a mechanism involving simultaneous movement of the two hydrogens.

The possibility that the large isotope effect is primarily due to quantum mechanical tunneling appears less likely for several reasons. (1) The Arrhenius plots are not curved. (2) The preexponential factors and values of $\Delta S^{\ddagger}$ are not significantly different for proton and deuteron tautomerism. (3) If the two protons move simultaneously, then the mass for tunneling would be 2 amu (rather than 1 amu as in single proton transfers), substantially decreasing the tunneling contribution to the rate of proton tautomerism relative to the case of movement of one proton.

Thus the porphyrin proton tautomerism rate data are consistent with a mechanism involving simultaneous movement of two hydrogens via a symmetrical transition state and do not compel interpretation in terms of a tunneling process. The porphyrins are believed to be the only class of compounds in which the exactly equivalent motion of two hydrogens has been observed to give a kinetic isotope effect.

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(11) Abbreviations used throughout are: (p-CF_3TPP), tetrakis(p-trifluoromethylphenyl)porphyrin dianion; (F_5TPP), tetrakis(pentafluorophenyl)porphyrin dianion; (p-i-PrTPP), tetrakis(p-isopropylphenyl)porphyrin dianion; and (p-Et_2NTPP), tetrakis(p-diethylaminophenyl)porphyrin dianion.
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Synthesis and Reactions of the Highly Mutagenic 7,8-Diol 9,10-Epoxides of the Carcinogen Benzo[*a*]pyrene

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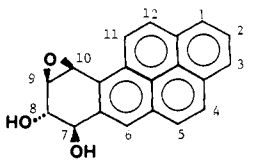
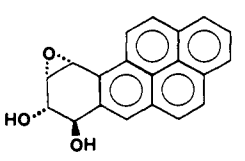
Abstract: Stereoselective syntheses are described for the preparation of the two possible diastereoisomeric epoxides at the 3,4-position of *trans*-1,2-dihydroxy-1,2-dihydronaphthalene and at the 9,10-position of *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]-pyrene. Both of the highly mutagenic diol epoxides from benzo[*a*]pyrene undergo trans addition at C-10 of the epoxide ring with methoxide, *p*-nitrothiophenolate, or aniline as nucleophiles. On solvolysis in water, the diol epoxide from benzo[*a*]pyrene, in which the benzylic hydroxy group is cis to the oxirane ring, undergoes mainly cis addition of water at C-10 to produce tetraols, while the other isomer, which cannot form an intramolecular hydrogen bond between the benzylic hydroxy group and the oxirane oxygen, suffers mainly trans hydration. The structures of the four tetraols were assigned by the NMR spectra of their acetates and by direct synthesis in some cases. The remarkable reactivity of the two diol epoxides from benzo[*a*]pyrene is emphasized by their ability to alkylate aqueous inorganic phosphate at pH 7.

In a recent preliminary report,[2] we have described the synthesis and stereochemical assignment of the two isomeric 9,10-epoxides of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene, which are known metabolites[3] of the environmental carcinogen benzo[*a*]pyrene. These two stereoisomers, (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (diol epoxide **1**) and (±)-7β,8α-dihydroxy-9α,10α-7,8,9,10-trahydrobenzo[*a*]pyrene (diol epoxide **2**), are of substantial chemical and biological interest.



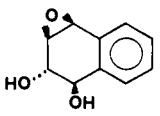
diol epoxide 1



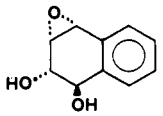
diol epoxide 2

These isomers are chemically interesting in that the benzylic hydroxy group at C-7 can intramolecularly hydrogen bond to the oxygen of the oxirane ring in diol epoxide **1**, whereas such an intramolecular hydrogen bond is not possible for diol epoxide **2**.[2,4,5] In structurally related epoxysterols[6,7] and the antileukemic agent triptolide,[8] intramolecular hydrogen bonding of this type causes as much as a 20-fold acceleration in rate for the attack of nucleophiles on the oxirane ring due to anchimeric assistance by a proximate *cis*-hydroxy group.

Comparison of the second-order rate constants for the attack of *p*-nitrothiophenolate on diol epoxides **1** and **2** in dry *tert*-butyl alcohol, a solvent in which both highly reactive epoxides are stable, established that diol epoxide **1** is >150-fold more reactive.[2,4,5] Even more impressive is the fact that diol epoxide **1** is ~500 times as reactive as 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, which lacks both of the hydroxy groups. The anchimeric assistance observed for **1** and **2** is not specific for the benzo[*a*]pyrene ring system, since the diol epoxides **3** and **4** from naphthalene show related effects.[2,4,5,9]



diol epoxide 3



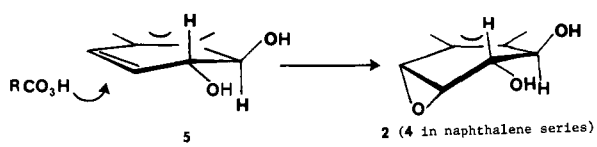
diol epoxide 4

Much of the biological interest in diol epoxides **1** and **2** stems from studies by Borgen et al.,[10] which indicated that *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (**5**) was extensively bound to DNA on metabolic activation in an in vitro system, and by the evidence of Sims et al.,[11] which indicated that a 7,8-diol-9,10-epoxide was responsible for this binding. Our initial studies[12,13] showed that the more reactive diol epoxide **1** was one of the most mutagenic compounds tested in Chinese hamster V79 cells. More recent studies have indicated that the more reactive diol epoxide **1** is a somewhat better mutagen than diol epoxide **2** toward bacterial cells, while the reverse is true for mammalian cells.[14] These comparisons are not corrected for the markedly shorter half-life of **1** in the biological media. Although other reports differ somewhat in the quantitative interpretation of the mutagenesis results in mammalian cells,[15,16] it is clear that both diol epoxide **1** and **2** must be highly mutagenic compounds.

Since both diol epoxides **1** and **2** must be considered as prime candidates for reactive ultimate carcinogens produced from benzo[*a*]pyrene, the present report describes the nature of the nucleophile adducts and solvolysis products of these compounds and presents the complete details of their synthesis. All of the compounds in the present study are racemic and are arbitrarily drawn with the benzylic hydroxy group of the dihydrodiol and subsequent products on the β-face of the molecules. The NMR spectra of the simple nucleophile adducts of diol epoxides **1** and **2** will be indispensable in the elucidation of the structures produced when these and other diol epoxides covalently bind to nucleic acids.

Results and Discussion

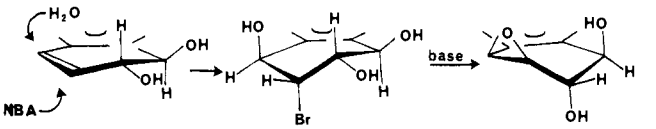
Synthesis. *trans*-Dihydrodiols at non-K-region positions of polycyclic hydrocarbons prefer the conformation in which both hydroxy groups are quasi-equatorial[17,18] when the diol does not form part of a hindered "bay region." Both the position and the conformation of these hydroxy groups are ideally suited to direct attack of peroxy acids on the face of the molecule opposite to the benzylic hydroxy group (cf. ref 2 and 4). Direct epoxidation of dihydrodiols to produce diol epoxide **2** (or **4**) proceeds without detectable formation of the isomeric diol epoxide **1** (or **3**). Although synthesis of **4** in the naphthalene series is readily achieved, the preparation of **2** from benzo[*a*]pyrene is made difficult by its high reactivity. Standard conditions for epoxidation of **5** with *m*-chloroperoxybenzoic acid



5 — 2 (4 in naphthalene series)

as well as *p*-nitroperoxybenzoic acid gave benzoate adducts as the major products (see Experimental Section). When **2** is prepared from **5** with a large excess of pure *m*-chloroperoxybenzoic acid in tetrahydrofuran, the reaction proceeds rapidly without formation of significant amounts of *m*-chlorobenzoate adducts,[19] presumably by enhancing the rate of oxidation relative to the rate of product decomposition in the slightly basic solvent. Although the dibenzoate of **5** could be epoxidized smoothly, the benzoate groups could not be removed without substantial decomposition of the epoxide.

