrodiols (e.g., bay region or peri substituents; cf. ref 14), vicinal *cis*- or *trans*-dihydrodiols on benzo rings such as **1b** and **1c** prefer the conformation in which the benzylic hydroxyl group is pseudoequatorial.[7b,12] Acid-catalyzed dehydration of **1a**, **1b**, and **1c** forms the 2-, 8-, and 11-hydroxybenz[a]anthracene, respectively, as the predominant product. The ratio of the two possible phenols produced from each of the bacterial *cis*-dihydrodiols may be related to their ground-state conformation since in each the pseudoaxial hydroxyl group is preferentially lost, presumably via a transition state involving *trans*-diaxial elimination of water. However, electronic considerations (Dewar perturbational molecular orbital calculations, $\Delta E_{deloc}/\beta$) also suggest that the carbocation leading to 2-hydroxy- (C_1 0.880 vs. C_2 0.869), 8-hydroxy- (C_9 0.838 vs. C_8 0.586), and 11-hydroxybenz[a]anthracene (C_{10} 0.813 vs. C_{11} 0.626) from their respective dihydrodiols is also favored. Thus, selective loss of the benzylic 1-hydroxyl group from **1a** is as expected by either argument. CD spectra of the isolated bacterial dihydrodiols are shown (Figure 2).



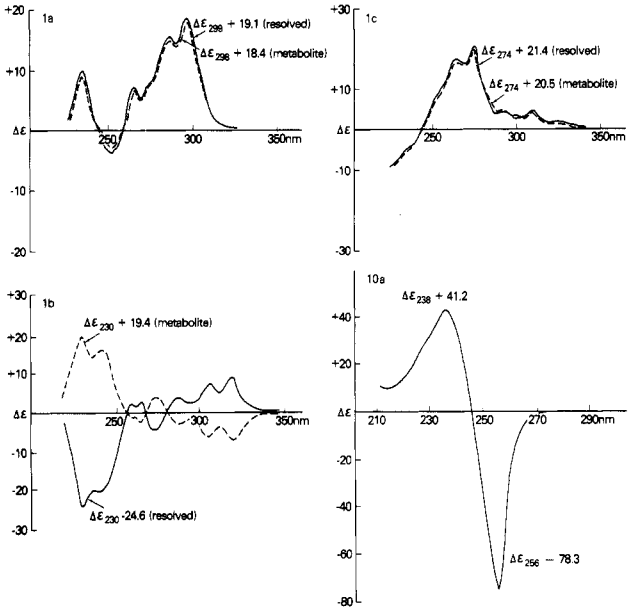
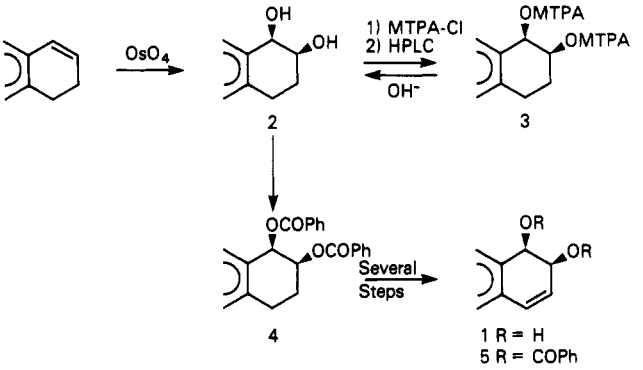


Figure 2. CD spectra (THF) of the metabolically formed (--) and synthetic (—) *cis*-dihydrodiols **1a**, **1b**, and **1c** of benz[a]anthracene. Practically identical spectra were obtained in ethanol. The CD spectrum (MeOH:$CHCl_3$) of *cis*-(1*S*)-hydroxy-(2*R*)-[(*p*-chlorobenzoyl)oxy]-1,2,3,4-tetrahydrobenz[a]anthracene (**10a**) is also shown.

Scheme I



Synthesis of Optically Active *cis*-Dihydrodiols. The same general approach was utilized for the synthesis of each of the *cis*-dihydrodiols in optically active form (Scheme I). Appropriate dihydrobenz[a]anthracenes[11] were allowed to react with osmium tetroxide to form *cis*-tetrahydrodiols which were resolved via separation of their diastereomeric diesters with (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA). HPLC separation of the diastereoisomeric mixtures **3a** and **3b** proceeded readily (α = 1.50 and 1.42) whereas the separation of **3c** proved more difficult (α = 1.12). Comparison of the specific rotations for the pairs of diastereomers indicated that the late eluting or more polar isomer under the chromatographic conditions described always had a more negative rotation and led to the negative *cis*-tetrahydrodiol upon hydrolysis. As will be shown later, this correlation of sign of rotation and polarity of the bis(esters) is not predictive of common absolute configuration for the carbon bearing the benzylic oxygen substituent. One enantiomer of each positional isomer of the *cis*-tetrahydrodiols was carried on to dihydrodiol by the following sequence: (i) benzoylation to the diester (**4**), (ii) introduction of the double bond either by treatment with DDQ[15] or by the sequence of

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(12) Jerina, D. M.; Selander, H.; Yagi, H.; Wells, M. C.; Davey, J. F.; Mahadevan, V.; Gibson, D. T. *J. Am. Chem. Soc.* **1976**, *98*, 5988–5996.
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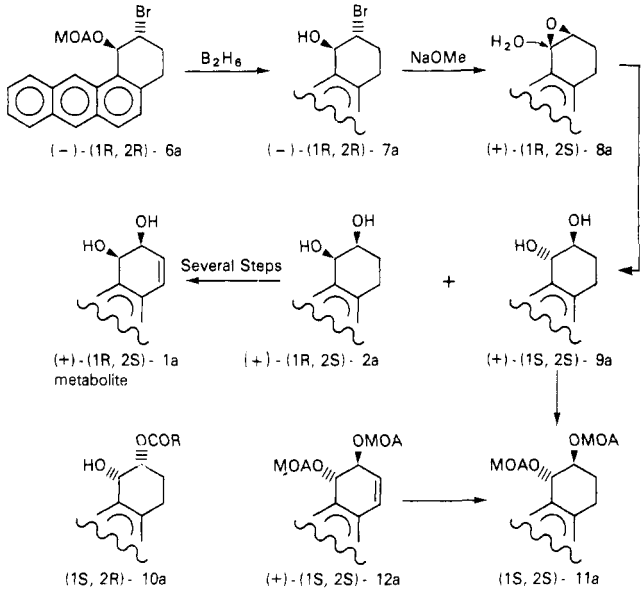
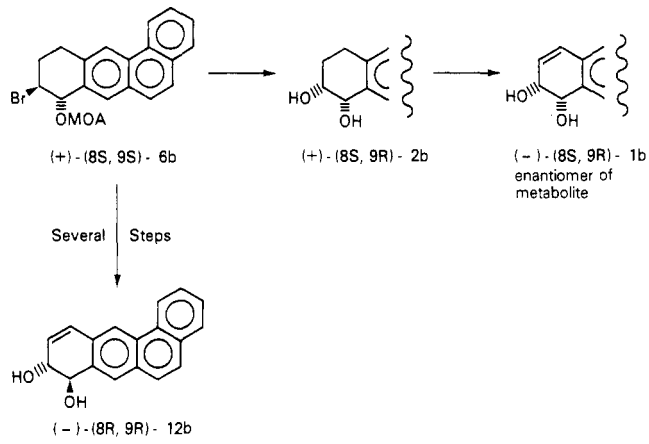
(15) Fu, P. P.; Harvey, R. G. *Tetrahedron Lett.* **1977**, *19*, 2059–2062.

