Structures of Covalent Nucleoside Adducts Formed from Adenine, Guanine, and Cytosine Bases of DNA and the Optically Active Bay-Region 3,4-Diol 1,2-Epoxides of Benz[a]anthracene

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Chemical structures of the principal covalent adducts formed from DNA upon reaction *in vitro* with the four optically active 3,4-diol 1,2-epoxides of benz[a]anthracene have been elucidated at the nucleoside level. In addition to adducts formed by cis and trans addition of the exocyclic amino groups of deoxyadenosine (dA) and deoxyguanosine (dG) and a trans deoxycytidine (dC) adduct, chemical characterization of a deglycosylated N-7 dG adduct formed in DNA by trans opening of the (4*S*,3*R*)-diol (2*R*,1*S*)-epoxide isomer is reported. Relative stereochemistries of the adducts (cis *versus* trans opening of the epoxides by the exocyclic amino groups) were deduced from the coupling constants of the methine protons of the tetrahydro benzo rings of the acetylated derivatives. Adducts having (*S*)-configuration at the attachment site on the hydrocarbon moiety have CD spectra that exhibit a positive band at 250–260 nm and a negative band at longer wavelengths, whereas (*R*)-configuration at this center gives rise to CD spectra with bands of approximately equal intensity and opposite sign. This allowed assignment of cis *versus* trans addition to the chiral epoxides for adducts that were not generated in sufficient quantity to obtain NMR spectra. Analysis of the patterns of adducts derived from benz[a]anthracene, benzo[c]phenanthrene, and benzo[a]pyrene shows that the comparative tumorigenicities of the diol epoxide isomers of each hydrocarbon do not correlate well with the extent of adduct formation, the ratio of cis *versus* trans addition to the epoxide, the propensity for forming adducts at dC or the N-7 position of dG, or the ratio of adduct formation at dA *versus* dG, although tumorigenicity may correlate with the ability to form dG adducts with (*S*)-configuration at the N-substituted benzylic carbon, especially those arising from trans addition to the epoxide.

Introduction

Bay-region diol epoxides metabolically formed *via* trans dihydrodiols have been established as ultimate carcinogens for over a dozen tumorigenic polycyclic aromatic hydrocarbons.[2a,b] Covalent modification of DNA by these and other electrophilic metabolites is presumed to be the first step in tumorigenesis or mutagenesis.[3a,b] Four optically active diol epoxides (enantiomers of a diastereomeric pair in which the benzylic hydroxyl group and epoxide oxygen are either cis, DE1, or trans, DE2, Figure 1) may be formed from a given hydrocarbon. The exocyclic amino group of purine residues in DNA is now recognized as the principal target for alkylation by these metabolites[3b] (Figure 2). Cis and trans addition of the exocyclic amino groups of deoxyguanosine (dG) and deoxyadenosine (dA) to the epoxide at the benzylic carbon of the four diol epoxide isomers can thus generate 16 different adducts. To date, definitive chemical characterization of the sets of DNA adducts formed *in vitro* from the four metabolically possible bay-region diol epoxide isomers of benzo[a]pyrene[4a] (BP), benzo[c]phenanthrene[4b] (BcPh), and most recently, dibenz[a,j]anthracene[4c] (DBajA) has been reported. The latter has been shown to generate a deoxycytidine (dC) adduct as well as purine adducts. Detailed data on the covalent bonding of selected bay-region diol epoxide isomers derived from several other hydrocarbons to DNA are also available.[5] Large differ-

(1) (a) Laboratory of Bioorganic Chemistry, NIDDK, NIH. (b) Department of Chemistry, American University. (c) Laboratory of Analytical Chemistry, NIDDK, NIH.

(2) (a) The prototype of a bay region is the hindered region between C_4 and C_5 of phenanthrene. The bay-region theory (Jerina, D. M.; Lehr, R. E.; Yagi, H.; Hernandez, O.; Dansette, P. M.; Wislocki, P. G.; Wood, A. W.; Chang, R. L.; Levin, W.; Conney, A. H. In *In Vitro Metabolic Activation In Mutagenesis Testing*; de Serres, F. J., Fouts, J. R., Bend, J. R., Philpot, R. M., Eds.; Elsevier/North-Holland Biomedical Press: Amsterdam, 1976; pp 159–177. Jerina, D. M.; Lehr, R. E. In *Microsomes and Drug Oxidations: 3rd International Syposium*; Ullrich, V., Roots, I., Hildebrandt, A. G., Estabrook, R. W., Conney, A. H., Eds.; Pergamon Press: Oxford, England, 1977; pp 709–720) predicted that benzo-ring diol epoxides of tumorigenic hydrocarbons in which the epoxide group formed part of a bay region would be ultimate carcinogens. (b) For reviews of metabolism and of tumorigenic activity, see: Thakker, D. R.; Levin, W.; Wood, A. W.; Conney, A. H.; Yagi, H.; Jerina, D. M. In *Drug Stereochemistry—Analytical Methods and Pharmacology*; Wainer, I. W., Drayer, D. E., Eds.; Marcel Dekker, Inc.: New York, 1988; pp 271–296. Jerina, D. M.; Yagi, H.; Thakker, D. R.; Sayer, J. M.; van Bladeren, P. J.; Lehr, R. E.; Whalen, D. L.; Levin, W.; Chang, R. L.; Wood, A. W.; Conney, A. H. In *Foreign Compound Metabolism*; Caldwell, J., Paulson, G. D., Eds.; Taylor and Francis Ltd.: London, 1984; pp 257–266.

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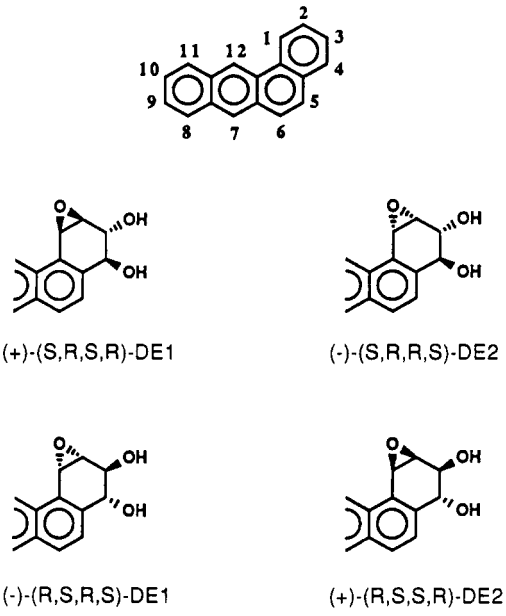

Figure 1. Numbering for benz[*a*]anthracene (BA) and the structures of its optically active bay-region 3,4-diol 1,2-epoxides. Absolute configurations are designated starting from the benzylic hydroxyl-bearing carbon and progress to the benzylic epoxide carbon.

ences in tumorigenicity are observed among the isomers of the bay-region diol epoxides from a given hydrocarbon:[2b] generally, the enantiomer whose configuration is (*R,S,S,R*) reading from the benzylic hydroxyl-bearing carbon to the benzylic epoxide carbon is the most tumorigenic, although other optical isomers, especially in the case of BcPh, can be quite tumorigenic as well. Thus, it is particularly important to establish a data base of the types and amounts of adducts formed from isomeric diol epoxides derived from different hydrocarbons. As yet, specific DNA adducts have not been identified as causative of cell transformation.[3b] In the present report, we describe structural characterization of the 16 possible cis and trans dG and dA adducts formed upon reaction *in vitro* of the exocyclic amino groups of these nucleoside residues in DNA with the four configurationally isomeric bay-region diol epoxides of benz[*a*]anthracene (BA), whose structures are shown in Figure 1. In addition, we report the second (after DBajA diol epoxide[4c]) complete characterization of a dC adduct and the characterization of a deglycosylated product of an N-7 dG adduct formed in DNA. The latter is formed in greater quantity than the dC adduct or the minor dG and dA adducts from the same diol epoxide isomer.