In order to prepare the opposite stereoisomers **1** and **3**, the directive influence of the hydroxy group was again put to good advantage. Reaction of the dihydrodiols with *N*-bromoacetamide (NBA) in the presence of water stereoselectively produced bromotriols by attack of bromine at the same face of the



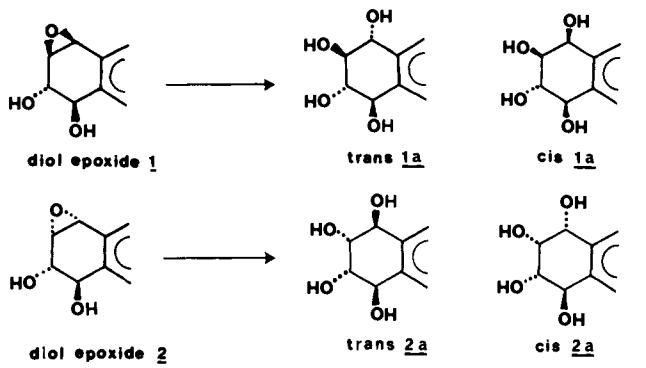
5 — 1 (3 in naphthalene series)

molecules as was attacked by peroxy acid. This reaction is of particular interest, since NBA attacks the same side of the molecule as was attacked by peroxy acid (cf. discussion in ref 2 and 4). In addition, oxidation of the benzylic 7-hydroxy group in either the starting material or the product is not a competitive process. Since the nonbenzylic hydroxy group is cis to bromine in the bromotriols, only the adjacent trans benzylic hydroxy group displaces bromide on treatment with base. Introduction of the epoxide function by this two-step procedure allowed synthesis of diol epoxide **1** and **3** without detectable amounts of the stereoisomers **2** and **4**. Since diol epoxide **1** is highly reactive and not readily purified, Amberlite resin in the hydroxide form was selected to effect the cyclization. By this approach, the resultant diol epoxide is obtained sufficiently clean so that little purification is necessary. The dibenzoate of **1** was also synthesized by this stereoselective two-step procedure.

Solvolysis. We have previously noted that the half-lifes for the mutagenic activity of diol epoxides **1** and **2** are very short. Diol epoxide **1** is extremely unstable in tissue culture medium at 37 °C and has a $t_{1/2}$ ~0.5 min. In contrast, diol epoxide **2** has a $t_{1/2}$ ~8 min for mutagenic or cytotoxic activity under these conditions.[14] The diol epoxides also rapidly decompose in phosphate-buffered saline.

In order to examine the solvolysis products of **1** and **2** in aqueous media, aqueous solutions of each of the tritiated diol epoxides, which contained 10% tetrahydrofuran and 0.09 M KCl, were maintained at constant pH's and 37 °C for 18 h. The only products detected by high-pressure liquid chromatography were tetraols (*cis*-**1a** and *trans*-**1a** from diol epoxide **1**, *cis*-**2a** and *trans*-**2a** from diol epoxide **2**), which result from the cis and trans addition of water at C-10 of the diol epoxides as shown in Scheme I. In 50% dioxane or THF: water around pH 5–11 (with or without 0.09 M KCl), diol epoxide **1** but not diol

Scheme I. Hydrolysis Products of Diol Epoxides 1 and 2.



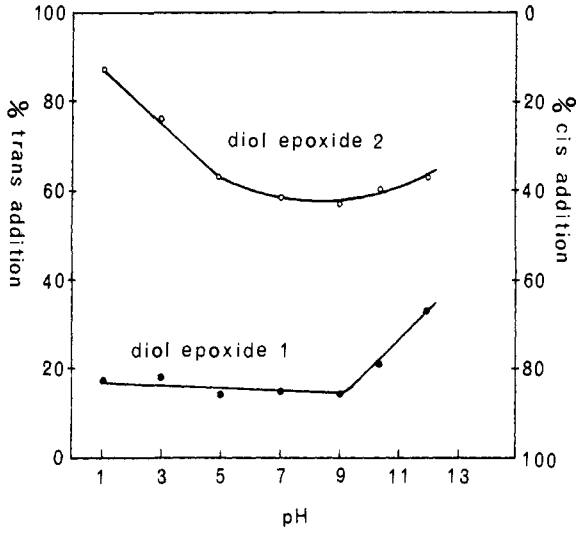


Figure 1. Percentage of cis *vs.* trans hydration of diol epoxides **1** and **2**. Solutions were prepared by addition of 50 nmol of [^{3}H] diol epoxide[3] (150 000 cpm in 1 μl of Me_2SO) to 1.8 ml of 0.1 M KCl and 0.2 ml of tetrahydrofuran. After storage for 18 h at 37 °C and at the indicated pH's, the tetraols were extracted into ethyl acetate and analyzed by HPLC on a 7.9 mm × 0.25 m Du Pont 5-μm ODS column as previously described;[3] *trans*-**2a** (22.5 min), *trans*-**1a** (24.0 min), *cis*-**2a** (26.0 min), and *cis*-**1a** (29.0 min). Experimental points represent the average of two to three determinations.

epoxide **2** underwent a significant amount of isomerization (~30%) to form a ketone, *trans*-7,8-dihydroxy-9-keto-7,8,9,10-tetrahydrobenzo[*a*]pyrene, whereas this product was not detected under highly acidic conditions.

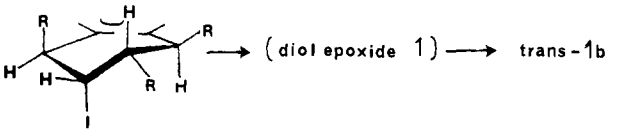
A plot of the percentage of each of these tetraols as a function of pH is shown in Figure 1. The difference in the cis/trans ratio of solvolysis products from the stereoisomeric diol epoxides is striking. Diol epoxide **1**, for which the intramolecular hydrogen bond is possible, is hydrated almost entirely (85%) by cis addition of water to form *cis*-**1a** from pH 1 to 9. Between pH 9 and 12, the amount of *trans*-**1a** increased from 15 to 33%, presumably due to nucleophilic attack by hydroxide. Battistini et al.[20] have demonstrated that a higher degree of carbocationic character is associated with the transition state, resulting in cis addition of water to 1-arylcyclohexene oxides when compared to the transition state for trans addition. A similar effect was observed for the hydration of phenanthrene 9,10-(K-region)oxide and its 3-bromo derivative.[21] The predominant cis hydration of diol epoxide **1** throughout the pH profile may result from enhanced formation of a carbocation at C-10 due to intramolecular hydrogen bonding between the oxirane ring and the 7-hydroxy group. Although kinetic evidence could not be found for such a hydrogen bond in the naphthalene series in water–alcohol, enhanced reaction rates were observed for diol epoxides **1** and **3** in *tert*-butyl alcohol.[2] For diol epoxide **2**, the pH profile is almost flat between pH 5 and 12 with trans hydration to form *trans*-**2** as the major pathway (~60%) of solvolysis. From pH 5 to 1, the amount of *trans*-**2** increased to 87%.