Scheme II



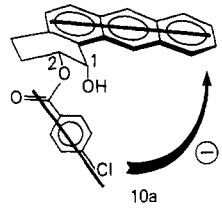
R = 4-Chlorophenyl, MOA = (−)-menthoxyacetyl

Scheme III



bromination and dehydrobromination[8,11] to give the dihydrodiol dibenzoates (**5**), (iii) hydrolysis of the esters to the dihydrodiols (**1**). Circular dichroism spectra of the synthetic and metabolically formed dihydrodiols are compared in Figure 2. Within experimental error, the metabolites appeared to be close to enantiomeric purity. Absolute configurations of the *cis*-dihydrodiols were then assigned and tied to the configurations of the corresponding trans isomers formed by mammals.

Correlation of Absolute Configuration. Conversion of (+)-*cis*-(1*R*,2*S*)-tetrahydrodiol **2a** to dihydrodiol **1a** provided a compound with an identical CD spectrum to that observed for the metabolite (Figure 2). Thus, the metabolite **1a** is assigned as (+)-*cis*-(1*R*,2*S*)-1,2-dihydroxy-1,2-dihydrobenz[*a*]anthracene, based on two independent lines of evidence (Scheme II). First, the enantiomers of **2a** were assigned through the use of an exciton chirality experiment.[16] For this purpose the enantiomer (−)-**2a**, which does not lead to the metabolite, was used. The exciton chirality CD interaction bands between the two benzoyloxy groups of a dibenzoate of (1*S*,2*R*)-**2a** would show a positive, long wavelength transition, based on inspection of molecular models in which the substituent at carbon-1 is pseudoaxial due to steric hindrance in the bay region. However, some ambiguity exists due to possible further interaction of these benzoyloxy groups with the anthracene chromophore. CD interaction between either of the two benzoyloxy groups with the anthracene chromophore should lead to a negative, long wavelength Cotton effect. Thus, an unequivocal conclusion regarding absolute configuration can be reached through examination of the CD spectrum of a monobenzoate. The mono-*p*-chlorobenzoates of (−)-**2a** were prepared. The major monoester (Scheme II, **10a**) resulted from selective reaction



10a

at the pseudoequatorial 2-hydroxyl group as evidenced by the large downfield shift of the NMR signal for 2-H in **10a** relative to the tetrahydrodiol (−)-**2a**. The CD spectrum of **10a** (Figure 2) shows two strong symmetric transitions at 256 nm ($\Delta\epsilon$ −78.3) and at 238 nm ($\Delta\epsilon$ +41.2) and passes through zero at 246 nm. Both the magnitude of the CD bands and their near symmetry are indicative that the exciton chirality rule may be applied with the conclusion that (−)-**2a** has 1*S*,2*R* absolute configuration due to the negative, long wavelength transition. The second method for assigning the enantiomers of **2a** consisted of an empirical correlation of the NMR characteristics of (−)-(menthyloxy)acetic acid esters (MOA) of trans diols[17] and bromohydrins[18] on saturated benzo rings of polycyclic aromatic hydrocarbons. Thus, the degree of magnetic nonequivalence between H_A and H_B in the $-OCH_AH_BCO_2-$ portion of (menthyloxy)acetates is greater for the esters of alcohols with *S* rather than *R* absolute configuration. Additionally, the *R*,*R* isomers are less polar on silica gel chromatography and have more negative values of $[\alpha]_D$. The NMR method of assignment of the bromohydrin MOA ester (−)-**6a** (Scheme II) as 1*R*,2*R* allows assignment of the epoxide (+)-**8a** as 1*R*,2*S* (see Table II). This epoxide is known to undergo acid-catalyzed hydrolysis to the *cis*-**2a** and *trans*-**9a** tetrahydrodiols by attack of water at the benzylic position.[19] The assignment of 1*R*,2*S* absolute configuration to the cis diol (+)-**2a** is consistent with the CD experiment described above. Additionally, the assignment of 1*S*,2*S* absolute configuration to the trans diol (+)-**9a** is consistent with the previous NMR assignment[20] of (+)-(1*S*,2*S*)-**12a**, the diMOA ester derived from the (+)-*trans*-(1*S*,2*S*)-1,2-dihydrodiol.

Assignment of the metabolite **1b** as (+)-*cis*-(8*R*,9*S*)-8,9-dihydroxy-8,9-dihydrobenz[*a*]anthracene is based on the configuration of the bromohydrin ester (+)-(8*S*,9*S*)-**6b** (Scheme III), which has been determined by X-ray crystallography.[21] For the present study, the less polar diastereomer of this ester, i.e., (−)-(8*R*,9*R*)-**6b** was treated with

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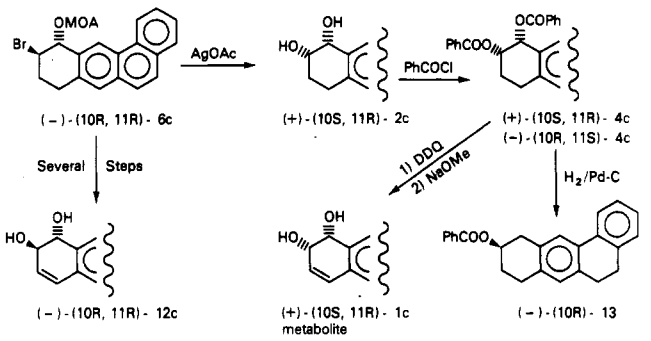
Table II. Physical Properties and Configurations of Bromohydrin MOA Esters 6a–c

compd	polarity[a]	$[\alpha]_D$ ($CHCl_3$)	NMR,[b] δ	configuration	ref
6a	less polar	−160°	3.91 (2 H, s, H_A and H_B)	1*R*,2*R*	this study
6a	more polar	+76°	3.94 (1 H, d, J = 16 Hz, H_A) 3.72 (1 H, d, J = 16 Hz, H_B)	1*S*,2*S*	
6b	less polar	−134°	4.05 (2 H, s, H_A and H_B)	8*R*,9*R*	21
6b	more polar	+44°	4.05 (1 H, d, J = 15 Hz, H_A) 3.93 (1 H, d, J = 15 Hz, H_B)	8*S*,9*S*	
6c	less polar	−74°	3.96 (2 H, s, H_AH_B)	10*R*,11*R*	25
6c	more polar	−9°	3.88 (1 H, d, J = 15 Hz, H_A) 4.03 (1 H, d, J = 16 Hz, H_B)	10*S*,11*S*	

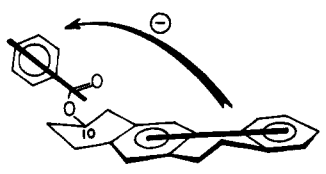
[a] The pairs of diastereomers were separated by HPLC on Du Pont Zorbax SIL columns eluted with 5% ether in cyclohexane. Values of α ranged from 1.17 to 1.20. [b] Chemical shift positions for the exocyclic methylene protons H_AH_B $-OCH_AH_BCO_2-$ at 220 MHz in deuteriobenzene.