Experimental Section

Materials and Methods. Calf thymus DNA (Sigma Chemical Co., St. Louis, MO) was dialyzed for 16 h against 10 mM Tris–HCl buffer, pH 7.4, before use. 2′-Deoxyguanosine 5′-monophosphate (5′-dGMP), 2′-deoxyadenosine 5′-monophosphate (5′-dAMP), and 2′-deoxycytidine 5′-monophosphate (5′-dCMP) as sodium salts, pancreatic deoxyribonuclease I, *Escherichia coli* alkaline phosphatase (as a suspension in 2.5 M $(NH_4)_2SO_4$), and snake venom phosphodiesterase from *Crotalus atrox* were obtained from Sigma Chemical Co. and were used as such. Optically active BA 3,4-diol 1,2-epoxides were prepared as described.[6]

Proton NMR spectra were measured at 300 or 500 MHz in acetone-d_6, except for the unacetylated deglycosylated N-7 dG adduct, which was measured in methanol-d_4. Chemical ionization (NH_3 gas) mass spectra were measured on a quadrupole mass spectrometer with a direct exposure probe. Circular dichroism (CD) spectra of the adducts in MeOH were normalized to 1.0 absorbance unit at the wavelength of maximum absorbance of the adduct. The pH titration curves[7] of the trans N^2 dG and deglycosylated N-7 dG adducts derived from the (−)-(*S,R,R,S*)-diol epoxide isomer of BA were determined at 25 °C by measurement of the CD spectra upon addition of aliquots of a methanolic stock solution of the adduct to aqueous HCl, NaOH, or buffer solutions to give final compositions of 1:9 MeOH/H_2O. The pH was measured in the final solutions. Reversiblity of the spectral changes observed at the pH extrema was verified by adjusting the pH of these solutions to 5–7 and redetermining their CD spectra. The pK_a values were determined from the pH dependence of θ_{obsd} at 258 nm, normalized to 1 abosrbance unit at 256 nm.

Preparation of Nucleoside Adducts. To 0.5 mL of calf thymus DNA (0.8 mg/mL in 0.01 M Tris–HCl, pH 7.4) was added 0.05 mL of a stock solution of optically pure BA diol epoxide (1 mg/mL in CH_3CN). After incubation at 37 °C for 1 h, each solution was extracted three times with 1 mL of EtOAc and twice with 1 mL of ether to remove noncovalently bound hydrolysis products (mainly tetraols). Residual ether was removed with a stream of N_2. Details of the enzymatic digestion of the adducted DNA to the nucleoside level using DNase I, snake venom phosphodiesterase (0.4 units/mg DNA), and alkaline phosphatase, as well as the recovery, separation, and quantitation of adducts, have been described previously.[8]

The (−)-(*R,S,R,S*)- and (+)-(*S,R,S,R*)-DE1 DNA adducts (Figure 3) were subjected to analytical HPLC on a Du Pont Zorbax ODS column (0.46 × 25 cm) eluted with H_2O/MeOH in a ratio of 32/68 at a flow rate of 1.0 mL/min. The (+)-(*R,S,S,R*)-DE2 DNA adducts (Figure 3) were separated on the same column and eluted with H_2O/CH_3CN/MeOH in a ratio of 74/24/2 at a flow rate of 1.0 mL/min. With the same column and the same operating conditions used to separate the (+)-(*R,S,S,R*)-DE2 DNA adducts, the dC adduct formed from (−)-(*S,R,R,S*)-DE2 appeared as a poorly separated late shoulder on the trans dG adduct peak. Separation of (−)-(*S,R,R,S*)-DE2 DNA adducts shown in Figure 3 was on a Du Pont Zorbax ODS column (0.94 × 25 cm) eluted at 3.0 mL/min with H_2O/CH_3CN/MeOH in a ratio of 72/26/2.

Sufficient amounts of adducts for characterization were generated by 20–40-fold scale up of the reaction, extraction, and digestion procedures. Adducts were recovered on C_{18} Sep-paks (Waters Associates), using one Sep-pak per 4.8 mg of DNA in the initial reaction mixture. Details of the HPLC procedures used for preparative isolation of the adducts obtained from DNA may be found in the supplementary material.

Minor adducts obtained upon reaction of the diol epoxides with DNA, such as the ones derived from dA and the cis exocyclic and N-7 adducts of (−)-(*S,R,R,S*)-DE2, were obtained in larger quantities via reactions of the diol epoxides with deoxynucleoside 5′-phosphates; these were pooled with the corresponding adducts obtained from DNA. Deoxyadenosine adducts of BA diol epoxides were generated by reaction of 4 mL of 5′-dAMP (0.1 M) in 0.01 M Tris–HCl (pH 7.4) with each optically active diol epoxide isomer (0.4 mL of 1 mg/mL diol epoxide in CH_3CN) for 16 h at 37 °C. Each reaction mixture was extracted three times with 8 mL of EtOAc and twice with 8 mL of ether, and residual ether was removed under N_2. Reaction mixtures were then adjusted to pH 8.6 and treated with 16 units of alkaline phosphatase for 4 or more h at 37 °C. The adducts were recovered on two Sep-paks. They were subsequently separated by preparative HPLC (supplementary material) and pooled with the corresponding adducts isolated from DNA that had identical retention times and spectra. In all cases, the major dA adducts formed from 5′-dAMP were also the major dA adducts formed from DNA.

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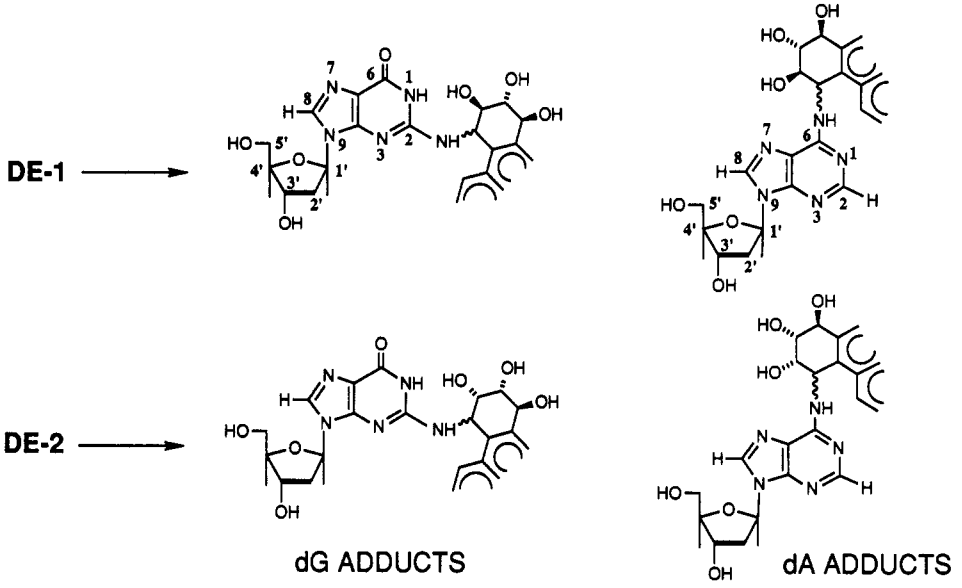

Figure 2. Structures and numbering of adducts formed upon covalent addition of the exocyclic amino groups of deoxyadenosine and deoxyguanosine to BA diol epoxides. Only one of the two possible sets of absolute configurations for the hydroxyl groups is shown.