Assignment of relative stereochemistry to the four tetraols from diol epoxides **1** and **2** was achieved by two independent methods. After preparative scale solvolysis of diol epoxide **1** in acetone–water, the major solvolysis product (*cis*-**1a**) was found to crystallize from methanol. The minor solvolysis product (*trans*-**1a**) was isolated from the above mother liquor by preparative high-pressure liquid chromatography. Both tetraols were converted to tetraacetates. Chromatography on Florisil allowed separation of the tetraacetates of *cis*-**2a** and *trans*-**2a**. With all four stereoisomers of the tetraols available, assignment of relative stereochemistry was possible from their NMR spectra (see later).

Spectral assignments on the tetraacetates were confirmed by synthesis of two of the stereoisomers. Oxidation of the dibenzoate of **5** with osmium tetroxide followed by cleavage of the osmate ester and removal of the benzoates should unequivocally produce *cis*-**1a**, *cis*-**2a**, or a mixture of both of these tetraols. Interestingly, stereoselective attack was once again observed, and only *cis*-**2a** could be isolated. Thus, osmium tetroxide and *N*-bromoacetamide selectively attack the same face of the dihydrodiol **5** whether free or as the dibenzoate. Nucleophilic addition of potassium acetate to diol epoxide **1** in the presence of a crown ether[22] produced *trans*-**1a** upon hydrolysis. Authentic samples of one of the tetraols from each of the diol epoxides ensures the validity of the spectral assignments.

Reactions with Nucleophiles. In the course of studying the metabolism of **5**, we have observed unusually high reactivity of diol epoxides **1** and **2**.[3] Both stereoisomers are sufficiently reactive to alkylate inorganic phosphate at pH 7 and 37 °C. On reverse phase high-pressure liquid chromatography with a linear gradient of 60–98% methanol in water, diol epoxide **2** appeared reasonably stable, while diol epoxide **1** was rapidly and completely converted into *cis*-**1a**, *trans*-**1a**, and two methanol adducts with longer retention times.[3] The high reactivity of the two diol epoxides as well as the interesting stereochemical problem associated with the course of nucleophilic addition prompted further study. The results of this study are summarized in Table I. Assignments of relative stereochemistry are based on the NMR spectra of the derived acetates, which are discussed in the next section.

Relative stereochemistry for the two methanol adducts obtained by direct injection of diol epoxide **1** onto the high-pressure liquid chromatograph was assigned by synthesis of the less retained adduct (*trans*-**1b**) through treatment of diol epoxide **1** with sodium methoxide. This same adduct was obtained by treatment of the dibenzoate of **5** with iodine and silver benzoate to form an iodotribenzoate (shown below), which, on treatment with sodium methoxide, formed *trans*-**1b**.

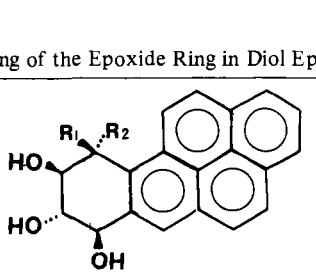


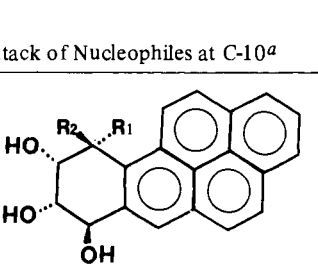
R = $OCOC_6H_5$

Formation of *trans*-**1b** is presumed to occur via diol epoxide **1**, although the stage at which the benzoates are lost is unknown. The intermediate iodobenzoate above is unusually stable in that it resisted conversion into a tetrabenzoate (see Experimental Section) with the relative stereochemistry of *trans*-**1a**. Only *trans*-**2b** was obtained from the less reactive diol epoxide-**2** with either sodium methoxide or acidic methanol. The reason for the failure of diol epoxide **2** to form any cis adduct in acidified methanol is unknown, but may be due to decreased polarity of this solvent relative to water.

As expected, the nucleophiles *p*-nitrothiophenolate and aniline effected trans opening of both diol epoxide **1** and **2** in *tert*-butyl alcohol. In marked contrast, phenol in *tert*-butyl alcohol was found to add cis to both diol epoxides. Although cis addition to diol epoxide **1** might have been anticipated under these slightly acidic conditions, no clear explanation is apparent to account for cis addition to diol epoxide **2**, which undergoes only trans addition (*trans*-**2b**) in acidic methanol. Both potassium phenolate and acetate add trans (*trans*-**1e,f**) to diol epoxide **1**, whereas diol epoxide **2** gave a complex mixture of unidentified products with potassium phenolate.

Table I. Comparison of Cis *vs.* Trans Opening of the Epoxide Ring in Diol Epoxides **1** and **2** after Attack of Nucleophiles at C-10[a]





Conditions	(from diol epoxide 1)[b]		(from diol epoxide 2)[b]	
0.1 M phosphate pH 7.4, 37 °C[c]	*cis*-1a (R_1 = OH) 85%	*trans*-1a (R_2 = OH) 15%	*cis*-2a (R_1 = OH) 7%	*trans*-2a (R_2 = OH) 93%
$NaOCH_3$		*trans*-1b (R_2 = OCH_3)		*trans*-2b (R_2 = OCH_3)
CH_3OH[d]	*cis*-1b (R_1 = OCH_3) 65%	*trans*-1b (R_2 = OCH_3) 35%		*trans*-2b (R_2 = OCH_3)
Sodium *p*-nitrothiophenolate		*trans*-1c (R_2 = $SC_6H_4NO_2$)		*trans*-2c (R_2 = $SC_6H_4NO_2$)
Aniline		*trans*-1d (R_2 = NHC_6H_5)		*trans*-2d (R_2 = NHC_6H_5)
Phenol	*cis*-1e (R_1 = OC_6H_5)		*cis*-2e (R_1 = OC_6H_5)	
Potassium phenolate		*trans*-1e (R_2 = OC_6H_5)		
Potassium acetate		*trans*-1f (R_2 = OAc)		

[a] Structures were assigned based on the NMR spectra of the derived acetates (Tables II and III). [b] Either R_1 or R_2 is designated for each structure. The undesignated R group is a hydrogen atom in each case. [c] Data from ref 3. See also Figure 1. [d] For diol epoxide 1, the data are for direct injection of diol epoxide 1 onto HPLC.[3] The ratio of methanol adducts was determined from peak height as measured by absorbance at 280 nm. For diol epoxide 2, the reaction was conducted in acidified methanol. Retention times for *trans*-2b (27.0 min), *trans*-1b (30.5 min), and *cis*-1b (32.0 min) were as indicated.

