was 70%. ^{1}H NMR δ 7.25 (m, 6 H), 5.22 (m, 1 H), 3.27 (t, 2 H), 2.30 (m, 2 H), 2.21 (m, 1 H), 1.98 (t, 1 H), 1.27 (m, 1 H), 1.18 (s, 3 H), 1.14 (d, 1 H), 0.74 (s, 3 H). The reported δ values[8] are 7.2 (s, 5 H), 5.3 (bs, 1 H), 3.33 (s, 2 H), 1.2 (s, 3 H), 0.73 (s, 3 H). The amount of product from γ substitution was less than 3% in some reactions. The amount of γ substitution, as indicated by olefinic hydrogen multiplets at δ 4.82 and 4.53 in the NMR spectrum, appeared to be larger when the reaction temperature was below 0 °C or when less than a two to one ratio of cuprous iodide to myrtenyl acetate was used.

Preparation of (1*R*)-2-(Bromomethyl)-6,6-dimethylbicyclo[3.1.1]hept-2-ene (1d) (Myrtenyl Bromide). In a modification of the published procedure,[23] phosphorus tribromide (1.13 mL, 0.012 mol) was injected into dry ethyl ether in a flame-dried nitrogen-flushed 50-mL round-bottomed flask, and the mixture was cooled to 0 °C. A catalytic amount of pyridine was injected followed by myrtenol (5.00 g, 0.032 mol). Reaction for 45 min and isolation gave undistilled (1*R*)-myrtenyl bromide, showing a clean NMR spectrum (5.78 g, 82%). ^{1}H NMR δ 5.645 (s, 1 H), 3.92 (AB pattern, 2 H), 2.41 (m, 1 H), 2.23 (m, 3 H), 1.28 (s, 3 H), 1.15 (d, 1 H), 0.80 (s, 3 H). Reported[24] ^{1}H NMR (100 MHz): δ 5.61 (m, 1 H), 3.85 (m, 2 H), 2.44 (dt, 1 H), 2.30 (m, 2 H), 1.31 (s, 3 H), 1.18 (d, 1 H), 0.83 (s, 3 H).

Preparation of 10-Phenyl-α-pinene from Myrtenyl Bromide. This procedure was based one used previously to couple allylic compounds.[25] Undistilled (1*R*)-myrtenyl bromide (5.92 g, 0.0275 mol) in ether (50 mL) was allowed to react under nitrogen with phenyllithium in cyclohehexane–ethyl ether (28 mL, 0.056 mol, injected slowly). As in the above preparation, distillation (1 mm, 126 °C) gave of a mixture of phenylpinene and biphenyl (3.97 g total) having a molar ratio of 1 to 0.096. The corrected yield of phenylpinene was 62%. ^{1}H NMR δ 7.245 (m, phenyl), 5.22 (m, vinyl H), 3.27 (AB pattern, 2 H), 2.30 (m, 2 H), 2.21 (m, 1 H), 1.98 (t, 1 H), 1.27 (m, 1 H), 1.183 (s, 3 H), 1.143 (d, 1 H), 0.742 (s, 3 H). The reported ^{1}H NMR values[8] are 7.2 (s, 5 H), 5.3 (bs, 1 H), 3.33 (s, 2 H), 1.2 (s, 3), and 0.73 (s, 3 H).

Registry No. **1b**, 19894-97-4; **1b** (X = OAc), 36203-31-3; **1c**, 91200-45-2; **1d**, 55527-89-4; **2**, 53369-17-8; **3**, 24041-60-9; **4**, 128301-02-0; **5**, 128244-23-5; **6**, 128244-24-6.

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Covalent Nucleoside Adducts of Benzo[*a*]pyrene 7,8-Diol 9,10-Epoxides: Structural Reinvestigation and Characterization of a Novel Adenosine Adduct on the Ribose Moiety

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The diastereomeric 7,8-diol 9,10-epoxides metabolically derived from the carcinogenic hydrocarbon benzo[*a*]pyrene react with the purine bases in nucleic acids to alkylate their exocyclic amino groups. *The major adducts formed from polyguanylic acid and the enantiomers of diol epoxide-1 (the diastereomer in which the benzylic 7-hydroxyl group and the epoxide oxygen are cis) have been shown to result from cis opening of the epoxide by the N-2 amino group of guanine, rather than trans opening as had been previously reported.* Four adducts resulting from alkylation of the exocyclic N-6 amino group of adenosine 5′-monophosphate by racemic diol epoxide-1 have been prepared and characterized. In addition, a major adduct formed from adenosine 5′-monophosphate and (−)-(7*R*,8*S*)-diol (9*R*,10*S*)-epoxide-1, but not from its (+) enantiomer, has been identified as a product of alkylation of the 2′-hydroxyl group of the sugar. We also report a quantitative reevaluation of the extent and distribution of covalent adduct formation from calf thymus DNA and both diastereomeric benzo[*a*]pyrene diol epoxides, as well as the identification of the principal DNA adducts formed from the enantiomers of diol epoxide-1. Tentative identification of several new minor adducts formed upon reaction of diol epoxide-2 with *denatured* DNA is described. The present results provide additional support for our previously proposed correlation between the signs of the circular dichroism bands of these adducts and their absolute configurations at the N-substituted benzylic carbon atom.

Introduction

Bay-region diol epoxides that are metabolically formed by the combined action of cytochrome P-450 and epoxide hydrolase have been shown or implicated as ultimate carcinogens from a large number of tumorigenic polycyclic aromatic hydrocarbons.[1] For a given hydrocarbon, two diastereomeric diol epoxides can be formed from the trans dihydrodiol metabolite by epoxidation of the benzo ring double bond from either face of the dihydrodiol; namely, diol epoxide-1, in which the epoxide oxygen and the benzylic hydroxyl group are cis, and diol epoxide-2, in which these groups are trans. Since the trans dihydrodiol can exist in two enantiomeric forms, four optically active isomeric diol epoxides are metabolically possible. These diol epoxides are reactive electrophiles whose cytotoxic, mutagenic, and tumorigenic activities presumably result from their ability to alkylate nucleic acids and/or other biological macromolecules. Covalent addition of these diol epoxides to DNA is well documented.[2-9] The major iso-

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lated adducts in most cases are derived from addition of an exocyclic amino group of a purine or pyrimidine base to the benzylic carbon of the epoxide, although adducts at other positions are possible. We have recently undertaken studies to develop a structural and spectroscopic data base of these nucleoside adducts formed upon covalent reaction ("bonding") with DNA of diol epoxides derived from several structurally diverse polycyclic aromatic hydrocarbons. In the course of these investigations we have observed a consistent relationship between the circular dichroism (CD) spectra of the nucleoside adducts and their absolute configurations at the benzylic carbon that is the site of nucleophilic attack by the purine or pyrimidine amino group. Specifically, *N*-2-deoxyguanosine (dG) adducts from diol epoxides of benzo[*c*]phenanthrene (BcPh),[5] benz[*a*]anthracene (BA),[10] and dibenz[*a,j*]-anthracene (DBA)[6] exhibit three principal CD bands at 240–250, 260–280, and 280–300 nm, with the central band the most intense. For adducts with *S* absolute configuration at the N-substituted benzylic carbon atom of the hydrocarbon moiety, this most intense band is always positive, whereas for adducts with *R* absolute configuration at this carbon, the sign of this band is negative. In the case of *N*-6-deoxyadenosine (dA) adducts, there are two major CD bands, at 240–275 and 280–290 nm. For dA adducts with *S* absolute configuration at the N-substituted benzylic carbon, the shorter wavelength band is positive and the longer wavelength band is negative, whereas for adducts with *R* absolute configuration at this carbon, these signs are reversed.