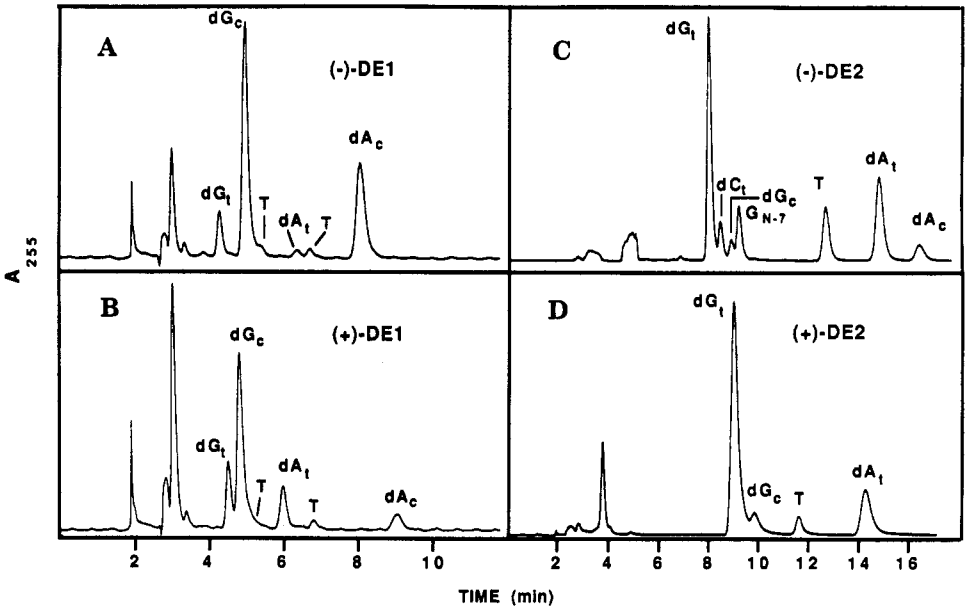

Figure 3. HPLC traces of adduct mixtures formed upon reaction of the optically active BA diol epoxides with DNA, followed by hydrolysis to the nucleoside level. For chromatographic conditions, see text; chromatograms A, B, and D were obtained by use of a 0.46- × 25-cm Du Pont Zorbax ODS column, whereas C was obtained by use of a 0.94- × 25-cm column of the same material. Adducts at the exocyclic amino group of deoxyadenosine, deoxyguanosine, and deoxycytidine are designated as dA, dG, and dC, respectively, the subscripts c and t are used to designate cis and trans opening of the epoxide ring at the benzylic position, T designates residual tetraol, and G_{N-7} is a deglycosylated adduct of dG at N-7.

Similarly, deoxyguanosine adducts formed by (−)-(*S*,*R*,*R*,*S*)-DE2 were generated by reaction with 5′-dGMP and workup as described. Preparative HPLC separation is described in the supplementary material. Again, the same dG adducts were formed from 5′-dGMP as were formed from DNA, so these were pooled for further analysis.

Since the (−)-(*S*,*R*,*R*,*S*)-DE2 dC adduct was also formed in limited amounts from DNA, several oligomers and polymers containing dC, namely $p(dC)_2$, $p(dC)_8$, $p(dC)_{19-24}$, poly dC, and poly(dI-dC)·poly(dI-dC) (Pharmacia, Piscataway, NJ), were allowed to react with (−)-(*S*,*R*,*R*,*S*)-DE2 in an attempt to produce larger amounts of adduct. Since the greatest amount of the desired adduct was produced from $(dC)_8$, this oligomer was used to generate sufficient amounts of the adduct for characterization. Yields of the desired adduct (identical to that from DNA by comparison of UV spectra and HPLC retention times) from each of the dC-containing oligomers ranged from 0.3 to 1.5 A_{255} units per mg and are tabulated in the supplementary material. Reactions between 1 mg/mL of oligomer or polymer and 0.1 mg/mL of diol epoxide were conducted in 1–2% DMSO in 0.01 M Tris–HCl, pH 7.0, for 16 h at 37 °C. Workup was as described for adducted DNA. The dC adduct was isolated by HPLC on a Du Pont Zorbax ODS column (0.46 × 25 cm) by isocratic elution at 1.0 mL/min with H_2O/CH_3CN/MeOH in a ratio of 71/27/2.

Acetylated Adducts. Adducts were acetylated with acetic anhydride in pyridine; acetylation of the deglycosylated N-7 dG adduct of (−)-(*S*,*R*,*R*,*S*)-DE2 required a catalytic amount of *p*-(dimethylamino)pyridine. The acetylated adducts wre dissolved in CH_2Cl_2 and purified by chromatography on a Du Pont Golden Series SIL column eluted at 3.0 mL/min with CH_2Cl_2/EtOAc/MeOH (94.5/5/0.5 for dA adducts, 87/10/3 for exocyclic dG adducts, 95/3/2 for the dC adduct and 88/10/2 for the deglycosylated N-7 dG adduct); absorbance was monitored at 255 nm.

Table I. Covalent Bonding Efficiency and Adduct Distribution of the dG, dC, and dA Adducts Formed in Calf Thymus DNA by the Bay-Region Diol Epoxides of Benz[a]anthracene[a]

diol epoxide enantiomer	avg % bonded	adduct distribution (%) dG cis[b]	dG trans[b]	dG N-7	dC trans[b]	dA cis[b]	dA trans[b]
(−)-(*R,S,R,S*)-DE1	10	54	9			35	2
(+)-(*S,R,S,R*)-DE1	8	62	16			6	16
(−)-(*S,R,R,S*)-DE2	10	5	45	12	7	6	24
(+)-(*R,S,S,R*)-DE2	25	8	71				21

[a] Average of four or more determinations. [b] Bonding at the exocyclic nitrogen.

Results and Discussion

Adduct Quantitation. Treatment of calf thymus DNA with each optically active isomer of the BA 3,4-diol 1,2-epoxides, followed by enzymatic hydrolysis to the nucleoside level, resulted in several covalently modified nucleosides which were separable by analytical HPLC (Figure 3). Assignments of structure (discussed below) to the peaks observed in the HPLC profiles are indicated in the figure. Differences in the ability to separate the trans dG and dC adducts were observed from one column to another of the same type from the same manufacturer. The elution position of the deglycosylated N-7 dG adduct also varied considerably.