Structure Assignments by NMR. The geometry of the benzo ring in 7,8,9,10-tetrahydrobenzoxp[*a*]pyrene should be closely related to that of cyclohexene, which can adopt either a half-chair or a boat conformation. Thus, for cyclohexene, rapid conformational inversion occurs at room temperature, since the half-chair form is favored by only 2.7 kcal/mol.[24] In substituted cyclohexenes, the coupling constants between the various methine hydrogens are best represented by a weighted average of the coupling constants for the above two conformations based on the percentage of time the molecule spends in each of these conformations. Relative stereochemistry for the 7,8,9,10-tetrasubstituted-7,8,9,10-tetrahydrobenzo[*a*]-pyrenes reported here could be assigned based on the coupling constants between the methine hydrogens of their acetate derivatives. The conduritols, a series of closely related stereoisomeric cyclohexene tetraols, provide a precedent for the analysis of this system.[25]

Three factors greatly simplify analysis of the NMR spectra of the acetylated adducts in Table I. First, as had been pointed out previously (cf. ref 2), nucleophiles preferentially attack aryl-substituted oxiranes of the present type at the benzylic carbon atom. Regioselective attack of nucleophiles at C-10 of diol epoxides **1** and **2** was substantiated by characteristic downfield shifts of the signal for the hydrogen at C-10 (see later) due both to its benzylic position and to edge deshielding by the proximate aromatic ring, since C-10 forms part of a "bay region" in the hydrocarbon.[18] Second, relative stereochemistry between the 7-, 8-, and 9-acetates is predetermined for each series in that it must be trans,trans for adducts derived from diol epoxide **1** and trans,cis for adducts derived from diol epoxide **2.** Third, the adducts will assume conformations in which the substituent at C-10 resides preponderantly or entirely in a quasi-axial environment in order to avoid adverse steric interaction with the "bay region" hydrogen at C-11.[18] This latter consideration does not apply for the conduritols and is primarily responsible for the conformational differences between the two systems. Pertinent conformational equilibria which reflect the above considerations for the substituted 7,8,9,10-tetrahydrobenzo[*a*]pyrenes are shown in Scheme II. All of the derivatives reported in Tables II and III have, without exception, small coupling constants for $J_{9,10}$ of 2.5–5 Hz. Since the dihedral angles between H_{10e}–H_{9e} and between

Scheme II. Possible Conformations for Nucleophile Adducts at C-10 of Diol Epoxides 1 and 2.

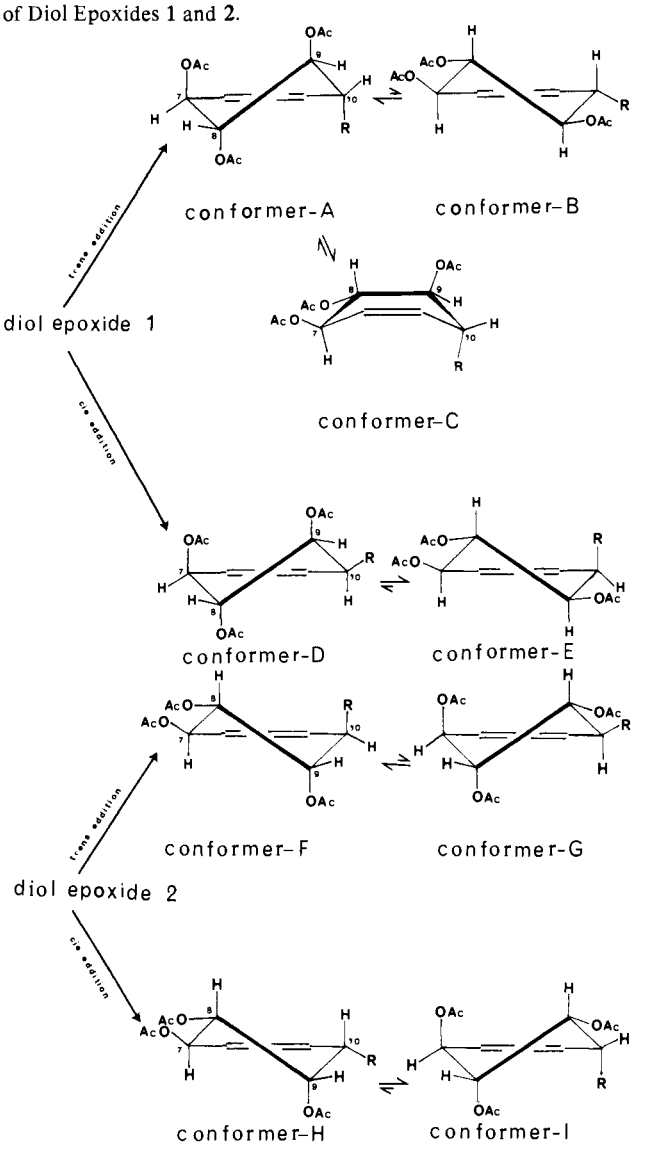


Table II. NMR Spectra of Acetates of Aqueous and Methanol Solvolysis Products from Diol Epoxides **1** and **2**[a]

Compds (as acetates)	Acetyl (methoxy) hydrogens	Methine hydrogens C-7	C-8	C-9	C-10	Aromatic hydrogens
cis-**1a**[b]	2.12, 2.17, 2.17, 2.28	6.64	6.02	5.55	7.34	7.84–8.30
		$^3J_{7a,8a}$ = 8.0, $^3J_{8a,9a}$ = 11.5, $^3J_{9a,10e}$ = 3.5				
trans-**1a**[b]	2.02, 2.10, 2.17, 2.37	6.80	5.42	5.68	7.07	7.85–8.35
		$^3J_{7a,8a}$ = 8.0, $^3J_{8a,9e}$ = 5.0, $^3J_{9e,10e}$ = 3.5				
cis-**2a**[b]	2.10, 2.13, 2.16, 2.19	6.65	5.66	5.95	7.33	7.85–8.30
		$^3J_{7e,8e}$ = 3.5, $^3J_{8e,9a}$ = 2.5, $^3J_{9a,10e}$ = 4.6				
trans-**2a**[b]	2.07, 2.15, 2.21, 2.35	6.96	5.89	5.95	7.12	7.85–8.30
		$^3J_{7a,8a}$ = 8.8, $^3J_{8a,9e}$ = 2.5, $^3J_{9e,10e}$ = 3.6				
trans-**1b**	1.91, 2.14, 2.33, 3.30 (OMe)	6.84	5.31	5.72	5.60	7.80–8.36
		$^3J_{7a,8a}$ = 8.8, $^3J_{8a,9e}$ = 3.8, $^3J_{9e,10e}$ = 3.8				
trans-**2b**	2.00, 2.12, 2.26, 3.80 (OMe)	6.83	5.86	6.08	5.34	7.90–8.30
		$^3J_{7a,8a}$ = 9.0, $^3J_{8a,9e}$ = 2.5, $^3J_{9e,10e}$ = 3.6				

[a] Spectra were measured in $CDCl_3$ at 100 or 220 MHz. Line positions are reported in δ (ppm) relative to internal tetramethylsilane, and coupling constants (J) are given in hertz. Decoupling was required for assignments in some spectra. [b] Spectra recorded at 220 MHz.

Table III. NMR Spectra of Acetates of the Nucleophilic Addition Products from Diol Epoxides **1** and **2**[a]

Compds (as acetates)	Acetyl or NH hydrogens	Methine hydrogens C-7	C-8	C-9	C-10	Aromatic hydrogens
trans-**1c**	1.92, 2.13, 2.38	6.86	5.42	5.66	5.81	7.65–8.30
		$^3J_{7a,8a}$ = 8.0, $^3J_{8a,9e}$ = 2.8, $^3J_{9e,10e}$ = 2.0				
trans-**2c**	2.04, 2.16, 2.34	6.90	6.34	5.75	5.79	7.80–8.46
		$^3J_{7a,8a}$ = 9.0, $^3J_{8a,9e}$ = 2.5, $^3J_{9e,10e}$ = 3.5				
trans-**1d**	1.99, 2.05, 2.26, 3.96 (NH, $J_{NH,10}$ = 8)	6.92	5.55	5.75	5.71	6.86–7.33
		$^3J_{7e,8e}$ = 4.5, $^3J_{8e,9e}$ = 4.0, $^3J_{9e,10e}$ = 4.0				8.0–8.30 (pyrene)
trans-**2d**	2.07, 2.14, 2.31, 4.06 (NH, $J_{NH,10}$ = 5)	6.81	6.09	5.85	5.59	6.90–7.36
		$^3J_{7a,8a}$ = 9.0, $^3J_{8a,9e}$ = 2.5, $^3J_{9e,10e}$ = 2.5				7.91–8.27 (pyrene)
cis-**1e**	1.84, 2.15, 2.34	6.74	6.30	5.56	6.79	7.05–7.36
		$^3J_{7a,8a}$ = 7.75, $^3J_{8a,9a}$ = 11.25, $^3J_{9a,10e}$ = 3.25				7.98–8.25 (pyrene)
cis-**2e**	1.84, 2.15, 2.20	6.76	6.11	5.64	6.81	7.08–7.41
		$^3J_{7e,8e}$ = 4.5, $^3J_{8e,9a}$ = 2.5, $^3J_{9a,10e}$ = 5.0				8.00–8.25 (pyrene)
trans-**1e**	1.92, 2.10, 2.37	6.94	5.41	5.82	6.51	6.78–7.40
		$^3J_{7a,8a}$ = 7.4, $^3J_{8a,9e}$ = 3.6, $^3J_{9e,10e}$ = 3.6				7.98–8.27 (pyrene)