Scheme IV



silver acetate in acetic acid to form the *cis*-tetrahydrodiol (−)-(8*R*,9*S*)-**2b**. The stereochemistry for such transformations is known to proceed without inversion at the benzylic center[22] as shown in Scheme III. Thus (+)-(8*S*,9*S*)-**6b** is stereochemically correlated with the *cis*-tetrahydrodiol (+)-(8*S*,9*R*)-**2b** which was utilized in the synthesis of (−)-(8*S*,9*R*)-**1b**, the enantiomer of the metabolite (Figure 2). It is noteworthy to point out that the NMR correlation technique (degree of magnetic nonequivalence for $-OCH_2CO_2-$ in MOA esters) on (+)-(8*S*,9*S*)-**6b** (see Table II) and on (+)-*trans*-(7*S*,8*S*)-7-[(menthyloxy)acetoxy]-8-bromo-7,8,9,10-tetrahydrobenzo[*a*]pyrene is supported by X-ray crystallographic studies in both of these cases.[21,23] In addition, (+)-**6b** has been chemically correlated to the mammalian metabolite (−)-*trans*-(8*R*,9*R*)-8,9-dihydroxy-8,9-dihydrobenz[*a*]-anthracene[21,24] ((−)-**12b**).

The metabolite (+)-*cis*-(10*S*,11*R*)-10,11-dihydroxy-10,11-dihydrobenz[*a*]anthracene (**1c**, Figure 2) has been synthesized from the trans bromohydrin ester (−)-(10*R*,11*R*)-**6c** (Scheme IV). Assignment of (−)-(10*R*,11*R*)-**6c** rests firstly on the NMR method[18] (see Table II) and secondly on an exciton chirality experiment[16] on (−)-(10*R*)-(benzoyloxy)-5,6,8,9,10,11-hexahydrobenz[*a*]-anthracene (**13**). This compound was obtained from the



13

(−)-(10*R*,11*S*)-tetrahydrodibenzoate **4c** (Scheme IV), by hydrogenolysis. The CD spectrum of (−)-**13** showed a pair of symmetric but opposite bands centered at 229 nm. The fact that the long wavelength band was negative requires 10*R* absolute configuration. Additionally, (−)-**6c** has been chemically related[25] to the mammalian metabolite (−)-*trans*-(10*R*,11*R*)-10,11-dihydroxy-10,11-dihydrobenz[*a*]-anthracene (**12c**) which was independently assigned by chiroptical methods.[24]

Conclusions

The present study identifies *cis*-1,2-, *cis*-5,6-, *cis*-8,9-, and *cis*-10,11-dihydrodiols as metabolites of benz[*a*]anthracene produced by the bacterium *Beijerinckia* B8/36. For all but the 5,6-dihydrodiol which was formed in trace quantities and is of unknown configuration, the dihydrodiols have *R* absolute configuration at their benzylic hydroxyl groups. Thus, for the three prominent metabolites, the bacterial dioxygenase(s) attacks different stereoheterotopic faces of the benz[*a*]anthracene hydrocarbon as is the case for a mammalian monoxygenase, cytochrome P450c.[26] The identified bacterial *cis*-dihydrodiols from naphthalene,[7b] anthracene,[6] and phenanthrene[6] also have *R* absolute configuration at their benzylic hydroxyl groups. Further studies of the present type are hoped to provide a basis for steric modeling of the catalytic binding site of the bacterial enzyme.

Experimental Section

Bacterial Metabolites. An ethyl acetate extract obtained from the incubation of 312 mg of benz[*a*]anthracene with *Beijerinckia* B8/36 was chromatographed on deactivated silica gel as described to provide 120 mg of crude dihydrodiol metabolites.[8] This unstable yellow oil slowly generates hydroxybenz[*a*]anthracenes even when stored below 0 °C. The oil was further purified by preparative TLC on silica gel plates eluted with chloroform:acetone (80:20); 70 mg were isolated from two close bands at R_f ~0.45. Preparative HPLC (cf. Figure 1) provided 35 mg of **1a**, 4 mg of **1b**, and 3 mg of **1c** after repeated rechromatography to free the samples from cross-contamination. Partially purified samples of **1a** were found to contain a very small amount of an earlier eluting component which had a retention time and UV spectrum identical with *cis*- (cf. ref 27) but not *trans*-5,6-dihydrodiol[11] of benz[*a*]anthracene. The three isolated dihydrodiols all had mass spectra (CI, NO–N_2) with M^+, m/z 262 (100%), $M^+ - 17$, m/z 245 (20–80%), and $M^+ - 18$, m/z 244 (20–80%).

The shapes of the UV spectra (75% methanol in water) of **1a** [263 (31 000), 297 (22 800), 364 (2080), 383 (3300), 406 nm (2440)],

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1b [266 (32 500), 306 (11 200), 320 nm (12 600)], and **1c** [275 (37 000), 310 nm (7900)] were practically identical with those reported for benz[*a*]anthracene *trans*-1,2-, *trans*-8,9-, and *trans*-10,11-dihydrodiols,[11] respectively. Additional evidence for the position of the hydroxyl groups in each isomer was obtained upon examination of their acid-catalyzed dehydration (5 N HCl for 15 min at 90 °C) to a pair of hydroxybenz[*a*]anthracenes which were separated by reverse-phase HPLC as described.[28] Based on absorbance of the column effluent at 256 nm and on retention times, 2-, 8-, and 11-hydroxybenz[*a*]anthracene (80%, 63%, and 83%, respectively) were the predominant isomers of the two possible phenols formed from **1a**, **1b**, and **1c**, respectively. The NMR spectra of the three *cis*-dihydrodiols are detailed in Table I. Particularly diagnostic features of these spectra consist of (i) the values of J_{diol} = 4.7–5.4 Hz expected for cis isomers,[7b,12] (ii) the chemical shifts of H_7 and H_{12} are appropriately at lower field due to their being meso-anthracenic,[29] bay region,[29] and/or proximate to a hydroxyl group,[13] and (iii) the pair of cis vinyl hydrogens (J = 10 Hz) in each isomer which indicates the dihydrodiols are on benzo rings. CD spectra of the metabolites (ethanol, THF) are shown in Figure 2.