The CD spectra of nucleoside adducts[9,11,12] derived from the 7,8-diol 9,10-epoxides of benzo[*a*]pyrene (BaP) are more complex than those of adducts containing other hydrocarbon moieties because of the multiple absorption bands of the pyrene chromophore. Examination of the CD spectra of the guanosine (G) adducts[11] derived from the two enantiomers of BaP diol epoxide-2 indicated that the sign of the most intense band (250 nm) was positive for diol epoxide-2 adducts with 10*S* absolute configuration and negative for diol epoxide-2 adducts with 10*R* absolute configuration. Results consistent with those for the guanosine adducts of BaP diol epoxide-2 have recently also been obtained for the corresponding *deoxy*guanosine adducts.[9]

The observed CD bands result from an exciton interaction between the purine and hydrocarbon chromophores.[13,14] Their sign depends on the skew sense of the electric dipole transition moments of these chromophores and hence is determined by the average orientation of these groups with respect to each other and should be independent of the orientation of the nonchromophoric hydroxyl substituents on the benzo ring (diol epoxide-1 vs diol epoxide-2 adducts) or on the sugar. Thus, the chiral centers on the sugar and the saturated benzo ring which

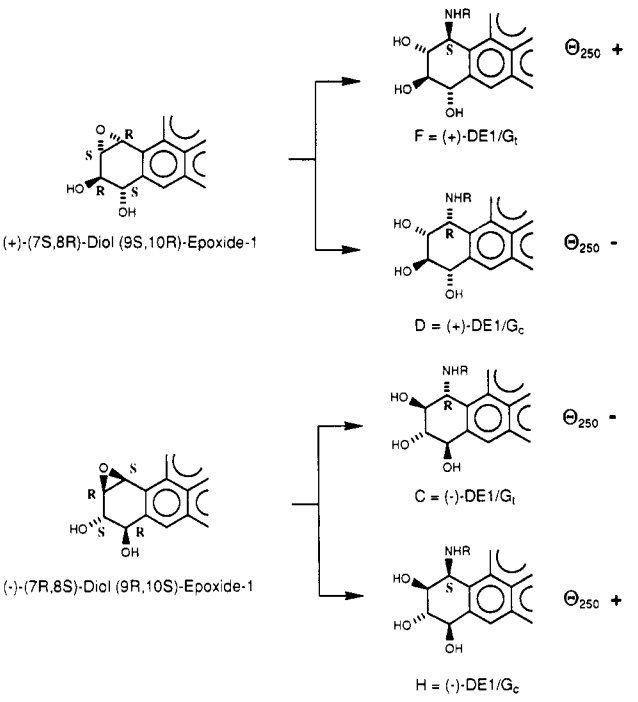

Figure 1. Partial structures of the nucleoside adducts derived from the optically active isomers of benzo[*a*]pyrene diol epoxide-1 and polyguanylic acid. Abbreviations for adducts derived from cis opening of the epoxide are designated with a subscript c, and those for adducts derived from trans opening are designated with a subscript t. The signs in parentheses in these abbreviations indicate the diol epoxide enantiomer from which individual adducts were derived. Signs of the observed Cotton effects at 250 nm are shown.

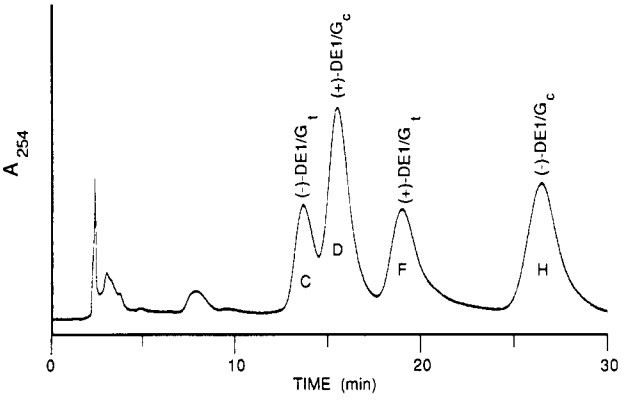

Figure 2. HPLC trace (254 nm) of the guanosine adducts derived from polyguanylic acid which had been modified with benzo[*a*]pyrene diol epoxide-1. For chromatographic conditions, see text. The capital letter designations for the peaks correspond to those in ref 11. Abbreviations are defined in the legend to Figure 1.

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have simple nonaromatic oxygen substituents contribute little to the observed CD spectra. However, we had previously reported[11] a relationship between absolute configuration at C-10 and the signs of the CD bands for guanosine adducts derived from racemic BaP diol epoxide-1 that was *opposite* to the relationship observed for the corresponding adducts from diol epoxide 2. Thus, in our original study, *positive* CD bands at 250 nm corresponded to chromatographic peaks that were assigned to the cis adduct derived from (+)-(7*S*,8*R*)-diol (9*S*,10*R*)-epoxide-1 and the trans adduct derived from (-)-(7*R*,8*S*)-diol (9*R*,10*S*)-epoxide-1, both of which should have 10*R* absolute configuration. Thus, there appeared to be a discrepancy in our previous assignment of structures and CD spectra to the diol epoxide-1 adducts. As reported here, reexamination of the NMR spectra of these adducts derived upon covalent addition of BaP diol epoxide-1 to poly G has shown that the assignment of cis vs trans addition of the purine amino group to diol epoxide-1 was reversed in the original study, although the previously reported[11] CD spectra correspond correctly to the chromatographic[11,15] peaks. The correct structures of the adducts and their retention times on HPLC are shown in Figures 1 and 2.

In the present study we also report the adenosine (A) adducts from BaP diol epoxide-1. Together with the corresponding diol epoxide-2 adducts prepared by Jeffrey et al.,[12] these adducts complete the set of BaP diol epoxide adducts from the purine *ribo*nucleosides. In the course of these studies, an unusual polar adduct formed from (-)- but not from (+)-diol epoxide-1 upon reaction with adenosine 5'-monophosphate at pH 7 was characterized. The data are most consistent with a structure that results from O-alkylation of the sugar at the 2'-hydroxyl group of adenosine by the diol epoxide. We also report a quantitative evaluation of the covalent bonding of BaP diol epoxides-1 and -2 to calf thymus DNA and identification by CD spectroscopy of the principal adducts formed from the diol epoxide-1 enantiomers, as well as several minor adducts formed upon reaction of BaP diol epoxide-2 with denatured DNA.

Experimental Section

Optically active[16] and racemic[17] BaP 7,8-diol 9,10-epoxides were prepared as described. Chemical ionization (NH_3) mass spectra were measured using a quadrupole mass spectrometer with a direct exposure probe. The pH titration curve for the adduct, (-)-DE1/A_s, obtained from (-)-BaP diol epoxide-1 by modification of the ribose moiety of adenosine was determined at 25 °C by measurement of CD spectra[18] upon addition of aliquots of a stock solution of the adduct to solutions whose pH was maintained either with HCl (0.1 M), NaOH (0.01 M), or buffers (0.05 M). The final composition of the mixtures was 1:9 CH_3OH/H_2O. Reversibility of the spectral changes observed at the pH extrema (1.1 and 11.8) was verified by adjusting the pH of these solutions to 5–8 and redetermination of the CD spectra, which were identical with those measured directly in buffer solutions near neutrality. The pK_a was determined from plots of Θ_{obs} at 245 nm vs pH.

Guanosine Adducts of Benzo[*a*]pyrene Diol Epoxide-1. Racemic diol epoxide-1 was allowed to react with poly G as described.[11] After base hydrolysis, the reaction mixture was applied to a C_{18} Sep-pak (Waters Associates), which was washed with 10 mL of H_2O and 10 mL of 25% CH_3OH in H_2O. The modified nucleotides were eluted with CH_3OH. Upon evaporation of the CH_3OH, the modified nucleotides were subjected to hydrolysis with *E. coli* alkaline phosphatase. HPLC of the reaction products on a Waters Associates C_{18} μBondapak column (4.6 × 250 mm), eluted with 45% CH_3OH in H_2O at a flow rate of 1.2 mL/min, yielded four major peaks (detected at 254 nm); t_R, 13.8, 15.6, 19.3, 27.1 min, in a ratio of ca. 14:34:22:30 (Figure 2). Use of optically active (+)-diol epoxide-1 confirmed that the two central peaks (D and F) arose from this enantiomer. The individual adducts were separated by preparative HPLC on the column described and acetylated (pyridine/acetic anhydride). Fully acetylated adducts were purified by HPLC on a Du Pont Zorbax SIL column (9.4 × 250 mm) eluted with CH_2Cl_2–EtOAc–CH_3OH, 90/7/3, at a flow rate of 5.5 mL/min. Retention times for all four acetates were ca. 6.5–7 min.

Adenosine Adducts of Benzo[*a*]pyrene Diol Epoxide-1. To an aqueous solution (20 or 50 mL) of adenosine-5'-monophosphate (10 mg/mL) at pH 7 was added 2–5 mg of the diol epoxide as a solution (1 mg/mL) in either acetone or THF in two equal portions, at time zero and 1 h. The final ratio of organic solvent to water was 1:10. Reaction was allowed to proceed at 37 °C for 12–18 h. At the end of this time tetrols were removed by repeated extraction of the solution with EtOAc and then with ether. Traces of organic solvents were removed by purging the solution with N_2, and the aqueous solution was adsorbed on a C_{18} Sep-pak, which was washed with H_2O to remove most of the unmodified nucleotide, and then eluted with CH_3OH. The methanolic eluate was concentrated to dryness. Enzymatic cleavage of the 5'-phosphate group was carried out with *E. coli* alkaline phosphatase (Sigma Chemical Co., Type III, 8–10 units) in Tris·HCl buffer (50 mM, pH 9.4, or 200 mM, pH 8.5) at 37 °C for 8–18 h. The reaction mixture was then adsorbed on a C_{18} Sep-pak, which was washed with H_2O and eluted with CH_3OH. For analytical HPLC, a Du Pont Zorbax ODS column (4.6 × 250 mm) was eluted at 1.5 mL/min with a linear gradient from 45% to 100% CH_3OH in H_2O in 30 min; absorbance was monitored at 340 nm. Adducts from (+)-diol epoxide-1 were purified by preparative HPLC on a Waters Associates C_{18} μBondapak column eluted with 45% CH_3OH in Tris-acetate buffer (pH 7, 50 mM) for 17 min, ramped to 75% CH_3OH in 1 min and then to 100% CH_3OH in 7 min, at a flow rate of 1.5 mL/min; t_R (minor adduct) 16 min, (major adduct) 23 min. For NMR spectroscopy, these adducts were acetylated (pyridine/acetic anhydride), and the acetates were purified by HPLC on a Du Pont Zorbax SIL column (9.4 × 250 mm) eluted with CH_2Cl_2–EtOAc–CH_3OH, 95/4/1, at a flow rate of 5.5 mL/min. Values of k' ca. 3.0 were observed for both acetylated adducts.