Table I shows the extent of covalent bonding of the four diol epoxide isomers to DNA and the relative amounts of each of the adducts formed. The extent of covalent addition compared to hydrolysis was estimated by comparing the total absorbance of adducts recovered from the Sep-paks to the absorbance of the diol epoxide that had been allowed to react with DNA. Material extracted from the diol epoxide–DNA reaction by organic solvent accounted for the difference between the absorbance of the diol epoxide and that recovered as DNA adducts. These organic soluble products consist largely of tetraols formed by DNA catalyzed hydrolysis of the diol epoxides, although in the case of DE1, some keto diol may also result from spontaneous rearrangement.[9] With DNA concentrations of 0.8 mg/mL, between 8 and 25% of each optically active diol epoxide is converted to adducts, consistent with the results obtained by Carberry *et al.*[10] with racemic mixtures of BA DE1 and DE2. The (4*R*,3*S*)-diol (2*S*,1*R*)-epoxide-2 isomer of BA is two to three times more efficient in bonding to DNA than the other optically active isomers, and all the isomers exhibit a preference (60–80% of total calf thymus DNA adducts) for adduct formation with dG residues. Individual diol epoxide isomers exhibit quite different patterns in the amount of cis *versus* trans adduct that is formed; these patterns are discussed in detail below. The pattern of adducts reported in Table I for the DE2 isomers derived from BA is similar to that for the DE2 isomers of 7-methyl BA,[5c] except that no dC or N-7 dG adducts or cis DE2 adduct with *S* configuration at C-1 were reported for 7-methyl BA. It is possible that these adducts were formed but not detected if they were cochromatographic with major adducts.

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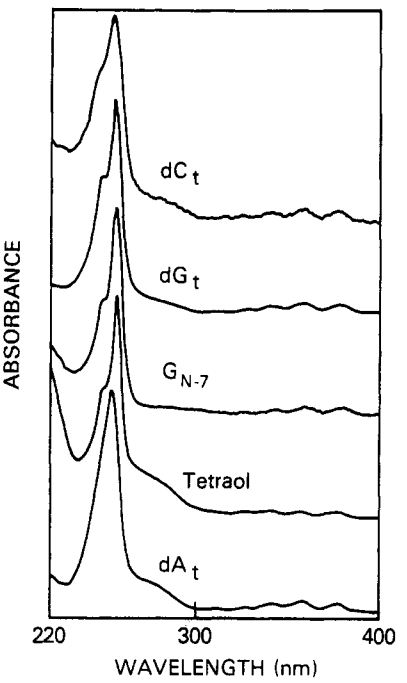

Figure 4. UV spectra of the exocyclic purine adducts, dC and N-7 dG adducts, and tetraol formed by trans opening of (−)-(*S,R,R,S*)-BA diol epoxide-2. Spectra of the exocyclic purine adducts formed by other diol epoxide isomers are essentially identical.

UV and Mass Spectra of Adducts. Although the dA, dC, and dG adducts of BA exhibit UV absorbance maxima at similar wavelengths (254, 255, and 256 nm, respectively, in methanol), they may be distinguished by their relative absorbances at 247 and 255 nm (Figure 4). The dC adduct exhibits a sizable absorbance at 247 nm (about 75%) compared to the absorbance at 255 nm. Deoxyguanosine adducts at the exocyclic N^2 show a better defined shoulder at 247 nm with about 65% of the absorbance at 255 nm. The deglycosylated N-7 dG adduct and tetraol show still less absorbance at 247 nm compared to 255 nm (about 55%). Deoxyadenosine adducts do not show a shoulder at 247 nm.

Mass spectra (CI, NH_3) of the acetylated adducts exhibited the expected (M + 1) peaks at *m/z* 740 for N^6 dA adducts, 796 for N^2 dG adducts, and 716 for the dC adduct as pentaacetates and at 598 for the deglycosylated N-7 dG adduct as a tetraacetate. Mass and UV spectra are particularly useful for distinguishing dG and dC adducts, which elute from C_{18} columns in the same region.

Adducts at the Exocyclic Amino Group: NMR Spectra. Ten purine adducts at the exocyclic amino group and one dC adduct were obtained from the BA diol epoxides in sufficient quantities for NMR spectral measurements. Adducts were converted to their pentaacetates, whose spectra were measured in acetone-d_6. These adducts exhibit characteristic patterns of chemical shifts and coupling constants for the methine protons of the tetrahydroaromatic moiety (Table II) which are very similar to those observed for the corresponding derivatives of DBajA[4c] and BP.[4a] For comparison, data for the corresponding tetraol tetraacetates are also included in Table II. In all of the BA adducts, the purine or pyrimidine substituent must be nearly pseudoaxial because of steric interference with the aromatic hydrogen (H-12) in the

Table II. ^{1}H NMR Data[a] for the Methine Protons of the Acetylated Deoxyribonucleoside Adducts Formed from Benz[a]anthracene Diol Epoxides-1 and -2 by Substitution at Exocyclic Purine and Pyrimidine Amino Groups

	methine hydrogens			
compd (as the acetate)	H-1	H-2	H-3	H-4
cis-1 tetraol[b,c]	7.15	5.38 ($J_{1,2}$ = 3.5, $J_{2,3}$ = 12.5, $J_{3,4}$ = 7.5)	5.84	6.38
(+)-(*S*,*R*,*S*,*R*)-DE1/dG$_c$	6.78	5.55 ($J_{1,2}$ = 4.5, $J_{2,3}$ = 11.8, $J_{3,4}$ = 8.1)	5.88	6.40
(−)-(*R*,*S*,*R*,*S*)-DE1/dG$_c$	6.84	5.58 ($J_{1,2}$ = 4.6, $J_{2,3}$ = 11.8, $J_{3,4}$ = 7.9)	5.87	6.39
(+)-(*S*,*R*,*S*,*R*)-DE1/dA$_c$[d]				
(−)-(*R*,*S*,*R*,*S*)-DE1/dA$_c$	7.15	5.65 ($J_{1,2}$ = 4.6, $J_{2,3}$ = 11.6, $J_{3,4}$ = 7.9)	6.21	6.32
trans-1 tetraol[b,c]	6.86	5.58 ($J_{1,2}$ = 4.0, $J_{2,3}$ = 7.15, $J_{3,4}$ = 7.15)	5.36	6.38
(+)-(*S*,*R*,*S*,*R*)-DE1/dG$_t$[d]				
(−)-(*R*,*S*,*R*,*S*)-DE1/dG$_t$[d]				
(+)-(*S*,*R*,*S*,*R*)-DE1/dA$_t$	6.72	5.73 ($J_{1,2}$ = 3.8, $J_{2,3}$ = 6.3, $J_{3,4}$ = 4.7)	5.56	6.41
(−)-(*R*,*S*,*R*,*S*)-DE1/dA$_t$[d]				
cis-2-tetraol[b,e]	7.10	5.74 ($J_{1,2}$ = 4.6, $J_{2,3}$ = 2.8, $J_{3,4}$ = 3.2)	5.48	6.26
(+)-(*R*,*S*,*S*,*R*)-DE2/dG$_c$[d]				
(−)-(*S*,*R*,*R*,*S*)-DE2/dG$_c$	6.80	5.83 ($J_{1,2}$ = 5.4, $J_{2,3}$ = 2.5, $J_{3,4}$ = 3.5)	5.61	6.31
(+)-(*R*,*S*,*S*,*R*)-DE2/dA$_c$[d]				
(−)-(*S*,*R*,*R*,*S*)-DE2/dA$_c$	7.02	5.80 ($J_{1,2}$ = 5.3, $J_{2,3}$ = 2.4, $J_{3,4}$ = 2.8)	5.68	6.31
trans-2-tetraol[b,c]	6.72	5.64 ($J_{1,2}$ = 3.5, $J_{2,3}$ = 2.5, $J_{3,4}$ = 8.5)	5.58	6.46
(+)-(*R*,*S*,*S*,*R*)-DE2/dG$_t$	6.12	6.12 ($J_{1,2}$ < 2, $J_{2,3}$ = 2.0, $J_{3,4}$ = 9.0)	5.73	6.51
(−)-(*S*,*R*,*R*,*S*)-DE2/dG$_t$	6.12	6.06 ($J_{1,2}$ = 3.3, $J_{2,3}$ = 2.4, $J_{3,4}$ = 9.0)	5.75	6.52
(+)-(*R*,*S*,*S*,*R*)-DE2/dA$_t$	~6.5	~5.96 ($J_{1,2}$ = ~2.5, $J_{2,3}$ < 2, $J_{3,4}$ = 8.6)	~5.98	6.53
(−)-(*S*,*R*,*R*,*S*)-DE2/dA$_t$[f]	6.54	5.95 ($J_{1,2}$ = ~3.0, $J_{2,3}$ = 2.5, $J_{3,4}$ = 9.0)	5.87	6.54
(−)-(*S*,*R*,*R*,*S*)-DE2/dC$_t$	~6.3	5.87 ($J_{1,2}$ < 3, $J_{2,3}$ < 3, $J_{3,4}$ = 9.0)	5.63	6.50