Synthesis of Racemic *cis*-Benz[*a*]anthracene Tetrahydrodiols 2a–c. A solution of osmium tetroxide (3.9 mmol) dissolved in pyridine (2 mL) was added to a solution of the dihydrobenz[*a*]anthracene[11] (3.9 mmol) in pyridine (15 mL) at 0 °C. The solution was stirred under nitrogen (30 min) at ambient temperature before addition of sodium bisulfite (2.0 g in 30 mL water) and methanol (60 mL) and was stirred for an additional 48 h. Addition of water (250 mL) and filtration yielded the *cis*-tetrahydrodiol product which was purified by recrystallization. Yields of **2a–c** were in the range of 53–85%. Spectral data were identical with the tetrahydrodiol enantiomers (see below). Further characterization was effected via the formation of the diesters **3a–c**.

Synthesis and Separation of the Bis(α-methoxy-α-(trifluoromethyl)phenylacetyl) (MTPA) Esters 3a–c of the *cis*-Benz[*a*]anthracene Tetrahydrodiols. (−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride[30] ((−)-MTPA-Cl, 2 mmol) was added slowly to the racemic tetrahydrodiol **2a**, **2b**, or **2c** (0.38 mmol) dissolved in pyridine (10 mL, 0 °C). The solution was stirred initially at 0 °C (2 h) and finally at ambient temperature (10 h). The bis(MTPA) ester product (**3a**, **3b** or **3c**) was treated with a saturated solution of $NaHCO_3$ (20 mL) and extracted with dichloromethane (3 × 40 mL). The organic layer was washed with $NaHCO_3$, dilute HCl, and water before being dried ($MgSO_4$) and concentrated.

(+)-(1*R*,2*S*)-3a and (−)-(1*S*,2*R*)-3a. The diastereoisomeric mixture **3a** was separated by HPLC (α = 1.50) using a Du Pont Zorbax SIL Column (9.45 × 250 mm) eluted with ether:cyclohexane (1:9). Microanalysis of the diastereoisomer mixture **3a**. Anal. Calcd for $C_{35}H_{25}O_6F_6$: C, 65.5; H, 4.3; F, 16.4. Found: C, 65.4; H, 4.3; F, 16.4.

(+)-(1*R*,2*S*)-**3a**: less polar (k' = 1.6); yield 38%; mp 161–162 °C; $[\alpha]_D$ +103° ($CHCl_3$); NMR δ (220 MHz, $CDCl_3$) 1.80–2.20 (2 H, m, 3-H), 2.95–3.20 (2 H, m, 4-H), 3.12 (3 H, s, OMe), 3.68 (3 H, s, OMe), 5.46 (1 H, m, $J_{1,2} = J_{2,3}$ = 2 Hz, $J_{2,3}$ = 12 Hz, 2-H), 6.40–7.84 (17 H, m, aryl H and 1-H), 8.16 (1 H, s, 7-H), 8.26 (1 H, s, 12-H).

(−)-(1*S*,2*R*)-**3a**: more polar (k' = 2.4); yield 40%; mp 156–158 °C; $[\alpha]_D$ −117° ($CHCl_3$); NMR δ (220 MHz, $CDCl_3$), 2.00–2.50 (2 H, m, 3-H), 3.04–3.28 (2 H, m, 4-H), 3.35 (3 H, s, OMe), 3.52 (3 H, s, OMe), 5.60 (1 H, m, $J_{1,2} = J_{2,3}$ = 2 Hz, $J_{2,3}$ = 12 Hz, 2-H), 6.66–8.10 (17 H, m, aryl H and 1-H), 8.24 (1 H, s, 7-H), 8.50 (1 H, s, 12-H).

(−)-(8*S*,9*R*)-3b and (−)-(8*R*,9*S*)-3b. The mixture of diastereoisomers **3b** was separated (α = 1.42) by using a Waters Prep LC system 500 and silica gel cartridge eluted with hexane:dichloromethane (2:1). Microanalytical data was obtained on the mixture of diastereoisomers **3b**. Anal. Calcd for $C_{35}H_{25}O_6F_6$: C, 65.5; H, 4.3. Found: C, 65.7; H, 4.3.

(−)-(8*S*,9*R*)-**3b**: less polar (k' = 6.2); yield 42%; $[\alpha]_D$ −24° ($CHCl_3$); NMR δ (100 MHz, $CDCl_3$), 2.00–2.42 (2 H, m, 10-H), 3.08–3.30 (2 H, m, 11-H), 3.25 (3 H, s, OMe), 3.37 (3 H, s, OMe), 5.48–5.70 (1 H, sextet, $J_{9,10}$ = 3.5 and 10 Hz, $J_{8,9}$ = 3 Hz, 9-H), 6.44 (1 H, d, $J_{8,9}$ = 3 Hz, 8-H), 6.86–7.84 (16 H, m, aryl H), 8.22 (1 H, s, 12-H), 8.44 (1 H, s, 1-H).

(−)-(8*R*,9*S*)-**3b**: more polar (k' = 8.3); yield 38%; $[\alpha]_D$ −112° ($CHCl_3$); NMR δ (100 MHz, $CDCl_3$) 2.02–2.26 (2 H, m, 10-H), 3.08–3.30 (2 H, m, 11-H), 3.26 (3 H, s, OMe), 3.38 (3 H, s, OMe), 5.42–5.72 (1 H, m, $J_{8,9}$ = 3 Hz, $J_{9,10}$ = 3.5 and 10 Hz, 9-H), 6.56 (1 H, d, $J_{8,9}$ = 3 Hz, 8-H), 6.84–7.85 (16 H, m, aryl H), 8.30 (1 H, s, 12-H), 8.46 (1 H, m, 1-H).

(+)-(10*S*,11*R*)-3c and (−)-(10*R*,11*S*)-3c. The diastereoisomeric mixture **3c** was separated by HPLC (α = 1.12) using a Spectra Physics (Model 3500B) instrument with a Whatman Magnum 9, Partisil 10 (9 × 250 mm) semipreparative column and cyclohexane:ether (96:4) as solvent. The purified mixture was characterized by HRMS, m/z 696.19303 ($C_{35}H_{25}O_6F_6$ requires 696.19463).

(+)-(10*S*,11*R*)-**3c**: less polar (k' = 6.1); yield 43%; mp 115–117 °C; $[\alpha]_D$ +95° ($CHCl_3$); NMR δ (250 MHz, $CDCl_3$), 2.22 (2 H, m, 9-H), 3.18 (2 H, m, 8-H), 3.33 (3 H, s, OMe), 3.55 (3 H, s, OMe), 5.69 (1 H, m, 10-H), 6.76 (1 H, d, $J_{10,11}$ = 3 Hz, 11-H), 6.98–7.90 (16 H, m, aryl H), 8.52 (1 H, m, 1-H), 8.66 (1 H, s, 12-H).