Isolation of a Covalent Adenosine Adduct Modified on a Sugar Hydroxyl Group by (-)-Benzo[*a*]pyrene Diol Epoxide-1. During the course of preparation of the adenosine adducts of the BaP diol epoxides, a major early eluting product (10.5 min), which possessed a slightly different UV spectrum from the other adducts, was observed. This product was derived from (-)-diol epoxide-1 but not from (+)-diol epoxide-1. This adduct was purified by HPLC on the Du Pont Zorbax ODS column, using the conditions described above for analytical chromatography on this column. The fully acetylated derivative (pyridine/acetic anhydride) prepared from the adduct was purified by HPLC on a Du Pont Golden Series SIL column (9.4 × 100 mm) eluted at 2 mL/min with CH_2Cl_2–EtOAc–CH_3OH, 87/10/3; t_R of the collected product (>80% of total A_{340} in the acetylation mixture), 1.85 min.

Covalent Modification of DNA by Benzo[*a*]pyrene Diol Epoxides. Quantitation of Adducts. In a typical experiment, racemic BaP diol epoxide-2 (0.8 mL of a solution of ca. 0.5 mg/mL diol epoxide in CH_3CN containing 1–2% DMSO) was added to 8 mL of dialyzed calf thymus DNA (0.8 mg/mL in 10 mM Tris·HCl buffer, pH 7.4) and allowed to react at 37 °C for 1 h. The reaction mixture was extracted four times with an equal volume of EtOAc and twice with ether to remove the tetrol hydrolysis products and then purged with N_2 to remove organic solvents. Sufficient 1 M $MgCl_2$ was added to give a final concentration of 10 mM, 485 units of bovine pancreatic DNase I (Type IV, Sigma Chemical Co.) were added per mL of the solution, and the mixture was incubated at 37 °C for 1.5 h. At the end of this time, the pH was adjusted to ca. 9.0 with 1 M Tris base, and 1%

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Table I. Chemical Shifts and Coupling Constants[a] for Selected Protons of the Acetylated Ribonucleoside Adducts Formed upon Modification of the Exocyclic Amino Group by Benzo[a]pyrene Diol Epoxide-1

	hydrocarbon methine hydrogens				sugar methine hydrogens		
compound[b] (as acetate)	H-7	H-8	H-9	H-10	H-1′	H-2′	H-3′
(+)-DE1/G_c (D)	6.64	5.98	5.63	6.96	6.26	5.94	5.71
		($J_{7,8}$ 8.2, $J_{8,9}$ 11.7, $J_{9,10}$ 4.3, $J_{1',2'}$ 4.6, $J_{2',3'}$ 5.8)					
(−)-DE1/G_c (H)	6.66	5.97	5.70	6.96	6.30	6.24	5.60
		($J_{7,8}$ 8.2, $J_{8,9}$ 11.8, $J_{9,10}$ 4.3, $J_{1',2'}$ 5.4, $J_{2',3'}$ 5.0)					
(+)-DE1/G_t (F)	6.61	5.58	5.89	6.49	6.21	6.29	5.63
		($J_{7,8}$ 5.3, $J_{8,9}$ 6.4, $J_{9,10}$ 3.9, $J_{1',2'}$ 5.5, $J_{2',3'}$ 5.3)					
(−)-DE1/G_t (C)	6.63	5.63	5.88	6.56	6.24	6.11	5.76
		($J_{7,8}$ 5.9, $J_{8,9}$ 6.4, $J_{9,10}$ 4.3, $J_{1',2'}$ 4.6, $J_{2',3'}$ 5.2)					
(+)-DE1/A_c	6.59	6.27	5.72	7.27	6.25	6.16	5.77
		($J_{7,8}$ 8.1, $J_{8,9}$ 11.9, $J_{9,10}$ 4.4, $J_{1',2'}$ 5.2, $J_{2',3'}$ 5.4)					
(+)-DE1/A_t	6.79	5.63	5.89	6.84	6.25	6.12	5.79
		($J_{7,8}$ 6.1, $J_{8,9}$ 7.1, $J_{9,10}$ 4.1, $J_{1',2'}$ 5.2, $J_{2',3'}$ 5.4)					

[a] Spectra were measured in acetone-d_6 at 300 MHz. [b] Letters in parentheses identify the chromatographic peaks (for the unacetylated compounds) shown in Figure 2.

NaN_3 solution was added to give a final concentration of 0.02% NaN_3. The reaction mixture was divided into four equal portions, which were incubated for 40 h at 37 °C with 0.023, 0.12, 0.58, and 1.75 units, respectively, of *Crotalus atrox* phosphodiesterase I (Type VII, Sigma Chemical Co.). These quantities of phosphodiesterase correspond to 0.014, 0.075, 0.37, and 1.1 unit per mg of DNA. *E. coli* alkaline phosphatase (10 units) was then added to each reaction mixture, and hydrolysis was allowed to proceed for 3 h at 37 °C. Upon completion of the enzymatic hydrolysis, the reaction mixtures were adsorbed on C_{18} Sep-paks which were washed with 50 mL of H_2O to remove unmodified nucleosides and then eluted with 15 mL of CH_3OH. The CH_3OH was evaporated, the residue was redissolved in a measured quantity (typically 0.5 mL) of 1/1 CH_3OH–H_2O, and the products were analyzed by HPLC on an Altex Ultrasphere C_{18} column (4.6 × 250 mm) eluted at 1 mL/min with 50% CH_3OH in H_2O for 10 min, followed by a linear gradient which increased the CH_3OH content by 1% per min for 25 or 35 min.

Results obtained with varying concentrations of snake venom phosphodiesterase indicated identical recoveries and distributions of adducts at the two highest concentrations of enzyme used, whereas the lowest concentration gave incomplete recovery of the deoxyadenosine adducts. Consequently, a phosphodiesterase concentration of 0.37 unit/mg DNA was routinely used in subsequent experiments.

Recovery of adducts relative to the precursor diol epoxide was quantitated by comparison of the areas of integration of the HPLC peaks (343 nm) with the areas obtained upon HPLC of a reaction mixture containing a measured quantity of the diol epoxide stock solution that had been hydrolyzed to tetrols in 1/9 dioxane-H_2O containing 1 mM $HClO_4$. Tetrol recovery from the DNA-catalyzed hydrolysis of the diol epoxide that occurs concurrently with adduct formation was also quantitated by HPLC of a measured sample of the tetrols obtained upon evaporation of the pooled organic extracts.

In experiments with denatured DNA, 2 mL of the DNA solution (0.8 mg/mL) was heated for 5 min at 100 °C and immediately cooled in ice. The diol epoxide-2 solution was added to the cold sample, and the mixture was then warmed to 37 °C and allowed to react for 45 min. An identical control experiment with native DNA was performed simultaneously. Subsequent processing of the samples followed the procedure described above, using 0.37 unit of *Crotalus atrox* phosphodiesterase per mg of DNA. For identification of products from the optically active isomers of diol epoxide-2, denatured DNA (10 mL, 0.8 mg/mL) was treated with 1.0 mL of either (+)- or (−)-diol epoxide-2 (1 mg/mL) in two equal portions at ca. 10-min intervals. For enzymatic hydrolysis, 0.23 units of the phosphodiesterase was used. After workup and chromatography of the nucleoside adducts as described, CD spectra of the components were measured, using when required 50- or 100-μL capacity microspectrophotometric cells (Hellma Cells Inc., Jamaica, NY) with a path length of 1 cm. For mass spectral determinations, isolated adducts were acetylated with acetic anhydride in pyridine. When required, the resultant peracetates were purified by chromatography on a Du Pont Zorbax SIL cartridge column (4.6 × 80 mm), eluted at 2 mL/min with either solvent A (CH_2Cl_2–EtOAc–CH_3OH, 20/10/1), or solvent B (CH_2Cl_2–EtOAc–CH_3OH, 50/10/1). Retention times were 2.2 min for the peracetate of (−)-DE2/dG_c (solvent A) and 1.8 min for the peracetate of (−)-DE2/dC (solvent B).