[a] Spectra were measured in $CDCl_3$ at 220 MHz. Line positions are reported in δ (ppm) relative to internal tetramethylsilane and coupling constants (J) are given in hertz.

H_{10e}–H_{9a} are very similar in both trans-9,10 and cis-9,10 derivatives, respectively, the values of $J_{9,10}$ cannot be diagnostic for assignment of relative stereochemistry.

The NMR spectra of the acetylated adducts which form on cis addition of water and phenol to diol epoxide **1** (*cis*-**1a** and *cis*-**1e**, Tables I–III) with 7e, 8e, 9e, and 10a substituents (conformer E, Scheme II) are quite similar to that of conduritol F. All three molecules prefer the half-chair conformation in which the C-10 benzylic (or equivalent) substituent is quasi-axial and the remaining three substituents are quasi-equatorial. The observed coupling constants of $J_{7,8} \sim 8.0$, $J_{8,9} \sim 11.5$, and $J_{9,10} \sim 3.5$ Hz for the adducts are in good agreement with the related coupling constants for conduritol F. Notably, this is the only conduritol for which the hydroxy group, which is equivalent to the substituent at C-10 in the adducts, prefers a quasi-axial environment, since its inverted form (similar to conformer D, Scheme II) suffers adverse steric interaction from three quasi-axial substituents.

Adducts which result from cis addition to diol epoxide **2** (*cis*-**2a** and *cis*-**2e**) favor the half-chair conformation in which the substituents at C-7, C-8, and C-10 are quasi axial (conformer I, Scheme II). Small coupling constants for $J_{7,8} \sim 4$, $J_{8,9} \sim 2.5$, and $J_{9,10} \sim 5$ Hz support the proposed conformation. The corresponding conduritol prefers the inverted form related to conformer H. Similarly, the adducts which result from trans addition to diol epoxide **2** (*trans*-**2a**, **2b**, **2c**, and **2d**) should prefer conformer F, in which the C-9 and C-10 substituents are quasi-axial and the C-7 and C-8 substituents are quasi-equatorial. Observed large coupling constants for $J_{7,8} \sim 9$ Hz and small coupling constants for $J_{8,9} \sim 2.5$ and $J_{9,10} \sim 2.5$–4 Hz favor this argument. The corresponding conduritol exists as a 1:1 mixture of the two equivalent chair forms.

The remaining class of adducts, formed by trans addition to diol epoxide **1** (*trans*-**1a**, **1b**, **1c**, **1e**), has rather unusual coupling constants ($J_{7,8} \sim 8$, $J_{8,9} \sim 2.8$–5, $J_{9,10} \sim 2$–3.8 Hz) and has no counterpart in the conduritols. Neither of the two

chair forms should be favored here, since conformer A would have substantial steric crowding due to the four axial substituents and conformer B has the substituent at C-10 quasi-equatorial in the highly hindered "bay region." A boat form (or a flattened chair at C-10; conformer C) is the most preferable of the three conformations and is consistent with the unusual value for $J_{8,9}$. The only exception in this series is the aniline adduct *trans*-**1d**, for which all three coupling constants are small ($J_{7,8}$ = 4.5, $J_{8,9}$ = $J_{9,10}$ = 4.0 Hz). The spectrum is compatible with conformer A, in which all four substituents are quasi-axial. Intramolecular hydrogen bonding between the amine hydrogen and the carbonyl of the acetate at C-8 might constitute a stabilizing force for this conformation. Reaction of diol epoxide **1** at the N-2 amino group of polyguanylic acid results in trans and cis adducts at C-10, in which the coupling constants are within ±1 Hz of those observed for *trans*-**1d** and *cis*-**1e**, respectively.[27] This provides an additional example of a trans adduct of diol epoxide **1** with a secondary amino substituent at C-10 which resides mainly in the unusual conformation A, where all the substituents are quasi-axial.

Conclusions

Although the diol group in diol epoxides **1–4** has a marked influence on chemical reactivity and on the steric course of reactions of the oxirane ring with nucleophiles, its importance in biological activity, such as mutagenesis, is presently unclear. For example, 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene is actually somewhat more mutagenic toward bacteria than is either diol epoxide **1** or **2**.[14a] Interestingly, diol epoxides **3** and **4** lack mutagenic activity toward bacteria.[5] The size and shape of the aromatic system as well as the position of the epoxide group of the diol epoxide must also be important factors in determining biological activity. Perturbational molecular orbital calculations by the method of Dewar[28] have indicated that carbonium ion formation at a benzylic position of a saturated, angular benzo ring (such as in a diol epoxide) occurs with much greater ease if the benzylic position is in a bay region of the hydrocarbon.[29] The ease of such carbonium ion formation shows significant correlation with the carcinogenicity of the parent hydrocarbon.[29,30,36]

Experimental Section

Proton NMR spectra were taken with Varian HA-100 and HR-220 instruments and chemical shift data are reported in parts per million (δ) downfield from tetramethylsilane as an internal standard with coupling constants (J) in hertz. Chemical ionization mass spectra (NO–N_2 gas) were run on a Finnigan Model 1015 gas chromatograph/mass spectrometer by the direct inlet mode unless otherwise noted, and only selected peaks are reported. Melting points were determined in unsealed capillary tubes and are uncorrected. Tetrahydrofuran, Me_2SO, and *tert*-butyl alcohol were purified by distillation from CaH_2 and stored under argon gas. The tritium labeled diol epoxides **1** and **2** were prepared from [^{3}H]BP (benzo[*a*]pyrene) 7,8-dihydrodiol as described.[3] The high-pressure liquid chromatography (HPLC) referred to in the legend of Figure 1 was conducted as described in ref 3.

(±)-7β,8α-Dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (diol epoxide 1). A mixture of 286 mg of *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (**5**),[19,23] 150 mg of *N*-bromoacetamide (freshly recrystallized from methylene chloride and *n*-hexane), 30 ml of tetrahydrofuran, 10 ml of water, and 1 drop of 12 N HCl was stirred at 4 °C for 3 h. The reaction was monitored by the disappearance of the absorption at 367 nm. Evaporation of the solvent afforded a solid, which was extracted with ethyl acetate (2 × 200 ml). The solvent was washed with water, dried ($MgSO_4$), and concentrated. The resulting colorless crystals (360 mg, 94%) were recrystallized from tetrahydrofuran–ethanol to give colorless prisms of (±)-9α-bromo-7β,8α,10β-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, mp 128–130 °C dec: NMR (see ref 2). Anal. Calcd for $C_{20}H_{15}BrO_3$: C, 62.68; H, 3.95. Found: C, 62.49; H, 3.96.