(−)-(10*R*,11*S*)-**3c**: more polar (k' = 6.8); yield 47%; mp 108–110 °C; $[\alpha]_D$ −160° ($CHCl_3$); NMR δ (250 MHz, $CDCl_3$), 2.25–2.42 (2 H, m, 9-H), 3.17 (2 H, m, 8-H), 3.28 (3 H, s, OMe), 3.52 (3 H, s, OMe), 5.67 (1 H, m, 10-H), 6.62 (1 H, d, $J_{10,11}$ = 3.6 Hz, 11-H), 7.04–7.89 (16 H, m, aryl H), 8.52 (1 H, m, 1-H), 8.63 (1 H, s, 12-H).

Hydrolysis of the Tetrahydro Bis(MPTA) Esters 3a–c To Yield the (+)- or (−)-*cis*-Benz[*a*]anthracene Tetrahydrodiols 2a–c. The bis(MTPA) ester **3a–c** (0.13 mmol) dissolved in THF (5 mL) was treated with a methanolic solution of 1 M NaOH (2 mL, 1:1) and stirred at ambient temperature (24 h). A saturated solution of NH_4Cl (3 mL) was added, and the sample was concentrated in vacuo. Water was added and the product was collected and recrystallized to yield the *cis*-tetrahydrodiol product **2a–c**.

(+)-*cis*-(1*R*,2*S*)-**2a**: yield 71%; mp 219–221 °C; $[\alpha]_D$ +18° (THF) from the less polar diastereoisomer.

(−)-*cis*-(1*S*,2*R*)-**2a**: yield 86%; mp 219–220 °C; $[\alpha]_D$ −17° (THF) from the more polar diastereoisomer; HRMS, m/z 264.11513 ($C_{18}H_{16}O_2$ requires 264.11502); NMR δ (100 MHz, THF-d_5/D_2O) 1.73–2.37 (2 H, m, 3-H), 2.85–3.09 (2 H, m, 4-H), 3.87 (1 H, sextet, $J_{1,2}$ = 4 Hz, $J_{2,3}$ = 4 Hz, $J_{2,3}$ = 13 Hz, 2-H), 5.35 (1 H, d, J = 4 Hz, 1-H), 7.09–8.13 (6 H, m, aryl H), 8.35 (1 H, s, 7-H), 8.87 (1 H, s, 12-H).

(+)-*cis*-(8*S*,9*R*)-**2b**: yield 97%; mp 186–188 °C; $[\alpha]_D$ +19° (THF) from the less polar diastereoisomer.

(−)-*cis*-(8*R*,9*S*)-**2b**: yield 96%; mp 186–188 °C; $[\alpha]_D$ −17° (THF) from the more polar diastereoisomer; HRMS, m/z 264.11513 ($C_{18}H_{16}O_2$ requires 264.11503); NMR δ (90 MHz, $CDCl_3$) 2.16 (2 H, m, 10-H), 3.09–3.29 (2 H, m, 11-H), 4.20 (1 H, m, 9-H), 4.95 (1 H, m, 8-H), 7.56–8.01 (6 H, m, aryl H), 8.45 (1 H, s, 12-H), 8.65 (1 H, m, 1-H).

(+)-*cis*-(10*S*,11*R*)-**2c**: yield 80%; mp 208–212 °C; $[\alpha]_D$ +146° (THF) from the less polar diastereoisomer; HRMS, m/z 264.11513 ($C_{18}H_{16}O_2$ requires 264.11503); NMR δ (90 MHz, $CDCl_3$) 2.00–2.30 (2 H, m, 9-H), 3.18–3.24 (2 H, m, 8-H), 4.20 (1 H, m, 10-H), 5.00 (1 H, m, 11-H), 7.55–7.89 (6 H, m, aryl H), 8.71 (1 H, m, 1-H), 8.80 (1 H, s, 12-H).

Synthesis of the (+)- or (−)-*cis*-Tetrahydrodiol Dibenzoate Esters 4a–c. Benzoyl chloride (0.7 mL) was added dropwise to a stirred solution of *cis*-tetrahydrodiol **3a–c** (0.38 mmol) in pyridine (2 mL) at 0 °C. The mixture was stirred under nitrogen at ambient temperature (6 h). Water was added (10 mL) and the *cis*-dibenzoate ester was extracted into dichloromethane (25 mL), which was washed with saturated $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated in vacuo. The *cis*-dibenzoates were purified either by TLC or recrystallization. In all cases the (+)-*cis*-tetrahydrodiol resulted in the formation of the (+)-*cis*-tetrahydrodiol dibenzoate.

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(29) Bartle, K. D.; Jones, D. W. *Adv. Org. Chem.* **1972**, *8*, 317–424.
(30) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549.

(+)-*cis*-(1*R*,2*S*)-**4a**: yield 99%; $[\alpha]_D$ +3° ($CHCl_3$); NMR δ (100 MHz, $CDCl_3$) 2.10–2.50 (2 H, m, 10-H), 3.00–3.30 (2 H, m 4-H), 5.44–5.70 (1 H, sextet, $J_{2,3}$ = 12 Hz, $J_{1,2}$ = $J_{2,3}$ = 3 Hz, 2-H), 7.08–8.16 (17 H, m, aryl H and 1-H), 8.36 (1 H, s, 7-H), 8.60 (1 H, s, 12-H).

(+)-*cis*-(8*S*,9*R*)-**4b**: yield 90%; mp 165 °C; $[\alpha]_D$ +105° ($CHCl_3$); HRMS, *m/z* 472.1681 ($C_{32}H_{24}O_4$ requires 472.16745); NMR δ (100 MHz, $CDCl_3$) 2.20–2.84 (2 H, m, 10 H), 3.26–3.50 (2 H, m, 11 H), 5.62–5.86 (1 H, sextet, $J_{9,10}$ = 9 Hz, $J_{8,9}$ = $J_{9,10}$ = 3 Hz, 9-H) 6.66 (1 H, d, $J_{8,9}$ = 3 Hz, 8-H), 7.20–8.24 (16 H, m, aryl H), 8.46 (1 H, s, 12-H), 8.62 (1 H, m, 1-H).

(+)-*cis*-(10*S*,11*R*)-**4c**: yield 84%; mp, 126–128 °C; $[\alpha]_D$ +264° ($CDCl_3$); HRMS, *m/z* 472.16810 ($C_{32}H_{24}O_4$ requires 472.16745); NMR δ (100 MHz, $CDCl_3$) 2.37–2.64 (2 H, m, 9-H), 3.16–3.44 (2 H, m, 8-H), 5.52–5.76 (2 H, sextet, $J_{9,10}$ = 10.7 Hz, $J_{9,10}$ = $J_{10,11}$ = 3.3 Hz, 10-H), 6.74 (1 H, d, $J_{10,11}$ = 3.3 Hz, 11-H), 7.08–8.10 (16 H, m, aryl H), 8.46 (1 H, m, 1-H), 8.62 (1 H, s, 12-H).