[a] Spectra were measured in acetone-d_6 at 300 MHz unless otherwise noted. Line positions are given in ppm, the acetone-d_6 peak (δ 2.04 ppm) being used as reference, and coupling constants (J) are given in hertz. [b] In $CDCl_3$. Chemical shifts and coupling constants for the methine protons of the *cis*-2 tetraol tetraacetate measured in acetone-d_6 and in $CDCl_3$ were very similar. [c] At 100 mHz: Thakker, D. R.; Levin, W.; Yagi, H.; Tada, M.; Ryan, D. E.; Thomas, P. E.; Conney, A. H.; Jerina, D. M. *J. Biol. Chem.* **1982**, *257*, 5103–5110. [d] Insufficient material for satisfactory NMR spectra. [e] This tetraol was prepared by reaction of osmium tetraoxide with the 3,4-dihydrodiol. [f] At 500 MHz.

bay region. Thus, the conformational flexibility of the tetrahydroaromatic ring is limited. Computer-generated structures (Figure 5) illustrate orientations of the methine hydrogens that are consistent with the coupling constants observed for these compounds. The coupling constants for both the cis and trans DE2 adducts in the BA series are very similar to those observed by Peltonen *et al.*[5c] in the 7-methyl BA series, and the dihedral angles calculated by these authors for the methine hydrogens in the 7-methyl BA cis DE2 adducts are in close agreement with those shown for the corresponding BA adducts in Figure 5 (for numerical values, see supplementary material).

For DE1 derivatives, the adducts formed upon cis and trans attack of the exocyclic amino group on the epoxide are easily distinguished from each other by the characteristically large coupling constants $J_{2,3}$ and $J_{3,4}$ for the cis adducts. In these adducts, pseudoaxial orientation of the nucleic acid base causes the ring to assume a chair conformation in which H-2, H-3, and H-4 are all pseudoaxial, whereas in the trans adducts all three of these hydrogens must be pseudoequatorial when the purine or pyrimidine substitutent is pseudoaxial (Figure 5). Thus, for the trans DE1 adducts $J_{2,3}$ and $J_{3,4}$ are small. Similarly, for the DE2 adducts, a large coupling constant $J_{3,4}$ is diagnostic of trans addition, which forces H-3 and H-4 into a pseudodiaxial orientation (Figure 5), whereas for the cis adducts these hydrogens are pseudodiequatorial, such that $J_{3,4}$ is small. In the DE1 series, H-3 of the cis dA adduct exhibits a downfield shift (~0.3 ppm) relative to the corresponding proton of the cis dG adduct. A similar deshielding effect on this proton in the dA relative to the dG adducts was observed for adducts derived from DBajA DE1 and was ascribed[4c] to the cis, diaxial orientation of the purine relative to H-3 in these adducts, which causes H-3 to be strongly influenced by the magnetic effects of the purine ring systems. Similarly, H-3 and the purine are cis and diaxial in the trans DE2 adducts. In this case also, this proton in the dA adducts is deshielded (0.12–0.25 ppm) relative to the dG adducts.

Characteristic singlets are observed in the aromatic region for H-7 and H-12 of the BA ring system as well as for the purine hydrogens H-8 of guanine (δ 7.9–8.0 ppm) and H-8 and H-2 of adenine (δ ~8.6 and 8.1–8.2 ppm). As previously observed for both BcPh[4b] and DBajA[4c] adducts, trans dA adducts derived from both DE1 and DE2 exhibit pronounced broadening of both purine resonances, when compared with the corresponding cis adducts. This line broadening was temperature sensitive in the DBajA series, and hence it is presumably due to slow interconversion of two or more conformers of these adducts.