A mixture of 1.07 g of the above bromotriol, 10 g of Amberlite IRA-400 resin in the hydroxide form (prepared by eluting the resin with 1 N KOH, water, and finally anhydrous tetrahydrofuran), and 200 ml of anhydrous tetrahydrofuran was stirred at room temperature under argon gas for 6 h. The reaction mixture was filtered and the filtrate was concentrated to ca. 10 ml and cooled. The resulting colorless feathers of diol epoxide **1** (0.68 g, 85%) were collected and washed with anhydrous ether, mp 226–228 °C dec: NMR (see ref 2); *m/e* 302 (M^+). Anal. Calcd for $C_{20}H_{14}O_3$: C, 79.46; H, 4.67. Found: C, 79.33; H, 4.76.

Diol Epoxide 1 Dibenzoate. A mixture of 200 mg of the dibenzoate of **5**, 73 mg of *N*-bromoacetamide (freshly recrystallized), 100 mg of sodium acetate, 50 ml of tetrahydrofuran, and 20 ml of water was stirred at room temperature under argon gas and in subdued light for 18 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate (100 ml), which was washed with water (2 × 30 ml), dried ($MgSO_4$), and evaporated to leave 267 mg of (±)-7β,8α-dibenzoyloxy-9α-bromo-10β-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene as colorless crystals which were recrystallized from chloroform–petroleum ether, mp 196–197 °C dec: NMR (220 MHz, $CDCl_3$) δ 7.26 (H_7), 6.13 (H_{10}), 6.33 (H_8), 5.14 (H_9), 7.20–7.60 (OCPh), and eight aromatic protons 7.80–8.40 ($J_{7,8}$ = 8.5, $J_{8,9}$ = $J_{9,10}$ = 3.0 Hz); *m/e* 590 and 592 (M^+). Anal. Calcd for $C_{34}H_{23}BrO_5$: C, 69.05; H, 3.9i. Found: C, 68.86; H, 3.94.

A mixture of 100 mg of the above bromohydrin, 3 g of Amberlite IRA-400 resin in the hydroxide form, and 25 ml of anhydrous tetrahydrofuran was stirred under argon gas at room temperature in subdued light for 3 days. Solids were removed by filtration, and the filtrate was evaporated to leave crystals of (±)-7β,8α-dibenzoyloxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, which were recrystallized from ether–petroleum ether to give 70 mg of colorless prisms, mp 169–170 °C: NMR (220 MHz, $CDCl_3$) δ 6.96 (H_7), 6.02 (H_8), 5.09 (H_{10}), 4.10 (H_9) ($J_{7,8}$ = 4.2, $J_{8,9}$ = 2.2, $J_{9,10}$ = 4.2, $J_{7,9}$ = 1.4 Hz); *m/e* 510 (M^+). Anal. Calcd for $C_{34}H_{22}O_5$: C 79.99; H, 4.34. Found: C, 79.85; H, 4.21.

(±)-7β,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (diol epoxide 2). A mixture of 100 mg of dihydrodiol **5**, 600 mg of *m*-chloroperbenzoic acid (purified), and 20 ml of dry tetrahydrofuran was stirred under argon gas for 1 h. The reaction mixture was diluted with 20 ml of ether, washed with 10% sodium hydroxide solution (3 × 20 ml) and then water, and dried (K_2CO_3). Evaporation of the solvent gave crystals (73.6 mg, 70%) of diol epoxide **2**, which were recrystallized from ethyl acetate, mp 214 °C: NMR (see ref 2); *m/e* 302 (M^+). Anal. Calcd for $C_{20}H_{14}O_3$: C, 79.46; H, 4.67. Found: C, 79.40; H, 4.74.

(±)-1β,2α-Dihydroxy-3β,4β-epoxy-1,2,3,4-tetrahydronaphthalene (diol epoxide 3). *N*-Bromoacetamide (1.76 mmol, 244 mg) was added portionwise at 0 °C under argon to a solution of *trans*-(±)-1,2-dihydroxy-1,2-dihydronaphthalene[17,18] (1.6 mmol, 286 mg) in 20 ml of tetrahydrofuran–water (3:1). After stirring for 3 h, acetone (5 ml) was added and the reaction mixture was allowed to warm to room temperature. The solvent was evaporated to dryness and the resulting oily residue was triturated with ethyl acetate (−25 °C) to provide 326 mg (79%) of (±)-3α-bromo-1β,2α,4β-trihydroxy-1,2,3,4-tetrahydronaphthalene as a white crystalline solid, mp 154–156 °C dec: NMR (see ref 2); *m/e* 257 and 259 ($M^+ - 1$), 223 and 225 ($M^+ - H_2O - OH$), 178 ($M^+ - Br$). Anal. Calcd for $C_{10}H_{11}BrO_3$: C, 46.35; H, 4.28. Found: C, 46.64; H, 4.38.

A mixture of 326 mg of the above bromotriol, ca. 2 g of Amberlite IRA-400 (OH form), and 10 ml of dry tetrahydrofuran was stirred at room temperature under argon. After stirring for 3 h the resin was removed by filtration and washed with dry tetrahydrofuran. Evaporation of the combined solvent gave 220 mg (95%) of diol epoxide **3** as a colorless syrup: NMR (see ref 2); *m/e* 178 (M^+), 177 ($M^+ - 1$), 160 ($M^+ - H_2O$), 132 ($M^+ - H_2O - CO$).

(±)-1β,2α-Dihydroxy-3α,4α-epoxy-1,2,3,4-tetrahydronaphthalene (diol epoxide 4). A solution of 85% *m*-chloroperoxybenzoic acid (Aldrich, 1 mmol, 203 mg) in dichloromethane (20 ml) was added dropwise at 0 °C to a solution of the dihydrodiol (1 mmol, 162 mg) in the same solvent (20 ml). After 2 h at 0 °C, the reaction mixture was allowed to warm to room temperature and stirring was continued for 2 h. The solvent was evaporated to dryness, and the residue was taken up in ether (ca. 10 ml) and allowed to stand in the cold for 0.5 h. The resulting precipitate was separated by filtration and washed with a small portion of cold ether to afford 108 mg (60%) of diol epoxide **4** as a white crystalline solid, mp 153–155 °C: NMR (see ref 2); *m/e* 178 (M^+), 160 ($M^+ - H_2O$), 132 ($M^+ - H_2O - CO$). Anal. Calcd for $C_{10}H_{10}O_3$: C, 67.40; H, 5.66. Found: C, 67.15; H, 5.69.

Synthesis of Tetraols. (a) *cis*-2a via OsO_4. To a solution of 494 mg of the dibenzoate of **5** in 20 ml of pyridine was added a solution of 260 mg of osmium tetroxide in 27 ml of pyridine at 5 °C. The reaction mixture was stirred at room temperature for 5.5 h. A solution of 2 g of sodium bisulfite in 25 ml of water was added to the reaction mixture and stirred for 1 h to decompose the osmate ester. The reaction mixture was extracted with ethyl acetate (3 × 150 ml), which was washed with water, dried ($MgSO_4$), and evaporated to provide crystals (508 mg, 92%) of (±)-7β,8α-dibenzoyloxy-9α,10α-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, which was recrystallized from methanol to give colorless prisms, mp 223–224 °C dec: NMR (100 MHz, $CDCl_3$–CD_3OD) δ 4.92 (H_9), 5.91 (H_{10}), 5.92 (H_8), 7.0–8.70 (H_7) and 18 aromatic protons ($J_{7,8}$ = 5.0, $J_{8,9}$ = 2.4, $J_{9,10}$ = 5.0 Hz); *m/e* 528 (M^+). Anal. Calcd for $C_{34}H_{24}O_6$: C, 77.26; H, 4.58. Found: C, 77.20; H, 4.52.