Nucleoside adducts from racemic BaP diol epoxide-1 (0.5 mg/mL, 0.8 mL) and native calf thymus DNA (0.8 mg/mL, 8 mL) were prepared analogously. With this diol epoxide, maximal recoveries of the two major dG adducts were observed only at the highest (1.1 unit/mg DNA) concentration of snake venom phosphodiesterase, and recoveries of these adducts appeared to decrease upon storage of the adduct sample. We ascribed this effect to poor solubility of these adducts. Consequently, a second experiment was performed using half the final concentration of diol epoxide-1. Enzymatic hydrolysis of the modified DNA was carried out as described, using 1.1 and 2.1 units of snake venom phosphodiesterase per mg of DNA. In both cases identical recoveries and adduct distributions were obtained, and these agreed well with those observed previously at the highest phosphodiesterase concentration upon prompt analysis of the adduct sample. Sufficient quantities of adducts for CD spectroscopy (in CH_3OH) were obtained by scale up of the described procedures. The origin of individual adducts was determined by comparison of the HPLC profile of the adducts from racemic diol epoxide-1 with the adduct profiles obtained upon reaction of the optically pure enantiomers with DNA.

Results and Discussion

Guanosine Adducts of Benzo[a]pyrene Diol Epoxide-1. Reassignment of Cis vs Trans Stereochemistry of Addition. An HPLC trace of the guanosine adducts derived upon reaction of poly G with racemic BaP diol epoxide-1 (Figure 2) closely resembles that previously reported[11] for these adducts under slightly different conditions. Peaks D and F were confirmed to be derived from (+)-diol epoxide-1. As previously reported, CD spectra of compounds C and D exhibited negative Cotton effects at 250 nm, whereas compounds F and H exhibited positive Cotton effects. Upon isolation and acetylation, these four products gave hexaacetates (m/z 838; MH^+). ^1H NMR data for the acetates are summarized in Table I. Since the bond to the nucleoside moiety in the bay region must be pseudoaxial, H-7, H-8, and H-9 are all pseudoaxial in a chair conformation of the cis adducts. Thus, the large coupling constants, $J_{8,9}$ and $J_{7,8}$, of ca. 12 and 8 Hz, respectively, in the acetylated adducts D and H are diagnostic of cis addition of the nucleoside to the oxirane of diol epoxide-1. Similarly, large coupling constants are observed for the nucleoside adducts formed upon cis opening of the epoxide ring of diol epoxide-1 diastereomers

derived from dibenz[a,j]anthracene[6] and benzo[c]-phenanthrene.[5]

In the case of adducts C and F, all the coupling constants for the methine protons of the tetrahydro benzo ring are small, consistent with trans addition of the purine to give a preferred conformation in which all the methine hydrogens are pseudoequatorial. Thus, we conclude that major adducts D and H, previously assigned as trans, are actually cis, whereas minor adducts C and F are trans. In the original report from this laboratory,[11] the major product isolated upon methylation, deglycosylation, and acetylation of a *mixture* of C, D, F, and H was found to be derived from trans addition, whereas the minor product was derived from cis addition. In both the present and the earlier report, the cis adducts D plus H comprise a slightly larger fraction of the total mixture than the trans adducts C and F. Thus, the discrepancy in our earlier assignments may have been a result of selective loss of cis adducts during the methylation/deglycosylation/acetylation sequence, which led to an erroneous conclusion based upon relative proportions of these adducts before and after this series of reactions, which convert D plus H as well as C plus F to single, racemic compounds. The present structural assignments by NMR, together with the CD spectra of the corresponding compounds, indicate that the sign (cf. Figure 1) of the major CD band at 250 nm is positive for BaP diol epoxide-1 adducts with 10*S* absolute configuration and negative for such adducts with 10*R* absolute configuration. This observation is consistent with the signs of the CD bands for adducts of BaP diol epoxide-2 with G[11] or dG[9] (which differ only in the configuration of the 7- and 8-hydroxyl groups relative to the epoxide) as well as previous observations of dG adducts in both the diol epoxide-1 and -2 series derived from BcPh,[5] BA,[10b] and DBA.[6] Thus, for all hydrocarbons studied to date, *guanosine or deoxyguanosine adducts having S absolute configuration* at the N-substituted benzylic carbon atom of the tetrahydroaromatic moiety exhibit a *positive ellipticity* for their most intense CD band (ca. 250–280 nm).

Adducts from Benzo[*a*]pyrene Diol Epoxide-1 at the Exocyclic Amino Group of Adenosine. Structures and CD spectra of the four nucleoside adducts at the exocyclic nitrogen formed upon reaction of poly A with BaP diol epoxide-2 have been reported,[12] as have the corresponding dA adducts[9,12] derived from DNA, poly(dA) or deoxyadenylic acid. Although the CD spectra of these adducts are complex, there exists (as in the case of the G and dG adducts) a clear correlation between the shapes of the CD spectra for both the A and dA adducts and their absolute configurations at the benzylic C-10 that is the site of nucleophilic addition; namely, the major CD bands at 244 and 279 nm are positive in the case of 10*S* adducts and negative in the case of 10*R* adducts. In the present study, we have prepared the four corresponding A adducts from diol epoxide-1 and 5′-AMP to complete the series of G and A adducts from the BaP diol epoxides. Figure 3 shows HPLC profiles of adenosine adducts derived from (+), (−), and racemic diol epoxide-1. Only two adduct peaks were observed from (+)-diol epoxide-1, whereas additional peaks were obtained from its (−) enantiomer that were also present in adduct mixtures derived from the racemic diol epoxide. Peak (−)-DE1/A_s, which constituted a major product from (−)-diol epoxide-1, was subsequently isolated and identified as an adduct modified on the sugar (see below). Upon acetylation, adducts (+)-DE1/A_c and (+)-DE1/A_t gave hexaacetates (*m/z* 822; MH^+), whose NMR spectra (Table I) were measured in acetone-d_6. As

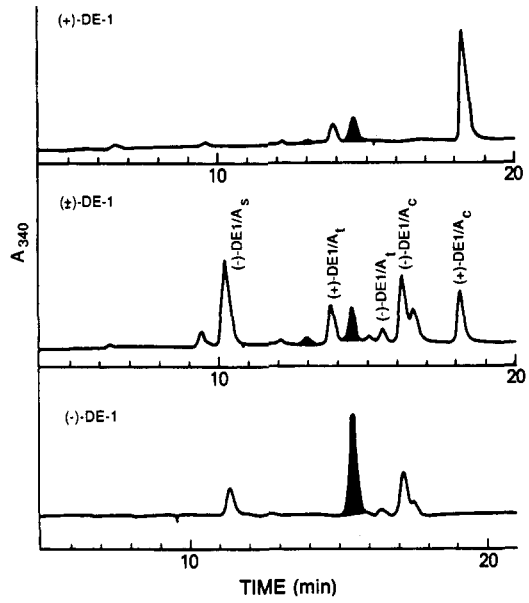

Figure 3. Analytical HPLC profiles (340 nm) of adenosine adducts derived from adenosine-5′-monophosphate which had been modified with optically active or racemic benzo[*a*]pyrene diol epoxide-1. For chromatographic conditions, see text. Tetrols produced upon hydrolysis of the diol epoxides are shaded; the chromatograms were aligned by using the major tetrol as an internal reference. Abbreviations are defined in the legend to Figure 1. Peak (−)-DE1/A_s is derived from O-alkylation of a sugar hydroxyl group.

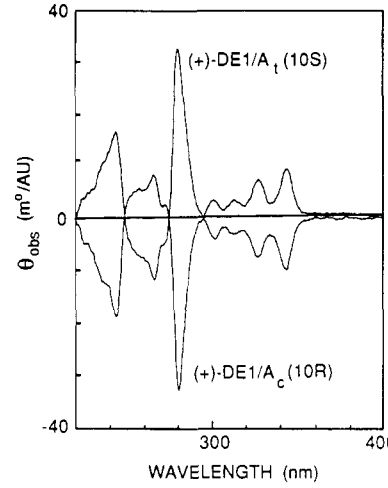

Figure 4. Circular dichroism spectra (in methanol, normalized to 1 absorbance unit at 278 nm) of the adenosine adducts formed upon covalent modification at the exocyclic amino group by the (+)-(7*S*,8*R*)-diol (9*S*,10*R*)-epoxide-1 derived from benzo[*a*]pyrene.

in the case of the G adducts, the large coupling constant $J_{8,9}$ of 11.9 Hz is diagnostic of cis addition at the benzylic position of the epoxide. Furthermore, in the case of the cis but not the trans adducts, the identity of the purine base influences the chemical shift of H-8 on the benzo ring, such that this hydrogen in the A adduct is deshielded (ca. 0.3 ppm) relative to the G adduct. A similar effect of the purine base in the case of the cis adducts obtained from dibenz[a,j]anthracene diol epoxide-1 and dG or dA was ascribed[6] to the proximity of the corresponding hydrogen (H-3 in these adducts) and the purine, which are in a cis, diaxial orientation.