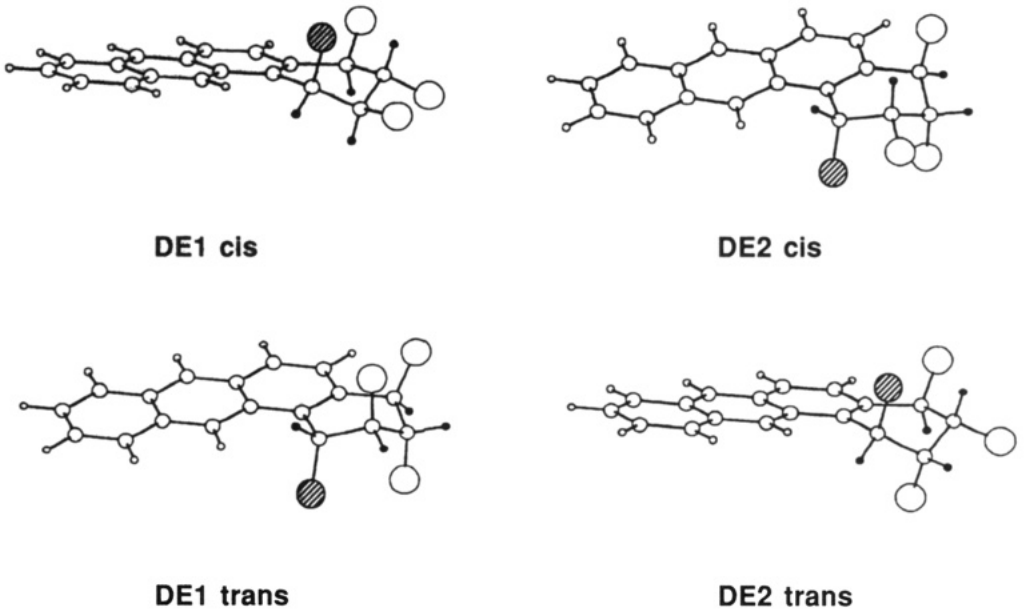

Figure 5. Computer-generated schematic conformational drawings of adducts formed upon cis and trans attack of an exocyclic purine amino group on BA (−)-diol epoxide-1 and (+)-diol epoxide-2. Structures for the triacetates of cis and trans adenine adducts without the sugar from DE1 and DE2 were generated by energy minimization in the gas phase by use of the computer program QUANTA/CHARMm (Polygen Corp., Waltham, MA). Although the calculations were done for the acetylated derivatives with adenine as the substituent at C-1, the purine and the acetoxy groups are omitted in the drawings for the sake of clarity. Large cross-hatched spheres represent the exocyclic amino nitrogens, large open spheres represent acetoxy oxygens, and small solid spheres represent the benzo ring methine protons. H–C–C–H dihedral angles and calculated ^3J values for these protons are given in the supplementary material.

It might be expected, and is generally true, that diastereomeric adducts formed from the enantiomers of a given diol epoxide and having the same relative (cis or trans) stereochemistry of addition to the epoxide should have virtually identical NMR spectra. However, the cis dG adducts from (+)-(*S*,*R*,*S*,*R*)- and (−)-(*R*,*S*,*R*,*S*)-DE1 of BA and DBajA[4c] exhibit small but distinctive differences in their aromatic chemical shifts, which must reflect subtle conformational differences that we cannot at present interpret. Specifically, the signals for the meso-anthracenic protons (H-12 of the BA adducts and H-14 of the DBajA adducts) and the purine H-8 exhibit downfield shifts of 0.05–0.13 ppm in the adducts derived from (−)-(*R*,*S*,*R*,*S*)-DE1 as compared to those from the enantiomeric (+)-(*S*,*R*,*S*,*R*)-DE1. This pattern is not obvious in any of the other diastereomeric pairs of cis or trans adducts. For the methine protons, the only significant difference in chemical shifts between BA adducts having the same relative configuration but derived from enantiomeric diol epoxides was observed with the trans dA adducts from (+)-(*R*,*S*,*S*,*R*)- and (−)-(*S*,*R*,*R*,*S*)-DE2, whose H-3 resonances differ by ca. 0.1 ppm.

As in the case of the DBajA diol epoxides,[4c] only one of the isomeric BA derivatives, (−)-(*S*,*R*,*R*,*S*)-DE2, gave quantities of a dC adduct sufficient for identification. Interpretation of the NMR spectrum of the pentaacetate (*m*/*z* 716) was complicated by the small coupling constants, $J_{1,2}$ and $J_{2,3}$, and by overlapping resonances for H-1 and H-1′, as well as for H-2 and the pyrimidine H-5. The latter signal (δ 5.85 ppm) as well as that for H-6 of the pyrimidine (δ 7.75 ppm) were assigned by decoupling. An exchangeable proton corresponding to the exocyclic NH was observed at δ 7.1 ppm. These chemical shifts were all very similar to those observed in the analogous dC adduct from (−)-(*S*,*R*,*R*,*S*)-DBajA DE2. As in the case of the corresponding DBajA adduct,[4c] a large value for $J_{3,4}$ (Table II) is diagnostic of trans addition to the epoxide.

Identification of a Ring-Substituted Deoxyguanosine Adduct from (−)-Benz[*a*]anthracene Diol Epoxide-2. Modification of deoxyguanosine residues in DNA by covalent bonding to N-7 occurs with simple epoxides such as propylene oxide[11] and styrene oxide,[12] as well as with the epoxides metabolically formed from aflatoxin B_1[13] and vinyl chloride.[14] The formation of labile adducts by polycyclic aromatic diol epoxides has been known for some time. A presumed N-7 guanine adduct formed upon reaction of DNA with racemic BP DE2 has been reported, but was not characterized.[15] Extensive formation of alkali-labile adducts by racemic BP DE2 has been observed. Quantitation of radiolabeled fragments indicated that the largest number of labile adducts were formed at dG, but smaller amounts were formed at dA and dC.[16] It has been assumed that the alkali-labile BP diol epoxide adducts on purines are N-7 adducts. Additional reports have suggested the formation of alkali-labile sites at dG in DNA by a non-bay-region diol epoxide of BA[17] and at dG predominantly, but also at dA and dC, by bay-region diol epoxides of BcPh.[18] Recently, we observed an adduct formed from the (−)-(7*S*,8*R*,9*R*,10*S*)-DE2 of BP upon reaction with *denatured* DNA that was tentatively assigned as an N-7 adduct, based on the mass spectrum

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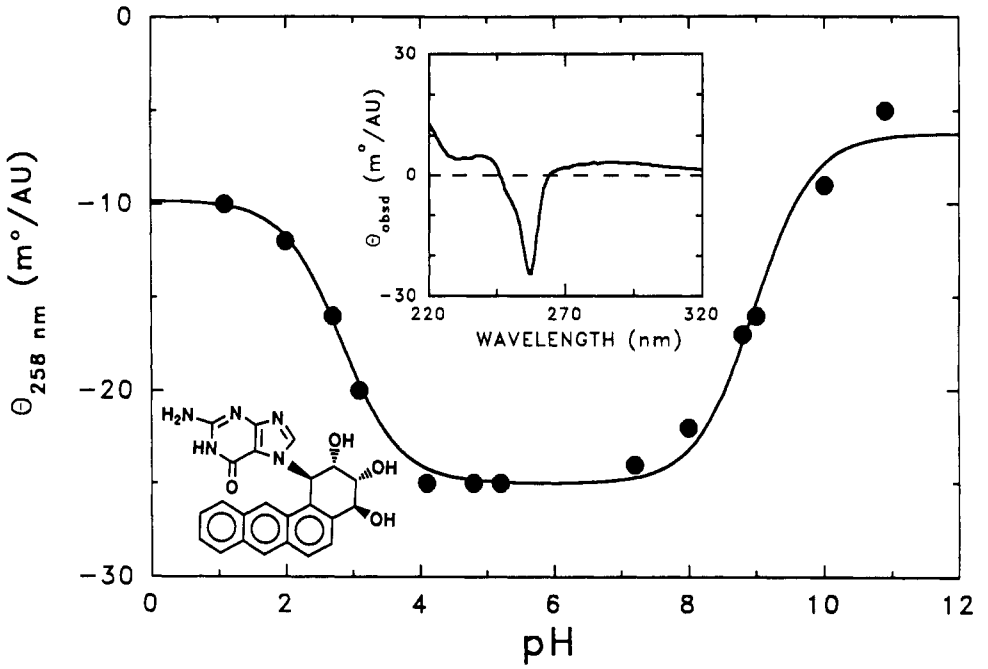

Figure 6. Effect of pH on the intensity of the CD signal at 258 nm for the adduct (−)-(*S,R,R,S*)-DE2/$G_{N\text{-}7}$ at 25 ° in 1:9 methanol/water. The solid line is calculated on the basis of pK_a values of 2.8 and 9.0. The inset shows the CD spectrum of this adduct in methanol, normalized to 1.0 absorbance unit at 256 nm.