Synthesis of Optically Active *cis*-Dihydrodiol Dibenzoates 5a–c. *cis*-(1*R*,2*S*)-5a. A mixture of (+)-(1*R*,2*S*)-*cis*-tetrahydrodiol dibenzoate **4a** (0.067 mmol), *N*-bromosuccinimide (0.074 mmol), and azobis(isobutyronitrile) (14 mg) was dissolved in CCl_4 and irradiated with a heat lamp. The temperature of the solution was maintained at ~65 °C for 45 min. The solution was cooled, filtered, and concentrated under vacuum to yield a yellow powder. This product, without further purification, was suspended in xylene (12 mL), finely powdered anhydrous $NaHCO_3$ (300 mg) was added, and the solution was refluxed for 0.5 h. Filtration and removal of the xylene under vacuum yielded an unstable yellow oil. Purification by preparative TLC gave **5a** (20% yield) which was identified on the basis of its NMR spectrum. As it was unstable, it was directly hydrolyzed to the dihydrodiol **1a**.

cis-(1*R*,2*S*)-**5a**: NMR δ (100 MHz, $CDCl_3$) 6.10–6.20 (1 H, dd, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 4 Hz, 2-H), 6.70–6.80 (1 H, dd, $J_{3,4}$ = 10 Hz, $J_{2,3}$ = 4 Hz, 3-H), 7.10–8.42 (18 H, m, aryl H and 1-H and 4-H), 8.30 (1 H, s, 7-H), 8.70 (1 H, s, 12-H).

(−)-*cis*-(8*S*,9*R*)-5b and (+)-*cis*-(10*S*,11*R*)-5c. A solution of *cis*-tetrahydrodibenzoate **4b** or **4c** (0.25 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.41 mmol) was refluxed in purified dioxane under nitrogen (48 h). The product mixture was cooled and purified by column chromatography (Al_2O_3) using benzene as solvent.

(−)-*cis*-(8*S*,9*R*)-**5b**: yield 72%; $[\alpha]_D$ −143° ($CHCl_3$); NMR δ (100 MHz, $CDCl_3$) 6.00–6.12 (1 H, dd, $J_{8,9}$ = 4 Hz, $J_{9,10}$ = 4 Hz, 9-H), 6.26 (1 H, dd, $J_{9,10}$ = 4 Hz, $J_{10,11}$ = 10 Hz, 10-H), 6.64 (1 H, d, $J_{8,9}$ = 4 Hz, 8-H), 6.98 (1 H, d, $J_{10,11}$ = 10 Hz, 11-H), 7.16–8.10 (16 H, m, aryl H), 8.42 (1 H, s, 12-H), 8.60 (1 H, m, 1-H).

(+)-*cis*-(10*S*,11*R*)-**5c**: yield 56%; mp 164–166 °C; $[\alpha]_D$ +647° ($CHCl_3$); HRMS, *m/z* 470.15213 ($C_{32}H_{22}O_4$ requires 470.15180); NMR δ (100 MHz, $CDCl_3$) 6.14 (1 H, dd, $J_{9,10}$ = 4 Hz, $J_{10,11}$ = 4 Hz, 10-H), 6.28 (1 H, dd, $J_{8,9}$ = 10 Hz, $J_{9,10}$ = 4 Hz, 9-H), 6.74 (1 H, d, $J_{10,11}$ = 4 Hz, 11-H), 6.96 (1 H, d, $J_{8,9}$ = 10 Hz, 8-H), 7.20–8.20 (16 H, m, aryl H), 8.68 (1 H, m, 1-H), 8.80 (1 H, s, 12-H).

Hydrolysis of the Dihydrodiol Dibenzoate Esters 5a–c To Yield (+)- or (−)-*cis*-Benz[*a*]anthracene Dihydrodiols 1a–c. A solution of sodium methoxide (0.11 mmol) in methanol (3 mL) was added to the dihydrodiol dibenzoate **5a**, **5b**, or **5c** in methanol:THF (3:5, 8 mL) at 0 °C. The reaction mixture was stirred at this temperature for 5 h before addition of saturated NH_4Cl (6 mL). Dilution with water and filtration yielded the dihydrodiol. All products showed spectral characteristics consistent with the corresponding metabolites (Figure 2 and Table I).

(+)-*cis*-(1*R*,2*S*)-**1a**: yield 2.5 mg (75%); $[\alpha]_D$ +81° (THF).

(−)-*cis*-(8*S*,9*R*)-**1b**: yield 11.2 mg (79%); $[\alpha]_D$ −37.2° (THF); enantiomeric to the metabolite.

(+)-*cis*-(10*S*,11*R*)-**1c**: yield 9 mg (54%); mp 135–137 °C; $[\alpha]_D$ +361° (THF).

Configurational Assignment of (+)- or (−)-*cis*-2a–c and *trans*-9a Tetrahydrobenz[*a*]anthracene Diols. The tetrahydrobenz[*a*]anthracene diols (**2a–c** and **9a**) were obtained in a high state of optical purity (>98%) via the corresponding resolved *trans*-[(menthyloxy)acetoxy]bromotetrahydrobenz[*a*]anthracene diastereoisomers **6a–c** whose absolute configurations have been deduced directly from NMR and X-ray studies (see Discussion) or from stereochemical correlation with chiral derivatives of known absolute configuration (Table II). While the diastereomeric bromohydrin MOA esters **6b** and **6c** have previously been separated and configurationally assigned,[21,25] the diastereomers of **6a** have been prepared in the usual[21] way from 3,4-dihydrobenz[*a*]anthracene and were separated by HPLC (Zorbax SIL, cyclohexane:ether (95:5), α = 1.20) in the present study to provide the less polar ((−)-**6a**, *k'* = 1.25, mp 112–114 °C, $[\alpha]_D$ −160° ($CDCl_3$)) and more polar ((+)-**6a**, *k'* = 1.5, mp 131–133 °C, $[\alpha]_D$ +76° ($CDCl_3$)) isomers. See Table II for NMR details.

Synthesis of (−)-*trans*-(1*R*,2*R*)-1-Hydroxy-2-bromo-1,2,3,4-tetrahydrobenz[*a*]anthracene (7a). A solution of (−)-**6a** (450 mg) in THF (5 mL) was treated with diborane (20 mL of 1 M solution in THF) at room temperature for 25 h. After addition of methanol (10 mL) and water (1 mL), the solvent was removed under vacuum and the residue was taken up in benzene. Purification through a Waters silica SEP-PAK with benzene as eluant gave bromohydrin (−)-**7a**: yield 355 mg (82%); $[\alpha]_D$ −128° ($CHCl_3$); NMR δ (100 MHz, $CDCl_3$) 2.10–3.46 (4 H, m, 3-H and 4-H), 4.68 (1 H, m, 2-H), 5.64 (1 H, m, 1-H), 7.10–8.10 (6 H, m, aryl H), 8.30 (1 H, s, 7-H), 8.62 (1 H, s, 12-H). The enantiomer (+)-**7a**, prepared similarly, had $[\alpha]_D$ +108° ($CHCl_3$).