CD spectra of (+)-DE1/A_c and (+)-DE1/A_t are shown in Figure 4. In these adducts, the signs of the CD bands are in agreement with those observed for the diol epoxide-2 adducts[9,12] of adenosine or deoxyadenosine with corre-

Table II. Chemical Shifts and Coupling Constants[a] for Selected Protons of Model Compounds and of an Adenosine Adduct from (−)-Benzo[a]pyrene Diol Epoxide-1, (−)-DE1/A$_s$, Formed by O-Alkylation of the 2′-Hydroxyl Group on the Sugar

compound	hydrocarbon methine hydrogens				sugar methine hydrogens				purine hydrogens
	H-7	H-8	H-9	H-10	H-1′	H-2′	H-3′	H-4′, H-5′, H-5″	
adenosine[b]					6.06	4.78	4.45	3.9–4.3	8.21, 8.32
						($J_{1',2'}$ 5.8, $J_{2',3'}$ 5.6, $J_{3',4'}$ 5.8)			
cis-1 methyl ether[c]	4.76	4.23	3.93	5.48					
		($J_{7,8}$ 8.1, $J_{8,9}$ 10.5, $J_{9,10}$ 2.9)							
(−)-DE1/A$_s$	4.78	4.35	3.90	5.96	6.04	5.09	4.45	3.2–4.0	7.94, 8.46
			($J_{7,8}$ 7.5, $J_{8,9}$ 10.2, $J_{9,10}$ 3.9, $J_{1',2'}$ 6.2, $J_{2',3'}$ 5.6, $J_{3',4'}$ 2.7)						
adenosine (pentaacetate)					6.40	6.10	5.78	4.3–4.6	8.69, 8.97
						($J_{1',2'}$ 4.8, $J_{2',3'}$ 5.4, $J_{3',4'}$ 4.4)			
cis-1 methyl ether[c] (triacetate)	6.62	6.08	5.49	5.75					
		($J_{7,8}$ 7.5, $J_{8,9}$ 11.3, $J_{9,10}$ 2.9)							
(−)-DE1/A$_s$ (heptaacetate)	6.64	6.07	5.57	6.28	6.55	5.62	5.24	4.0–4.4	8.83, 9.04
			($J_{7,8}$ 7.1, $J_{8,9}$ 11.2, $J_{9,10}$ 1.9, $J_{1',2'}$ 4.9, $J_{2',3'}$ 5.6, $J_{3',4'}$ 4.6)						

[a] Spectra were measured at 300 MHz in acetone-d_6 unless otherwise noted. Assignments were made by decoupling as required. [b] In D_2O. [c] At 500 MHz.

sponding absolute configurations at C-10. The structures of the adducts from the enantiomeric diol epoxide, (−)-DE1/A$_c$ and (−)-DE1/A$_t$, whose NMR spectra were not measured, were assigned on the basis of their CD spectra which were essentially equal and opposite to those shown in the figure. These observations also lend support for the use of CD in the tentative assignment[19] of absolute configuration to several dG and dA adducts derived from DNA upon reaction with 7,8,9,10-tetrahydro BaP 9,10-epoxide. Specifically, dG adducts with positive CD bands at 247 nm and a dA adduct[20] with a positive band at 280 nm have 10*S* absolute configuration.

Relationship of CD Spectra with the Absolute Configuration of PAH Adducts at the Exocyclic Nitrogen of Purine Nucleosides. On the basis of this and previous studies, we propose the following empirical "rule" relating the signs of the major long wavelength band in the CD spectrum with absolute configuration of these adducts: For guanine nucleoside adducts at the exocyclic nitrogen (N-2), or adenine nucleoside adducts at the exocyclic nitrogen (N-6), the sign of the most intense CD band in the long-wavelength region (ca. 250–280 nm in the adducts observed to date) is positive when the absolute configuration at the N-substituted benzylic carbon of the tetrahydroaromatic moiety is *S* and negative when this configuration is *R*. If two bands in this region are of approximately equal intensity, then the shorter wavelength band is (as above) positive for *S* and negative for *R* absolute configuration at the N-substituted benzylic carbon atom; the corresponding long wavelength bands are reversed in sign. For example, the dA adducts of dibenz[*a*,*j*]anthracene diol epoxides[6] exhibit CD bands at 270 and 290 nm that are of similar intensity; in this case, the shorter wavelength band (which is slightly weaker) is negative for adducts with *R* absolute configuration at the N-substituted benzylic carbon and positive for adducts with *S* absolute configuration.

Adenosine[7] and deoxyadenosine[21] adducts of 7,12-dimethylbenz[*a*]anthracene (DMBA) 3,4-diol 1,2-epoxide-1 also exhibit two CD bands of approximately equal intensity. Two such adducts with opposite absolute configurations at C-1 result from cis opening (as assigned by NMR) of the racemic diol epoxide by the exocyclic nitrogen of adenylic acid or poly A; the corresponding trans adducts, whose NMR spectra were not reported, were identified by their CD spectra. On the basis of CD and NMR spectra, the elution orders on reverse phase HPLC for the deoxyadenosine adducts of DMBA diol epoxide-1 were found to be the same as those for the corresponding adenosine adducts. Assignment of absolute configuration to the major (trans) dA adduct of diol epoxide-1 observed upon incubation of the hydrocarbon with fetal mouse cells was based on its formation from optically active DMBA (4*S*,3*S*)-dihydrodiol.[22] Thus, this trans adduct was derived from DMBA (4*S*,3*R*)-diol (2*S*,1*R*)-epoxide-1 and must have 1*S* absolute configuration. As predicted by the proposed rule, this adduct exhibits a positive CD band at 260 nm and a negative band at ca. 275 nm. Based on limited available data, the same correlation of the signs of CD spectral bands with absolute configuration at the N-substituted benzylic carbon atom also appears to apply for deoxyribonucleoside adducts derived from 5-methylchrysene-1,2-diol 3,4-epoxide-2, in which the most intense band (at ca. 260 nm for dG and ca. 275 nm for dA adducts) is positive for trans adducts with 4*S* absolute configuration and negative for those with 4*R* absolute configuration.[8]

The existence of the correlations described requires that the average conformation preferred by these adducts (in terms of the skew sense between the aromatic rings whose electronic transitions give rise to the observed exciton interactions) must involve overlap between the purine and hydrocarbon moieties and must be the same for adducts with a given absolute configuration at the N-substituted benzylic carbon atom. Since these molecules, naively considered, would appear to be quite flexible, this conclusion is of considerable interest because of its implication that they must have a nonrandom preferred conformation in solution.

Characterization of a Novel Adenosine Adduct of (−)-Benzo[a]pyrene Diol Epoxide-1 Derived from Alkylation of the Sugar. As noted above, an adduct, (−)-DE1/A$_s$, t_R 10.5 min, that was not formed from (+)-diol epoxide-1, constituted a substantial fraction of the adduct yield derived from reaction of adenosine 5′-monophosphate with the (−) enantiomer. The CD spectrum of the unacetylated nucleoside adduct (inset, Figure 5) was similar in intensity but different in shape when compared with the spectra of the adducts derived from alkylation of the

(19) Kinoshita, T.; Lee, H. M.; Harvey, R. G.; Jeffrey, A. M. *Carcinogenesis* **1982**, *3*, 255–260.

(20) The authors' assignment of 10β absolute stereochemistry to the dA adduct with a positive CD band at 280 nm is correct, but this corresponds to the 10*S* configuration, and not 10*R* as had been incorrectly stated in the paper.

(21) Cheng, S. C.; Prakash, A. S.; Pigott, M. A.; Hilton, B. D.; Lee, H.; Harvey, R. G.; Dipple, A. *Carcinogenesis* **1988**, *9*, 1721-1723.

(22) Vericat, J. A.; Cheng, S. C.; Dipple, A. *Carcinogenesis* **1989**, *10*, 567–570.