of its fully acetylated derivative, which indicated the absence of the sugar moiety.[4a] Loss of the sugar prior to or during isolation of the adduct was suggestive of a ring-substituted adduct, which would be expected to undergo relatively facile cleavage of the purine–sugar bond. Most recently, a guanine adduct at N-7 formed from racemic BP DE2 upon reaction with deoxyguanosine has been characterized.[19] This adduct was not formed from DNA, although a N-7 adenine adduct was formed.[20]

In the present study we report the characterization of a polycyclic aromatic diol epoxide adduct formed at the N-7 of dG in DNA. The adduct is formed by the (−)-(*S,R,R,S*)-DE2 isomer of BA; the same configurational isomer of BcPh was also found to form a greater amount of labile adducts[18] than the (*R,S,S,R*)-isomer. This BA adduct, ($G_{N\text{-}7}$, t_R ~9 min, in the upper right panel of Figure 3), was formed in relatively large quantity, which facilitated its isolation and identification. Absence of the sugar was indicated by the mass spectrum of the acetylated adduct, which exhibited a molecular ion at *m/z* 598, corresponding to the tetraacetate of a deglycosylated guanine adduct, and by the lack of sugar proton resonances in the NMR spectra of both the free and acetylated adducts. Depurination resulting in loss of the sugar is well documented for N-7 derivatives of guanosine and deoxyguanosine, whereas there is no chemical rationale for facile depurination of guanosine derivatives substituted at O^6, N-1, or N-3. Thus, the likely site of alkylation is at N-7. Alkylation at C-8 is excluded by observation of the H-8 resonance (see below). The CD spectrum of the unacetylated adduct (Figure 6) was similar to but less intense than that observed for the corresponding (−)-(*S,R,R,S*)-DE2/dG_t adduct. The pH dependent changes in the CD spectrum of this adduct (Figure 6) indicated the presence of two ionizable groups with pK_a values of 2.8 and ≥9.0. For a guanine adduct at N-7, the first pK_a represents deprotonation of the positively charged imidazole ring at N-9, whereas the second ionization corresponds to loss of a proton from N-1 to give an amide anion. Reported pK_a values for the analogous 7-methylguanine are 3.5 and 10.0.[21] Although our observed pK_a values are consistent with the proposed structure, it should be noted that an ionization at high pH cannot be used to exclude substitution at N-1 or N-3 in a *deglycosylated* derivative of guanine, since such derivatives can undergo loss of a proton from the neutral imidazole ring; pK_a values are 3.1 and 10.5 for 1-methylguanine[21] and 4.0 and 9.7 for 3-benzylguanine.[22] However, as noted above, substitution at these positions is not consistent with facile loss of the sugar.

For a trans exocyclic N^2 dG adduct of (−)-BA DE2, pK_a values of 2.2 and 9.2, corresponding to deprotonation of the positively charged imidazole ring at N-7 and of the amide group at N-1, respectively, were also observed. These are in agreement with previously determined values for other adducts at the exocyclic amino group of guanine nucleosides.[4b,c,7]

NMR spectral data for the unacetylated and acetylated adduct, (−)-(*S,R,R,S*)-DE2/$G_{N\text{-}7}$, are summarized in Table III. Coupling constants for the methine protons of the tetrahydro benzo ring of the hydrocarbon are similar to those observed for other adducts in which trans addition to the epoxide ring has occurred (cf. Table II). In particular, $J_{3,4}$ is large, consistent with a conformation in which the purine as well as H-3 and H-4 are pseudoaxial. Of particular interest is the large upfield shift of H-8 of guanine in this adduct (δ 7.16 ppm) and its tetraacetate (δ 7.5 ppm) relative to the unsubstituted nucleoside (δ 8.0 ppm in Me_2SO-d_6). Molecular modeling in which the

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Table III. Chemical Shifts and Coupling Constants (Hz) for a Deglycosylated N-7 Adduct of Deoxyguanosine Formed from Benz[a]anthracene Diol Epoxide-2 and DNA

	methine hydrogens				aromatic hydrogens					
compd	H-1	H-2	H-3	H-4	H-5	H-6	H-7(12)[a]	H-8(11)	H-9(10)	purine hydrogen H-8
(−)-(*S,R,R,S*)-DE2/$G_{N\text{-}7}$[b]	6.76	4.59	3.86	4.96	7.84	8.18	8.14	7.78	7.43	7.16
							8.51		8.00	
					($J_{1,2}$ = 3.3, $J_{2,3}$ = 2.3, $J_{3,4}$ = 8.3, $J_{5,6}$ = 8.8, $J_{8,9}$ = $J_{10,11}$ = 8.4)					
(−)-(*S,R,R,S*)-DE2/$G_{N\text{-}7}$ (tetraacetate)[c,d]	7.02	6.12	5.54	6.41	~7.5	8.28	8.34	7.85	~7.5	~7.5
							8.66	8.08		
					($J_{1,2}$ = 3.3, $J_{2,3}$ = 2.5, $J_{3,4}$ = 8.6, $J_{5,6}$ = 8.9, $J_{8,9}$, $J_{10,11}$ = 8.5, 8.1)					

[a] Although H-12 would normally be expected to resonate downfield relative to H-7, the assignments of these two protons in this adduct are uncertain because of possible effects of the purine substituent. [b] At 500 MHz in methanol-d_4. [c] Exchangeable NH proton resonances were not observed. [d] At 300 MHz in acetone-d_6.

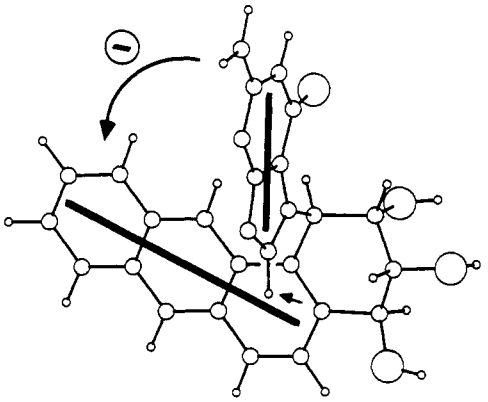

Figure 7. Minimum energy structure (calculated in the gas phase by use of the program QUANTA/CHARMm) for the adduct (−)-(*S,R,R,S*)-DE2/$G_{N\text{-}7}$. Note the location of H-8 (small arrow) of the guanine base above the plane of an aromatic ring, consistent with the pronounced shielding of this proton observed in the NMR spectrum. Heavy lines indicate the transition moments that give rise to the negative CD Cotton effect at 258 nm.