Synthesis of (+)-(1*R*,2*S*)-1,2-Epoxy-1,2,3,4-tetrahydrobenz[*a*]anthracene (8a). A solution of (−)-(1*R*,2*R*)-**7a** (74 mg) in dry THF (10 mL) was stirred with Amberlite (IRA 400, OH^- form) ion exchange resin under an atmosphere of argon at ambient temperature (6 h). The resin was filtered off and the solvent was removed in vacuo to yield (+)-(1*R*,2*S*)-tetrahydro epoxide **8a** (35.6 mg, 64%): $[\alpha]_D$ +177° ($CHCl_3$); NMR δ (100 MHz, $CDCl_3$) 1.70–2.12 (2 H, m, 3-H), 3.68–3.10 (2 H, m, 4-H), 3.86–4.00 (1 H, m, 2-H), 4.86 (1 H, d, $J_{1,2}$ = 4.5 Hz, 1-H), 7.32–8.08 (6 H, m, aryl H), 8.40 (1 H, s, 7-H), 8.80 (1 H, s, 12-H). The enantiomer (−)-**8a**, prepared similarly, had $[\alpha]_D$ −171° ($CHCl_3$).

Solvolysis of (+)-(1*R*,2*S*)-8a To Yield (+)-*cis*-(1*R*,2*S*)-1,2-Dihydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene (2a) and (+)-(1*S*,2*S*)-*trans*-1,2-Dihydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene (9a). The (+)-(1*R*,2*S*)-enantiomer of epoxide **8a** (54 mg) dissolved in dioxane (70 mL) was slowly added to a mixture of dioxane (125 mL), water (375 mL), and acetic acid (0.25 mL). After stirring for 2 h at room temperature, the solution was extracted with 3 × 200 mL of ethyl acetate, and the organic phase was dried (Na_2SO_4) and concentrated to a solid residue. The resulting mixture (ca. 1:1) of *cis*-**2a** and *trans*-1,2-diols **9a** was separated on a Waters Associates HPLC instrument equipped with a Du Pont Zorbax ODS column (21.2 × 250 mm) which was eluted with methanol; k'_{trans} = 0.56 and k'_{cis} = 0.78 with α = 1.39. The resulting (+)-*cis*-(1*R*,2*S*)-1,2-dihydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene (**2a**) (27 mg, 47% yield) had $[\alpha]_D$ +19 °C (THF) and mp 219–221 °C; HRMS, *m/z* 264.11513 ($C_{18}H_{16}O_2$ requires 264.11513). The (+)-*trans*-(1*S*,2*S*) isomer ($[\alpha]_D$ +9.3° (THF)), (+)-**9a**, as its diMOA ester **11a** was found to be chromatographically identical with *trans*-(1*S*,2*S*)-1,2-[(dimenthyloxy)acetyl]-1,2,3,4-tetrahydrobenz[*a*]anthracene obtained by reduction (H_2 with 10% Pd on carbon in ethyl acetate for 2 h, progress of reduction monitored by successive UV spectra) of (+)-*trans*-(1*S*,2*S*) 1,2-[(dimenthyloxy)acetyl]-1,2-dihydrobenz[*a*]anthracene (**12a**). The sample of (+)-**12a** was obtained by resolution of racemic *trans*-dihydrodiol in an earlier study.[20] As was the case for the diesters of the *trans*-dihydrodiols[20] **12a**, the diester of the *trans*-(1*S*,2*S*)-tetrahydrodiol **11a** is more polar (*k'* = 3.3) and elutes after the (1*R*,2*R*)-diester (*k'* = 2.5) on HPLC (9.45 × 250 mm Du Pont Zorbax SIL column eluted with 10% ether in cyclohexane, α = 1.33) and the methylene hydrogens of the $-OCOCH_2O-$ groups show the most magnetic nonequivalence in their NMR spectra in deuteriobenzene for the more polar (1*S*,2*S*)-diastereomer.

Synthesis of *cis*-(1*S*)-Hydroxy-(2*R*)-[(*p*-chlorobenzoyl)oxy]-1,2,3,4-tetrahydrobenz[*a*]anthracene (10a). The resolved (−)-*cis*-(1*S*,2*R*)-tetrahydrodiol **2a** (18.0 mg), of opposite absolute configuration to that used for the synthesis of the metabolically formed *cis*-dihydrodiol **1a**, was dissolved in anhydrous pyridine (2 mL) and 12 mg of *p*-chlorobenzoyl chloride was added slowly. The mixture was stirred under nitrogen at ambient temperature for 3 h. Ethyl acetate (25 mL) was added, and the organic phase was washed with 1 M HCl (3 × 10 mL) and Na_2CO_3 solution and dried (Na_2SO_4). Evaporation of the solvent yielded the crude product as an oil which was separated by preparative HPLC (Du Pont Zorbax SIL column, 9.45 × 250

mm, ethyl acetate:hexane (1:1), $\alpha = 5$) into major and minor *p*-chlorobenzoate positional isomers as oils.

cis-(1*S*)-Hydroxy-(2*R*)-[(*p*-chlorobenzoyl)oxy]-1,2,3,4-tetrahydrobenz[*a*]anthracene (**10a**): $k' = 0.2$ (17 mg, 54%); NMR δ (100 MHz, $CDCl_3$) 2.10–2.70 (2 H, m, 3-H), 3.04–3.24 (2 H, m, 4-H), 5.42 (1 H, m, $J_{2,3} = 12$ Hz, $J_{1,2} = 3$ Hz, $J_{2,3} = 3$ Hz, 2 H), 5.80 (1 H, d, $J_{1,2} = 3$ Hz, 1-H), 7.12–8.18 (10 H, m, aryl H), 8.36 (1 H, s, 7-H), 8.84 (1 H, s, 12-H); mass spectrum (CI, isobutane), *m/z* M^+ 402 (31%), $M^+ + 2$ 404 (11%), $M^+ - (p\text{-}ClC_6H_5CO_2)$ 247 (100%); UV (MeOH) λ_{max} (ϵ) 247 (72 900), 256 (109 000), 358 (5730), 377 (5450). The CD spectrum (MeOH:$CHCl_3$) is shown in Figure 2.

cis-(1*S*)-[(*p*-chlorobenzoyl)oxy]-(2*R*)-hydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene (**10b**): $k' = 1$ (1.4 mg, 5%); NMR δ (100 MHz, $CDCl_3$) 2.10–2.70 (2 H, m, 3-H), 3.04–3.28 (2 H, m, 4-H), 4.10 (1 H, m, 2-H), 7.16–8.04 (11 H, m, aryl and 1-H), 8.38 (1 H, s, 7-H), 8.50 (1 H, s, 12-H); mass spectrum (CI, isobutane), *m/z* $M^+ - (p\text{-}ClC_6H_5CO_2)$ 247 (100%).

Synthesis of (−)-*cis*-(8*R*,9*S*)-8,9-Dihydroxy-8,9,10,11-tetrahydrobenz[*a*]anthracene (2b) from (−)-(8*R*,9*R*)-6b. A mixture of (−)-**6b** (100 mg) and silver acetate (100 mg) in glacial acetic acid (20 mL) containing water (0.1 mL) was refluxed for 3 h. After cooling, the reaction mixture was filtered and the residue was washed with ethyl acetate. The organic phases were combined. Removal of solvent yielded a product which was dissolved in THF (10 mL) and hydrolyzed using 1 M KOH in methanol (10 mL, 1:1) over 1 h. A saturated solution of NH_4Cl (5 mL) was added to the reaction mixture, and the organic solvent was removed under vacuum. Dilution of the product mixture with water precipitated the required product **2b**, which was filtered, washed with water, and dried.