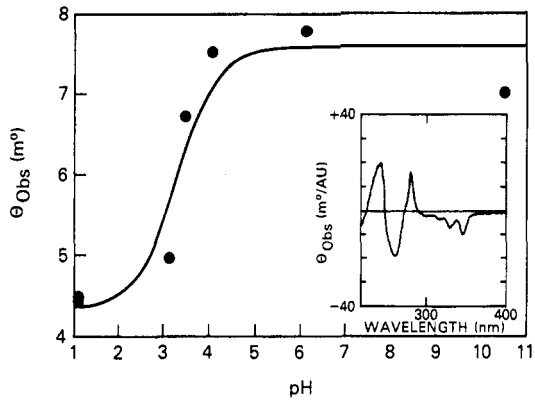

Figure 5. Dependence on pH of the observed CD signal at 245 nm for the adduct (-)-DE1/A_s in 1:9 methanol/water. The complete CD spectrum of this adduct (in methanol, normalized to 1 absorbance unit at 246 nm) is shown in the inset.

exocyclic amino group (cf. Figure 4). The pH dependence of the observed ellipticity at 245 nm (Figure 5) gave a titration curve[18] with a pK_a of 3.2. This pK_a is inconsistent with substitution at N-1, since reported pK_a values for 1-alkyladenosines are >8,[23a] whereas the pK_a for protonation at the unsubstituted N-1 of adenosine[23b] is 3.5–3.6. Given the facile deglycosylation of N-3 or N-7 alkylated adenine nucleosides in DNA and deoxyadenylic acid, addition of the diol epoxide moiety at N-7 or N-3 appears unlikely:[24] if the hydrocarbon substituent were at N-3 or N-7, the bond to the sugar would not be expected to survive the conditions used for preparation of this adduct. Attachment to C-8 with concomitant loss of a hydrogen at this position is excluded by the observation of two singlets in the NMR spectrum (Table II) assignable to purine protons H-2 and H-8. Thus, we conclude that the most likely site of substitution was on one of the free hydroxyl groups, 2′-OH or 3′-OH, of the sugar.

The product[25] formed upon cis attack of methanol on the benzylic carbon of BaP diol epoxide-1 in acid, indicated in Table II as cis-1 methyl ether, as well as the corresponding triacetate derivative, gave NMR spectra with chemical shifts for the methine protons H-7, H-8, and H-9 as well as coupling patterns that were virtually identical with those observed for (-)-DE1/A_s. Somewhat surprisingly, the chemical shift for H-10 appears about 0.5 ppm farther downfield in the adenosine adduct than in the simple model ether. This deshielding may result from an electronic effect of adjacent oxygen substituents in the sugar derivative, and/or from edge interaction with the adenine ring. Since a Dreiding model for the adduct is quite flexible, the orientation of the adenine relative to this hydrogen remains open to speculation.

Evidence for substitution at the 2′- rather than the 3′-hydroxyl group was obtained by preparation of the same adduct from adenosine-3′,5′-diphosphate (200 mg) and (±)-BaP diol epoxide-1 (2 mg). Following enzymatic hydrolysis, the products of this reaction were subjected to

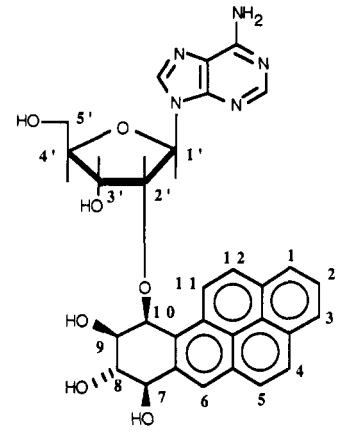

Figure 6. Proposed structure of the adduct (-)-DE1/A_s.

reverse-phase HPLC, and the compound (t_R 10.5 min) corresponding to (-)-DE1/A_s (cf. Figure 3) was isolated. The UV and CD spectra of this compound were identical with those of the adduct prepared from adenosine-5′-monophosphate. Upon conversion to its heptaacetate (m/z = 864 (MH^+)), the adduct generated from the 3′,5′-diphosphate gave an NMR spectrum that was identical with that of the adduct obtained from the 5′-monophosphate. Since, in the absence of any rearrangement of the phosphate group,[26] only the 2′-hydroxyl group is available for reaction in the 3′,5′-diphosphate, this result supports a structure for this adduct (Figure 6) in which addition has occurred at the 2′-hydroxyl group.

The NMR spectra of (-)-DE1/A_s before and after acetylation (Table II) are also more consistent with O-alkylation of the 2′- rather than the 3′-hydroxyl group. The mass spectrum of the acetylated derivative indicated that it was a heptaacetate, and thus two sites of acetylation must be on the purine moiety. For comparison, NMR data for the corresponding pentaacetate of adenosine as well as for the unacetylated nucleoside are given in Table II. Two points are worthy of note. (1) Values of $J_{7,8}$ and $J_{8,9}$ in both the acetylated and unacetylated adducts are large and are consistent with cis attack upon the benzylic C-10 position of the epoxide. (2) Acetylation of OH-2′ and OH-3′ of adenosine results, as expected, in substantial downfield shifts of the corresponding methine proton signals (1.3 ppm). The changes in δ upon acetylation of (-)-DE1/A_s are appreciably smaller: 0.53 and 0.79 ppm downfield for H-2′ and H-3′, respectively. In particular, the small downfield shift for H-2′ is consistent with the conclusion that acetylation did not (and could not) occur at this position, i.e. that this is the site of the benzylic ether group derived from the diol epoxide. Interpretation is complicated slightly by the fact that $\Delta\delta$ for H-3′ upon acetylation is also somewhat smaller than expected: 0.79 ppm. This is a result of the fact that *in the acetates*, H-3′ of the adduct is more shielded than H-3′ of adenosine, whereas this is not the case with the unacetylated compounds. Adduct formation on the 2′-hydroxyl group has been observed in the reaction of guanosine with the K-region 5,6-oxide of 7,12-dimethylbenz[*a*]anthracene (DMBA) under basic conditions.[14] As in the present case, a surprising degree of stereospecificity is exhibited by the DMBA oxide. Adducts derived from trans addition of the 2′-hydroxyl group to the 5- and 6-positions of the arene oxide were both obtained but only from the (5*R*,6*S*)-oxide

(23) (a) Macon, J. B.; Wolfenden, R. *Biochemistry* **1968**, *7*, 3453–3458. Martin, D. M. G.; Reese, C. B. *J. Chem. Soc. C* **1968**, 1731–1738. (b) Ts'o, P. O. P. In *Basic Principles in Nucleic Acid Chemistry*; Ts'o, P. O. P., Ed.; Academic Press: New York, 1974; Vol. I, pp 453–584.

(24) Lawley, P. D.; Brookes, P. *Biochem. J.* **1963**, *89*, 127–138. Kriek, E.; Emmelot, P. *Biochim. Biophys. Acta* **1964**, *91*, 59–66. Margison, G. P.; O'Connor, P. J. *Biochim. Biophys. Acta* **1973**, *331*, 349–356. Lawley, P. D. In *Screening Tests in Chemical Carcinogenesis* (IARC Scientific Publications, No. 12); Montesano, R., Bartsch, H., Tomatis, L., Eds.; IARC: Lyon, 1976; pp 181–208. Lawley, P. D.; Brookes, P. *Biochem. J.* **1964**, *92*, 19c–20c.

(25) Thakker, D. R.; Yagi, H.; Lu, A. Y. H.; Levin, W.; Conney, A. H.; Jerina, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3381–3385.

(26) Adenosine-2′- and -3′-monophosphates undergo this rearrangement in acidic solutions, but not in base. Brown, D. M.; Todd, A. R. *J. Chem. Soc.* **1952**, 44–51, 52–58.

enantiomer, although the reactant arene oxide was racemic. The same two adducts, and not their diastereomers, were among the products formed from DMBA upon modification of RNA of liver cells in culture.[27] Although adducts of BaP diol epoxides formed by alkylation of ribose have not to our knowledge been demonstrated in vivo, our present observation, together with the precedent of DMBA 5,6-oxide, suggests that modification of cellular RNA by this route may be possible.

Reinvestigation of DNA Adduct Formation from Benzo[a]pyrene Diol Epoxides. Quantitation of Adduct Recoveries and Identification of Adducts from Diol Epoxide-1. In 1979, Meehan and Straub[3] reported the chromatographic separation and identification of several adducts derived upon bonding of each of the enantiomers of benzo[a]pyrene diol epoxide-2 to DNA, and the more recent study by Cheng et al.[9] has provided detailed NMR and CD spectral data for all the possible adducts derived upon alkylation of the exocyclic amino groups of dG and dA by this diastereomer. To date, however, comparable studies of the corresponding diol epoxide-1 adducts have been unavailable. Furthermore, the covalent bonding efficiencies (relative to DNA-catalyzed[28] hydrolysis) of the four optically isomeric benzo[a]pyrene diol epoxides, in comparison to each other and to diol epoxides derived from other parent hydrocarbons, have been difficult to assess quantitatively.