A mixture of 100 mg of the above dibenzoate, 3 ml of 10% aqueous potassium hydroxide solution, and 30 ml of tetrahydrofuran was heated at 55 °C for 18 h. The products were extracted into ethyl acetate (3 × 100 ml), which was washed with NaCl solution, dried ($MgSO_4$), and evaporated to provide crystals (60 mg). Analysis of this material by HPLC indicated that *cis*-**2a** was the sole tetraol present. The tetraacetate of *cis*-**2a**, (±)-7β,8α,9α,10α-tetraacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, was prepared with pyridine–acetic anhydride and was recrystallized to give colorless prisms (methanol), mp 206–207 °C: NMR (see Table II); *m/e* 488 (M^+). Anal. Calcd for $C_{28}H_{24}O_8$: C, 68.85; H, 4.95. Found: C, 69.09; H, 4.91.

(b) *trans*-1a via Potassium Acetate. Diol epoxide **1** (25 mg) was suspended in 25 ml of dry acetonitrile under argon. Dibenzo-18-crown-6 (Aldrich, ca. 5 mg) was added followed by potassium acetate (17 mg, dried at i00 °C for 18 h). The resulting suspension was heated slowly to reflux and became homogenous in approximately 10 min. After 25 min, formation of a tan colored precipitate was observed. Reflux was continued for 1 h, the mixture was allowed to cool to room temperature, and the solvent was evaporated to dryness. The residue was acetylated with pyridine–acetic anhydride, and the crude product was chromatographed on Florisil using chloroform as the developing solvent. The resulting pale yellow solid (9 mg) was identified as (±)-7β,8α,9β,10α-tetraacetoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene: *m/e* 488 (M^+); NMR (Table II). After hydrolysis with lithium borohydride, only *trans*-**1a** could be detected by HPLC.

(c) *cis*- and *trans*-2a by Oxidation of 5. A mixture of 235 mg of the dihydrodiol **5**, 200 mg of *m*-chloroperbenzoic acid, and 250 ml of chloroform was stirred at 0 °C for 48 h. After washing with 10% sodium hydroxide solution and water, the chloroform was dried ($MgSO_4$) and concentrated to leave a solid (250 mg). A small portion of the solid was silylated and examined by mass spectrometry; a major signal at *m/e* 674 and a very small signal at *m/e* 446 indicated that most of the diol epoxide **2** which had formed had been further converted to a chlorobenzoate adduct (see ref 2). Ammonolysis of another small portion of the solid in methanol followed by HPLC analysis indicated the presence of both *cis*-**2a** (40%) and *trans*-**2a** (60%). Repeated crystallization of the solid from ethanol provided 25 mg of colorless leaflets of (±)-10α-(4-chlorobenzoyloxy)-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, mp 214 °C dec: NMR (100 MHz, tetrahydrofuran-d_8) δ 4.21, (H_8), 4.72 (H_9), 5.42 (H_7), 7.35 (H_{10}), 22 aromatic protons 7.2–8.5 ($J_{7,8}$ = 5.0, $J_{9,10}$ = 5.0, $J_{8,9}$ = 3.5 Hz). Anal. Calcd for $C_{27}H_{19}ClO_5$: C, 70.67; H, 4.17. Found: C, 70.38; H, 4.15. After ammonolysis, only *cis*-**2a** was detected on HPLC.

Oxidation of **5** (50 mg) with *p*-nitroperoxybenzoic acid (57 mg) in tetrahydrofuran (5 ml) was complete after 15 min at room temperature. Workup, ammonolysis, and acetylation with pyridine–acetic anhydride provided 46 mg of *cis*- and *trans*-**2a** as their tetraacetates. Preparative thick-layer chromatography on Florisil employing cyclohexane–dioxane (8:2) as the eluent allowed isolation of 20 mg of the tetraacetate of *trans*-**2a** after crystallization from methanol, mp 220–222 °C: NMR (Table II); *m/e* 488 (M^+). Anal. Calcd for $C_{28}H_{24}O_8$: C, 68.84; H, 4.95. Found: C, 68.57; H, 5.19. The tetraacetate of *cis*-**2a** (10 mg) had a lower mobility: NMR (Table II).

Hydrolysis of Diol Epoxides 1 and 2. To a mixture of 50% acetone–water (50 ml) was added 40 mg of either diol epoxide in 5 ml of tetrahydrofuran. After agitation at 37 °C overnight, tetraols were extracted (3 × 50 ml) into ethyl acetate; diol epoxide **1** → 85% *cis*-**1a** and 15% *trans*-**1a**, diol epoxide **2** → 40% *cis*-**2a** and 60% *trans*-**2a** by HPLC. Fractional crystallization from methanol allowed isolation of 10 mg of *cis*-**1a** free of *trans*-**1a**. A small quantity of *trans*-**1a** was isolated by preparative HPLC. Both isomers were acetylated and their NMR spectra recorded (Table II). The tetraols from diol epoxide **2** were acetylated, separated by preparative TLC on Florisil (as above), and their NMR spectra recorded (Table II). To confirm the identity of the separated components in the HPLC effluent, all four of the tetraacetates were hydrolyzed (NH_3–CH_3OH) to tetraols and their retention times confirmed by HPLC.

Products were also examined in both 50% dioxane:water and 50% THF:water in the pH range of 5–11 as measured by the glass electrode with and without 0.09 M KCl. About 70% of the products from diol epoxide **1** are tetraols, the ratio of which depends upon the pH. Almost all of the remaining 30% consisted of *trans*-7,8-dihydroxy-9-keto-7,8,9,10-tetrahydrobenzo[*a*]pyrene which was identified directly by its tetrahydrobenzo[*a*]pyrene chromophore in the UV and its mass spectrum (M^+ 18 320; CI, NH_3) after isolation by HPLC. The ketone was further characterized as its enol triacetate (pyridine:acetic anhydride) which shows M^+ at 428 (CI, NO–N_2) and a typical 7,8-dihydrobenzo[*a*]pyrene UV spectrum as well as by reduction with sodium borohydride to a known mixture of triols (see ref 31). Under none of the conditions examined did diol epoxide **2** isomerize to this ketone.

(±)-7β,8α,9β-Trihydroxy-10α-methoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*trans*-1b). A mixture of 515 mg of silver benzoate, 266 mg of iodine, and 100 ml of benzene was stirred at room temperature until all of the iodine color disappeared. To the resulting mixture was added 494 mg of the dibenzoate of dihydrodiol **5** and the suspension was refluxed for 7 h under a current of argon gas. The reaction mixture was filtered, and the filtrate was evaporated to a reddish oil which was recrystallized from chloroform–petroleum ether to give 720 mg of (±)-7β,8α,10β-tribenzoyloxy-9α-iodo-7,8,9,10-tetrahydrobenzo[*a*]pyrene as pale yellowish prisms, mp 213–215 °C: NMR (100 MHz, $CDCl_3$) δ 5.35 (H_9), 5.62 (H_8), 7.42 (H_7), and 7.56 (H_{10}) ($J_{7,8}$ = 8.7, $J_{8,9}$ = $J_{9,10}$ = 3.5 Hz). Anal. Calcd for $C_{41}H_{27}IO_6$: C, 66.33, H; 3.66. Found: C, 66.11; H, 3.77. Silver benzoate in boiling xylene failed to transform this compound into a tetrabenzoyloxy derivative.