The *cis*-tetrahydrodiol **2b** was separated from a small proportion of the trans isomer by preparative TLC (silica gel, $CHCl_3$:MeOH, 19:1, 3 elutions, upper band is **2b**). Recrystallization of the *cis*-tetrahydrodiol **2b** from MeOH yielded 15 mg (30%), mp 186–188 °C, $[\alpha]_D$ −19° (THF), with identical spectral characteristics to the enantiomer obtained from the resolved diester **3b**.

Synthesis of (+)-*cis*-(10*S*,11*R*)-10,11-Dihydroxy-8,9,10,11-tetrahydrobenz[*a*]anthracene (2c) from (−)-(10*R*,11*R*)-6c. Reaction of (−)-(10*R*,11*R*)-**6c** (100 mg) with silver acetate (100 mg) in glacial acetic acid, using similar conditions and workup to that indicated for the synthesis of (−)-**2b**, gave the *cis*-tetrahydrodiol **2c** (12 mg, 25%). Recrystallization from acetone gave crystals, mp 208–210 °C, $[\alpha]_D$ +136° (THF), which had identical spectra to the (+)-enantiomeric form of **2c** derived from (+)-**3c**.

Synthesis of (−)-(10*R*)-(Benzoyloxy)-5,6,8,9,10,11-hexahydrobenz[*a*]anthracene (13) from (−)-4c. A mixture of (−)-*cis*-(10*R*,11*S*)-**4c**, 10% Pd on charcoal (35 mg), and ethyl acetate (7 mL) was stirred under an atmosphere of hydrogen at room temperature for 96 h. The catalyst was removed by filtration and washed with acetone. The combined solvent was removed under vacuum and the residue purified by preparative TLC (silica gel, hexane:dichloromethane (1:1) to yield (−)-(10*R*)-(benzoyloxy)-5,6,8,9,10,11-hexahydrobenz[*a*]anthracene **13** (7 mg, 28%): $[\alpha]_D$ −35.8° ($CHCl_3$); UV (MeOH:$CHCl_3$) $\lambda_{max}(\epsilon)$ 226 (25 500), 268 (13 800), 305 (5100); CD (MeOH/dioxane, 9:1) $\Delta\epsilon_{237}$ −13.6, $\Delta\epsilon_{229}$ 0, $\Delta\epsilon_{221}$ +10.6.

Acknowledgment. These studies were supported in part by Grants CA-19078 from the National Cancer Institute, DHEW and F-440 from the Robert A. Welch Foundation (to DTG), and by Grant CA-25185 from the National Institutes of Health (to MK). We also wish to express our gratitude to Professor R. E. Lehr at the University of Oklahoma for performing the cited perturbational molecular orbital calculations.

Registry No. **1a**, 91423-01-7; **1b**, 91423-02-8; **1c**, 91423-03-9; (+)-(1*R*,2*S*)-**2a**, 91422-90-1; (−)-(1*S*,2*R*)-**2a**, 91423-71-1; (+)-(8*S*,9*R*)-**2b**, 91423-72-2; (−)-(8*R*,9*S*)-**2b**, 91423-73-3; (+)-(10*S*,11*R*)-**2c**, 91384-62-2; (+)-(1*R*,2*S*)-**3a**, 91365-97-8; (−)-(1*S*,2*R*)-**3a**, 91422-87-6; (−)-(8*S*,9*R*)-**3b**, 91365-98-9; (−)-(8*R*,9*S*)-**3b**, 91422-88-7; (+)-(10*S*,11*R*)-**3c**, 91365-99-0; (−)-(10*R*,11*S*)-**3c**, 91422-89-8; (+)-(1*R*,2*S*)-**4a**, 91422-91-2; (+)-(8*S*,9*R*)-**4b**, 91422-92-3; (+)-(10*S*,11*R*)-**4c**, 91422-93-4; (−)-(10*R*,11*S*)-**4c**, 91423-00-6; *cis*-(1*R*,2*S*)-**5a**, 91422-94-5; (−)-(8*S*,9*R*)-**5b**, 91422-95-6; (+)-(10*S*,11*R*)-**5c**, 91422-96-7; (−)-(1*R*,2*R*)-**6a**, 90997-20-9; (+)-(1*S*,2*S*)-**6a**, 91050-74-7; (−)-(8*R*,9*R*)-**6b**, 77550-50-6; (−)-(10*R*,11*R*)-**6c**, 79298-97-8; (−)-(1*R*,2*R*)-**7a**, 91422-97-8; (+)-(1*S*,2*S*)-**7a**, 91422-98-9; (+)-(1*R*,2*S*)-**8a**, 89618-16-6; (−)-(1*S*,2*R*)-**8a**, 89618-15-5; (+)-(1*S*,2*S*)-**9a**, 91422-99-0; (1*S*,2*R*)-**10a**, 91366-00-6; (1*S*,2*R*)-**10b**, 91366-01-7; **13**, 91365-96-7; benz[*a*]anthracene, 56-55-3; 1,2-dihydrobenz[*a*]anthracene, 60968-08-3; 8,9-dihydrobenz[*a*]anthracene, 60968-17-4; 10,11-dihydrobenz[*a*]anthracene, 34501-50-3.

Application of 1-*tert*-Butoxy-3-[(trimethylsilyl)oxy]buta-1,3-diene in the Preparation of Functionalized β-Hydroxycyclohexanone Derivatives, Including Valuable Precursors of Daunomycinone Analogues

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Received March 12, 1984

The usefulness of 1-*tert*-butoxy-3-[(trimethylsilyl)oxy]buta-1,3-diene as a valuable precursor for the synthesis of 3-hydroxycyclohexanone derivatives has been demonstrated. With a variety of electron-poor alkenes high yields of Diels–Alder adducts were obtained which were transformed into 3-*tert*-butoxycyclohexanones by mild acid hydrolysis. After protection of the keto group by acetalization with ethylene glycol the *tert*-butoxy group was converted into a hydroxy group in high yield with trifluoroacetic acid. Also with quinone dienophiles the cycloadducts were formed in a high yield. In this case the keto group, obtained by hydrolysis of the cycloadduct, was transformed by reaction with ethynylmagnesium bromide, after which the *tert*-butoxy group again was removed with trifluoroacetic acid. A short and efficient synthesis of 4-demethoxydaunomycinone along this route has been given.

Among the large variety of 1,3-dioxygenized butadienes[1] the 1-alkoxy-3[(trimethylsilyl)oxy]buta-1,3-dienes **1a** and **1b** have been extensively investigated[2,3] in the reactions with electron-poor double bond systems (**2**). Acid hy-

0022-3263/84/1949-3628$01.50/0