In particular, sequences containing certain adducts (notably those derived from deoxyadenosine) are known to be partially resistant to nucleolytic cleavage by snake venom phosphodiesterase,[29,30] a key step in the enzymatic digestion of covalently modified DNA to the nucleoside level. As a result, recoveries of certain dA adducts are dependent on the amount of phosphodiesterase used for digestion at low enzyme concentrations, and plateau only at high enzyme concentrations. Thus, to ensure maximal recovery of such adducts, a phosphodiesterase concentration in this plateau region must be used. Furthermore, material balance experiments are required to determine whether all or most of the precursor epoxide can be accounted for as reaction products under a given set of experimental conditions. In the absence of radiolabeled diol epoxides, recoveries of diol epoxides as nucleoside adducts and as tetrols formed by DNA-catalyzed hydrolysis can reasonably be estimated by UV absorption spectroscopy, if the assumption is made that the nucleic acid bases make an insignificant contribution to the observed UV absorption of the adducts at the analytical wavelength. It should be pointed out that calculations[31] of adduct recovery based exclusively on the *difference* between the quantity of tetrols formed upon hydrolysis of a given amount of a diol epoxide in acid and in the presence of DNA may be misleading in the case of diol epoxides that are inefficient covalent bonders to DNA, since such calculations are based on the measurement of a small difference between large numbers. Furthermore, at neutral pH, diol epoxide-1 diastereomers may give variable quantities of ketone rearrangement products[17,32] that are unstable and/or are not detected chromatographically so that incomplete tetrol recoveries from neutral reaction mixtures containing DNA may not accurately reflect the amount of diol epoxide covalently bound to DNA. The foregoing considerations, discussed[33] in connection with studies of DNA bonding by diol epoxides of BaP and other hydrocarbons, have been taken into account in the present investigation.

Table III. Covalent Bonding Efficiency and Adduct Distribution for Benzo[a]pyrene 7,8-Diol 9,10-Epoxides with Calf Thymus DNA[a]

enantiomer	percent bound to DNA	adduct distribution, % (t_R)[b] dG trans	dG cis	dA trans	dA cis
(−)-(*R,S,R,S*)-DE1	5	4 (20.1)	82 (24.5)	≤2[c]	9 (32.2)
(+)-(*S,R,S,R*)-DE1	3–4	10 (21.1)	72 (22.8)	≤2[c]	15 (34.0)
(−)-(*S,R,R,S*)-DE2[d]	2–3	50 (20.1)	9	36 (28.6)	3 (27.7)
(+)-(*R,S,S,R*)-DE2	13–15	93 (22.2)	2.5	3.5 (26.8)	<1 (29.7)
(−)-(*S,R,R,S*)-DE2[e,f]	9	37	15	21	7
(+)-(*R,S,S,R*)-DE2[e]	16	85	6	5	3

[a] At pH 7.4, 37 °C. Absolute configurations of the tetrahydro benzo ring carbon atoms are designated in sequence, beginning at C-7. Because of the presence of very minor, unidentified peaks in the profiles, the identified products do not always sum to exactly 100%. [b] Retention time (min) under the chromatographic conditions described in the text. [c] Upper limit; no HPLC peak that could be assigned to this adduct was observed. [d] Approximately 2% of an adduct with a retention time corresponding to compound X (Figure 8), tentatively assigned as a deglycosylated dG adduct, was also formed from this isomer. [e] Reaction with denatured DNA. [f] Approximately 2% of a dC adduct and 12–20% of adduct X (Figure 8) were also observed.

A material balance, based on recovery of products as measured by HPLC detection at 343 nm (see the Experimental Section), indicated that, after completion of the bonding reaction with native calf thymus DNA, 85% of racemic BaP diol epoxide-2 was recovered as tetrols upon extraction of the DNA solution and 1.4% as tetrols that were present in the mixture of modified nucleosides obtained after enzymatic hydrolysis. Nucleoside adducts corresponded to 9% of the total yield of products from the diol epoxide. Thus, ca. 95% of the parent diol epoxide could be accounted for. Of the covalent adducts formed, substantially more adducts were derived from the (+) than the (−) enantiomer; 15% of the (+)-diol epoxide-2 was converted to adducts whereas only 2.3% of the (−) enantiomer was covalently bound to the DNA. Experiments with the individual enantiomers confirmed this result: 13% of the (+) enantiomer and 2.1% of the (−) enantiomer were converted to covalent adducts (cf. Table III). Total observed recoveries of tetrols plus adducts were 96% and 105% respectively from the (+) and (−) enantiomers. Hence, there is no evidence for any *substantial* conversion of diol epoxide-2, upon reaction with native DNA, to adducts[34] of undetermined structure that are undetectable by the methods used, unless such adducts could be readily

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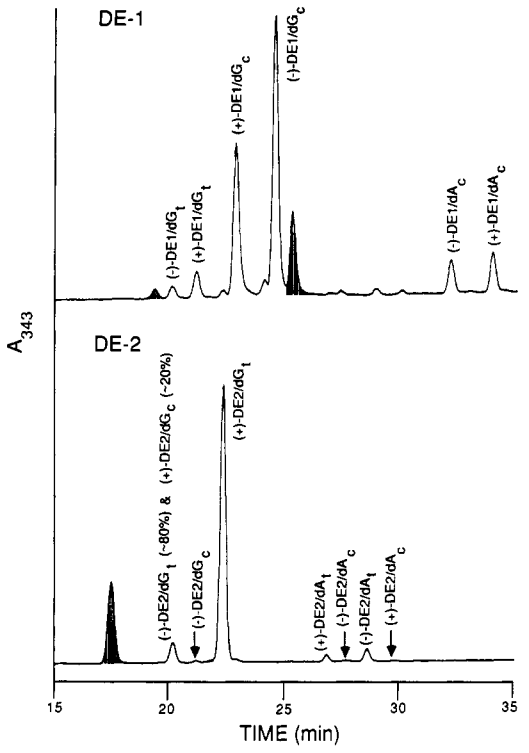

Figure 7. HPLC profiles (Altex Ultrasphere C_{18} column, 4.6 × 250 mm, eluted at 1 mL/min with 50% methanol in water for 10 min, followed by a linear gradient which increased the methanol content by 1% per minute) of the nucleoside adducts formed upon covalent bonding of racemic BaP diol epoxides-1 and -2 to native DNA. Abbreviations are defined in the legend to Figure 1. Shaded peaks correspond to tetrol hydrolysis products.

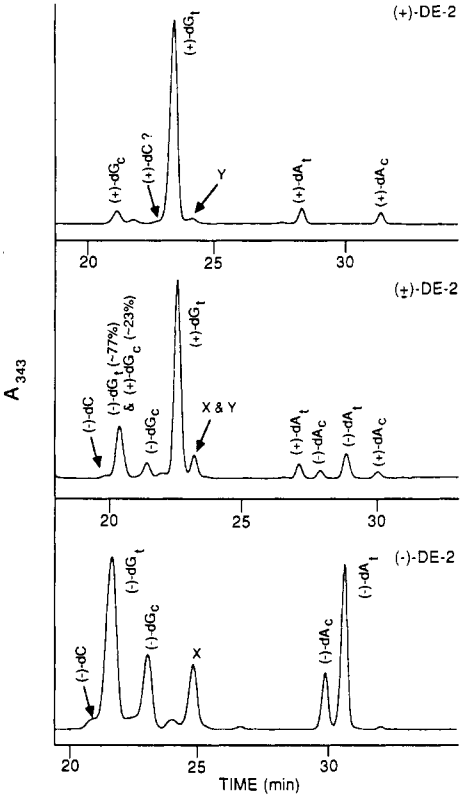

Figure 8. HPLC profiles of the nucleoside adducts formed upon covalent bonding of racemic and optically active BaP diol epoxide-2 to denatured DNA. Chromatographic conditions are the same as those described for Figure 7. Abbreviations are defined in the legend to Figure 1. Because of small variations in retention times, the time scales of the chromatograms have been aligned to show more clearly the peak assignments.

converted to tetrols under the experimental conditions. The HPLC profile (Figure 7) of the nucleoside adducts formed from BaP (±)-diol epoxide-2 and native calf thymus DNA is qualitatively similar to that previously observed by Meehan and Straub.[3] As in the earlier study, the predominance (≥90% of total adducts from native calf thymus DNA) of the adduct (+)-DE2/dG$_t$ is the most notable feature of the profiles and tends to obscure the distribution of minor adducts. The product profile from optically pure (+)-diol epoxide-2 is virtually identical to that reported by Cheng et al.[9] In the present study, as well as in that of Cheng et al.,[9] the principal adducts from (-)-diol epoxide-2 are a trans dG adduct, a cis dG adduct, and a trans dA adduct. However, the adduct distribution observed by us (Table III) differs from that found by Cheng et al., who observed a ratio of (-)-DE2/dG$_t$:(-)-DE2/dG$_c$:(-)-DE2/dA$_t$ of 63:22:15. Since experimental details of the digestion protocol were not given, it is conceivable that the lower recovery of the dA adduct relative to our observations may have resulted from incomplete digestion by snake venom phosphodiesterase,[29,30] as discussed above. Both these recent results contrast with the earlier observation[3] of a *large preponderance* of the major dA, relative to the major dG adduct, from this enantiomer when optically pure diol epoxide was used as the reactant, but (somewhat surprisingly) not when the racemic diol epoxide was used. In our experience, similar ratios of these adducts were obtained from both (-)- and (±)-diol epoxide-2, as would be expected if there is no interaction between sites of addition on the DNA. Since both the present and the earlier study employed ratios of diol epoxide per nucleotide (ca. 1:12 and 1:250, respectively), that should result in the covalent modification of fewer than 1 in 50 base pairs, interaction between adduct sites is highly unlikely, and the explanation for the earlier observation of inconsistent product ratios from the racemic and optically active diol epoxides is unclear.