The above iodotribenzoate (240 mg) was then treated with sodium methoxide (70 mg) in 4 ml of tetrahydrofuran and 10 ml of methanol for 18 h. After workup and acetylation with pyridine–acetic anhydride, the triacetate of *trans*-**1b** was obtained as an oil in 95% yield: NMR (Table II); *m/e* 460 (M^+). Diol epoxide **1** (20 mg), when reacted with sodium methoxide (20 mg) for 3 h under the above conditions, also produced *trans*-**1b** (24 mg after acetylation). HPLC (Table I), either before acetylation or after ammonolysis of the triacetate, established *trans*-**1b** as the minor methanol adduct (less retained) formed when diol epoxide **1** is injected onto the ODS column.

(±)-7β,8α,9α-Trihydroxy-10β-methoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*trans*-2b). Diol epoxide **2** (20 mg), when reacted either with sodium methoxide as above or with a solution of 20 ml of methanol which contained 4 ml of tetrahydrofuran and 1 drop of 60% $HClO_4$, afforded 26–28 mg of the triacetate of *trans*-**2b** after workup and acetylation; NMR (Table II); *m/e* 460 (M^+). Analysis by HPLC, either before acetylation or after ammonolysis of the triacetate, show a single peak (R_t 27.0 min).

(±)-7β,8α,9β-Triacetoxy-10α-(4-nitrothiophenyl)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (triacetate of *trans*-1c). A solution of 29.4 mg of sodium thiophenolate was added dropwise to a solution of 50 mg of diol epoxide **1** in 100 ml of dry *tert*-butyl alcohol over 1 h at room temperature under argon gas in subdued light. The reaction mixture was stirred for 18 h, and the solvent was replaced by 100 ml of ethyl acetate, which was washed with water and dried ($MgSO_4$). Evaporation of the solvent gave 60 mg of a glass which was acetylated with pyridine–acetic anhydride. Workup provided a solid which was recrystallized from chloroform–petroleum ether to give 60 mg of yellow prism, mp 251–252 °C dec: NMR (Table III); *m/e* 523 (M^+ – CH_3COOH), 429 (M^+ – $SC_6H_4NO_2$). Anal. Calcd for $C_{32}H_{25}NO_8S$: C, 65.86; H, 4.32; N, 2.40. Found: C, 65.72; H, 4.20; N, 2.38.

(±)-7β,8α,9α-Triacetoxy-10β-(4-nitrothiophenyl)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (triacetate of *trans*-2c). Reaction was carried out exactly as above on 50 mg of diol epoxide **2** to yield 70 mg of the triacetate of *trans*-**2c** as orange-red prisms (chloroform–petroleum ether), mp 252–253 °C dec: NMR (Table III); *m/e* 583 (M^+), 523 (M^+ – CH_3COOH), 429 (M^+ – $SC_6H_4NO_2$). Anal. Calcd for $C_{32}H_{25}NO_8S$: C, 65.86; H, 4.32; N, 2.40. Found: C, 65.58; H, 4.37; N, 2.23. Analysis of the NMR spectra of the crude acetylated product

in this and the preceding experiments gave no indication of cis addition in either case. The conditions here approximate the kinetic conditions under which diol epoxide **1** showed a much higher rate.

(±)-7β,8α,9β-Triacetoxy-10α-anilino-7,8,9,10-tetrahydrobenzo[*a*]pyrene (triacetate of *trans*-1d). The diol epoxide **1** (25 mg) was dissolved in 25 ml of dry tetrahydrofuran and diluted with 25 ml of dry *tert*-butyl alcohol. To this solution was added dropwise 10 mg (1.3 mol equiv) of aniline in 10 ml of dry *tert*-butyl alcohol under argon at room temperature. The initially turbid solution was stirred for 20 h, and the solvent was replaced by pyridine–acetic anhydride. Workup provided 55 mg of solid which was purified by preparative TLC on Florisil with chloroform as eluent. The resulting triacetate of *trans*-**1d** (40 mg) was recrystallized from chloroform–petroleum ether, mp 233–236 °C dec; NMR (Table III); *m/e* (CI, NH_3 gas) 522 ($M^+ + 1$), 446 ($M + NH_4 - NH_2C_6H_5$), 429 ($M^+ + 1 - NH_2 - C_6H_5$), 153 (base peak). Attempts to acetylate the amine in the triacetate of *trans*-**1d** with warm pyridine–acetic anhydride resulted in decomposition.

(±)-7β,8α,9α-Triacetoxy-10β-anilino-7,8,9,10-tetrahydrobenzo[*a*]pyrene (triacetate of *trans*-2d). Diol epoxide **2** (25 mg) was allowed to react with aniline, and the product was acetylated and chromatographed as above to provide 25 mg of the triacetate of *trans*-**2d** as an oil: NMR (Table III); *m/e* (CI, NH_3 gas), 522 ($M^+ + 1$, base peak), 446 ($M + NH_4^+ - NH_2C_6H_5$), 429 ($M^+ + 1 - NH_2C_6H_5$).

(±)-7β,8α,9β-Triacetoxy-10β-phenoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (triacetate of *cis*-1e). Diol epoxide **1** (25 mg) was dissolved in 25 ml of dry tetrahydrofuran and diluted with 25 ml of dry *tert*-butyl alcohol. To this solution was added dropwise 10 mg (1.3 mol equiv) of freshly sublimed phenol in 10 ml of dry *tert*-butyl alcohol under argon at room temperature. After reaction for 30 h at room temperature, the solvent was replaced by pyridine–acetic anhydride. Workup, preparative TLC on Florisil plates developed with chloroform, and crystallization from chloroform–petroleum ether provided 25 mg of the triacetate of *cis*-**1e**, mp 225–227 °C dec: NMR (Table III); *m/e* 522 (M^+). Anal. Calcd for $C_{32}H_{26}O_7$: C, 73.55, H, 5.02; Found: C, 73.55; H, 5.22.

(±)-7β,8α,9α-Triacetoxy-10α-phenoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (triacetate of *cis*-2e). In an experiment identical with that described above, 25 mg of diol epoxide **2** was converted into 23 mg of the triacetate of *cis*-**2e**, mp 192–194 °C dec: *m/e* 522 (M^+). Anal. Calcd for $C_{32}H_{26}O_7$: C, 73.55; H, 5.02. Found: C, 73.81; H, 5.33.

(±)-7β,8α,9β-Triacetoxy-10α-phenoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (triacetate of *trans*-1e). Diol epoxide **1** (25 mg) was dissolved in 25 ml of dry tetrahydrofuran and diluted with 25 ml of dry acetonitrile. To this solution, 12 mg (1.1 mol equiv) of freshly prepared potassium phenolate was added at 0 °C under argon along with 5 mg of dibenzo-18-crown-6 (Aldrich). The mixture was stirred at room temperature for 20 h. Workup, acetylation, and preparative TLC on Florisil plates developed with chloroform followed by recrystallization from chloroform–petroleum ether provided 11 mg of the triacetate of *trans*-**1e**, mp 221–223 °C dec; NMR (Table III); *m/e* 522 (M^+). Analysis of the acetylated product by TLC gave no indication that the triacetate of *cis*-**1e** was present prior to purification.

References and Notes

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(36) NOTE ADDED IN PROOF. Since submission of this manuscript, we have completed the synthesis of the five trans dihydrodiols which are probable mammalian metabolites of benzo[*a*]anthracene[32] and have converted most of these dihydrodiols into diastereomeric pairs of diol epoxides[33] by the methods described in the present study. As anticipated from perturbational molecular orbital calculations,[29] the 3,4-diol 1,2-epoxides were the most reactive[33,34] and the most mutagenic.[35] These results provide strong support for our concept of "bay region" epoxides of dihydrodiols as ultimate carcinogenic forms of the polycyclic aromatic hydrocarbons.[29,30]