Reaction of racemic BaP diol epoxide-1 with native calf thymus DNA, followed by enzymatic hydrolysis to the nucleoside level, was carried out under the conditions used with diol epoxide-2. A material balance experiment indicated 85% recovery of tetrols in the organic extract after the bonding reaction, 0.7% recovery of tetraols with the modified nucleosides after enzymatic hydrolysis, and 4.1–4.6% recovery of starting material as nucleoside adducts. Thus, in this case, 90% of the parent diol epoxide was recovered as identifiable products.

The absolute configurations at C-10 were determined for the deoxyribonucleoside adducts of diol epoxide-1 identified in Figure 7 by comparison of their CD spectra (in MeOH) with the corresponding spectra of the guanosine and adenosine adducts. Since the origin of the DNA adducts from (+)- or (-)-diol epoxide-1 was known from reactions with the individual enantiomers, the stereochemistry of addition (cis or trans) to the epoxide could thus be assigned. The product distribution from the two enantiomers corresponds to an overall adduct yield of 3.9% from the (+) and 5.1% from the (-) isomer. Thus, efficiency of covalent adduct formation from all the optically active isomeric diol epoxides is low and similar except in the case of the (+)-(*R,S,S,R*)-diol epoxide-2. An analogous small preference for bonding to DNA of the *R,S,S,R* isomer, which is the most tumorigenic enantiomer[35] of those

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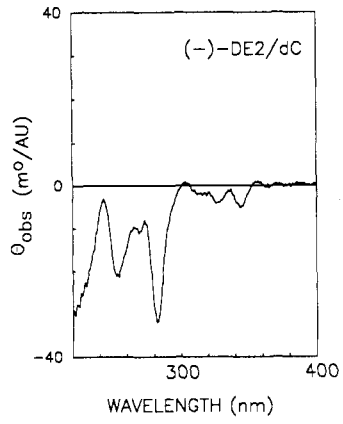

Figure 9. Circular dichroism spectrum (normalized to 1 absorbance unit at 281 nm) in methanol–water of a proposed deoxycytidine adduct of BaP (−)-diol epoxide-2.

optically active diol epoxides that have been studied to date, was also observed with diol epoxides derived from other parent hydrocarbons.[6,10]

Reaction of racemic BaP diol epoxide-2 with denatured (5 min at 100 °C) calf thymus DNA gave a slightly higher overall adduct yield (13% of total diol epoxide) and an approximately 2-fold increase (from ca. 9% to 16%) in the fraction of total adducts that were formed from dA (cf. Figure 8). The ratio of (+)-DE2/dG_t to (−)-DE2/dG_t was also decreased about 3-fold (from 13:1 to 4:1) upon denaturation of DNA. These effects are qualitatively similar to the effects of DNA secondary structure previously noted by Meehan and Straub[3] with single- and double-stranded ΦX174 DNA, as well as with native and denatured calf thymus DNA. However, they are less pronounced: Meehan and Straub observed a 4-fold increase in the percent of dA adducts (from 10% to 40%), as well as a 17-fold decrease in the ratio of (+)-DE2/dG_t to (−)-DE2/dG_t (from 20:1 to 20:17) when single-stranded DNA was used.

The identity of the dG and dA addducts from diol epoxide-2, as shown in Figures 7 and 8, was confirmed by measurement of their CD spectra. Elution orders of these adducts agreed with those reported by Cheng et al.[9] It should be noted that the elution order of the two central dA adduct peaks derived from (−)-diol epoxide-2 is reversed in both these current studies relative to that observed earlier by Jeffrey et al.,[12] so that the major (trans) dA adduct from this enantiomer is eluted later than the minor (cis) adduct under the present chromatographic conditions.

Several minor adducts, not formed in appreciable quantities upon reaction with native DNA, were observed upon reaction of diol epoxide-2 with denatured DNA (Figure 8). A dC adduct from (−)-diol epoxide-2 was tentatively identified by the mass spectrum of its pentaacetate (m/z 740, MH^+). The CD spectrum of this adduct showed a strong negative band at 281 nm (Figure 9). By analogy with a trans dC adduct[6] of (−)-dibenz[a,j]anthracene (4S,3R)-diol (2R,1S)-epoxide-2, this is suggestive of 10R absolute configuration, and thus we suggest that this adduct arose from trans addition of the exocyclic N-4 to the epoxide. A trace component with an equal and opposite CD spectrum was also observed upon reaction of the (+)-diol epoxide-2 enantiomer (Figure 8). Upon treatment with acetic anhydride in pyridine, the compound indicated as X in Figure 8 gave a pair of acetates (t_R 1.4 and 3.2 min in solvent B; see the Experimental Section), whose CD spectra were very similar in both shape and sign; thus they presumably are not cis and trans isomers. The mass spectra of these acetates exhibited (MH^+) peaks at m/z 622, corresponding to tetraacetates of dG adducts. Upon storage, the less polar of these two acetates appeared to convert to the more polar. We speculate that these acetates are formed from a single compound, which could be a deglycosylated N-7 dG adduct.[34] The apparent conversion of the less polar to the more polar acetate may involve rearrangement that results in a change in the position of acetyl substitution; alternatively, the less polar compound may be an unstable pentaacetate that converts to the observed tetraacetate both on standing and under the conditions used for mass spectrometry. A compound (Y) with a CD spectrum opposite in sign and similar, although not identical, in shape to the CD spectrum of X was formed in trace quantities from (+)-diol epoxide-2 and denatured DNA (Figure 8).

Of the diol epoxides whose bonding to DNA has been extensively studied, those derived from the hydrocarbon BaP are as a group the most selective for deoxyguanosine.[33] Approximately 80–90% of the isolated adducts from three of the isomers (both enantiomers of diol epoxide-1 and (+)-diol epoxide-2) result from alkylation of dG. The only exception is (−)-diol epoxide-2, which gave an approximately equal distribution of dG and dA adducts. It is also of interest that diol epoxide-2 exhibits an extremely high selectivity for trans adduct formation, whereas diol epoxide-1 yields predominantly cis adducts. Recent chemical studies[36] have shown that these two BaP diol epoxides solvolyze at neutral pH by different mechanisms. Diol epoxide-1 undergoes rate-determining formation of an α-hydroxy carbocation that can subsequently be trapped by nucleophiles in a fast (in some cases diffusion-controlled) step. In contrast, diol epoxide-2 undergoes bimolecular substitution reactions with nucleophiles such as azide and *N*-acetylcysteine anion at a rate that must be much faster than the carbocation trapping pathway, with the result that rapid trapping of the carbocation is not observed for diastereomer-2. It is tempting to speculate that these two diastereomers may exhibit analogous behavior when noncovalently complexed to DNA: in this case direct nucleophilic attack on the epoxide within the complex would account for the trans adduct formation that predominates from diol epoxide-2, whereas carbocation trapping by an exocyclic amino group could lead to cis as well as trans adducts from diol epoxide-1. The observation that adduct formation is at least somewhat competitive (2–15% of the total reaction) with DNA-catalyzed hydrolysis suggests that enforced proximity of the nucleophilic group and the diol epoxide moiety in the noncovalent complex must provide a sufficient entropic advantage to overcome the low pK_a values[37] and weak nucleophilicities of the exocyclic purine amino groups.

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Registry No. (+)-DE1, 63323-29-5; (−)-DE1, 63357-09-5; (+)-DE1/G_t, 77340-98-8; (+)-DE1/G_c, 77341-00-5; (−)-DE1/G_t, 77341-01-6; (−)-DE1/G_c, 77340-97-7; (+)-DE1/A_c, 129783-62-6; (+)-DE1/A_t, 129783-63-7; (−)-DE1/A_t, 129783-64-8; (−)-DE1/A_c, 129783-65-9; (−)-DE1/A_a, 129678-51-9; (±)-DE2, 58917-67-2; (±)-DE1, 58917-91-2; (−)-DE2, 63323-30-8; (+)-DE2, 63323-31-9; 5′-adenylic acid, 61-19-8; polyguanylic acid, 25191-14-4.

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