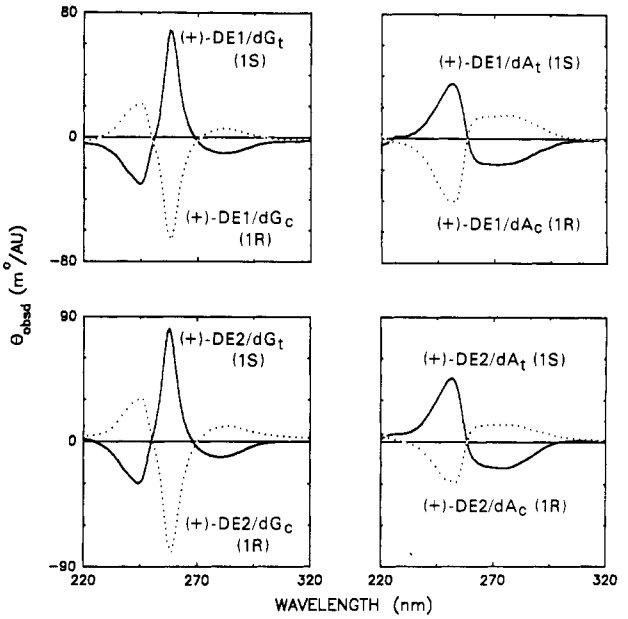

Figure 8. CD spectra in methanol (normalized to 1.0 absorbance unit at 257 nm for dG adducts and 255–256 nm for dA adducts) of the deoxyguanosine and deoxyadenosine adducts formed by attack of their exocyclic amino groups on (+)-BA DE1 and DE2. The corresponding derivatives of the (−)-enantiomers of these diastereomeric diol epoxides exhibited CD spectra (see supplementary material) that were essentially mirror images of those shown.

torsion around the N-7 → C-1 bond was varied over a range of 360° indicated that conformations with H-8 located approximately above C-4a of the anthracene moiety are at an energy minimum in the gas phase (Figure 7). This result is consistent with the observed shielding of H-8.[23]

Circular Dichroism Spectra and Absolute Configuration. Figure 8 shows the circular dichroism (CD) spectra in methanol of unacetylated adducts obtained upon reaction of (+)-(*S,R,S,R*)-DE1 and (+)-(*R,S,S,R*)-DE2 with the exocyclic amino groups of dA and dG. Each of the eight analogous spectra (supplementary material) for the adducts from (−)-(*R,S,R,S*)-DE1 and (−)-(*S,R,R,S*)-DE2 is essentially the mirror image of the spectrum of the adduct derived from the corresponding (+)-enantiomer. The major band for the dG adducts is at longer wavelength (close to 260 nm) and is more intense than that observed for the dA adducts (close to 250 nm).

An empirical relationship between absolute configuration and the signs of the CD bands has been demonstrated for adducts resulting from addition of the exocyclic amino groups of dA or dG to the bay-region diol epoxides of several hydrocarbons.[4,5] Assignment of absolute configuration to many of the present BA adducts was possible on the basis of the known absolute configurations of the starting epoxides and the relative configurations of the adducts (cis *versus* trans) as determined by NMR spectroscopy. In these cases, the same empirical relationship observed for adducts derived from BcPh,[4b] DBajA[4c] and methyl-substituted BAs[5b,c] is also followed. For dA adducts, a positive short-wavelength CD band (250–260 nm) and a negative long-wavelength band (270–290 nm) are observed for adducts with (1*S*)-configuration, whereas bands of approximately equal intensity and opposite sign are observed with adducts whose absolute configuration is (1*R*). For dG adducts, the most intense, central band (250–270 nm) is positive for (1*S*)- and negative for (1*R*)-configuration. Use of this relationship between CD spectra and absolute configuration, together with the known

(23) Several differences exist between the conformation which we suggest here for the N-7 dG adduct from BA DE2 and that proposed by RamaKrishna *et al.* for the analogous compound derived from BP DE2 (ref 19). Specifically, these authors have assigned the chemical shift at δ 8.11 ppm to H-8 of guanine in this BP adduct (in Me_2SO), as contrasted to our assignment of this resonance at δ 7.16 ppm (in MeOD), and have further proposed that the orientation of the purine substituent is pseudequatorial, whereas we believe that the purine in the BA adduct is close to pseudoaxial. The latter conformation is more consistent with the chemical shift for H-8 and the methine proton coupling constants for the BA adduct, as well as with the couplings shown in ref 19 for these protons in the BP adduct. The proposal of a pseudoequatorial conformation of the purine in this adduct was based on an upfield shift of H-11, presumably due to the purine ring current, which would only be possible if this substituent were pseudoequatorial. However, this shielding could alternatively result from interaction of H-11 with the anisotropic carbonyl group in a conformer in which the orientation of guanine is more nearly pseudoaxial.

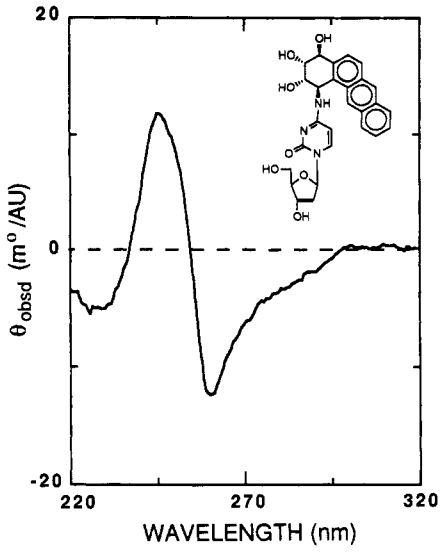

Figure 9. CD spectrum in methanol (normalized to 1.0 absorbance unit at 256 nm) of the dC adduct formed by trans attack of the exocyclic amino group on C-1 of (−)-(*S,R,R,S*)-BA DE2.

absolute configurations of the parent diol epoxides, made it possible to assign cis or trans relative stereochemistry to BA adducts that were not obtained in sufficient quantities for NMR spectroscopy: (−)-(*R,S,R,S*)-DE1/dG_t, (−)-(*R,S,R,S*)-DE1/dA_t, (+)-(*S,R,S,R*)-DE1/dG_t, (+)-(*S,R,S,R*)-DE1/dA_c, (+)-(*R,S,S,R*)-DE2/dG_c, and (+)-(*R,S,S,R*)-DE2/dA_c.

The two BA adducts lacking an observed counterpart with an opposite CD spectrum were the (−)-(*S,R,R,S*)-DE2 dC (Figure 9) and deglycosylated N-7 dG (Figure 6) adducts, which were shown by NMR to have arisen by trans opening of the parent epoxide. The CD spectrum of the N-7 dG adduct bears some resemblance to the spectrum of the (−)-(*S,R,R,S*)-DE2 exocyclic dG_t adduct but its intensity (θ_{obsd} per A_{256} unit) is about 1/3 as great. The Cotton effect at 258 nm results from an exciton interaction between the ^1B_b transition of the anthracene chromophore (parallel to its long axis, λ at 252 nm)[24] and the transition of guanine that is parallel to its long axis (λ at 248 nm).[25] The observed *negative* sign of the long wavelength Cotton effect (ca. 260 nm) and the positive sign of the short wavelength Cotton effect (ca. 240 nm) are thus in accordance with the negative skew sense[24] between the transition moments in the preferred conformation calculated for this adduct (Figure 7). The CD spectrum of the dC adduct (Figure 9), in which attachment of the BA moiety is at the exocyclic amino group, like that of the dC adduct derived from DBajA[4c], is less intense than the spectra of the corresponding purine adducts (Figure 8).

Reaction Preference and Carcinogenicity of Bay-Region Diol Epoxides. The adducts formed by the four diol epoxide isomers derived from each of four different hydrocarbons have now been fully characterized. The present work, as well as previous studies of the covalent binding to purified DNA of the four bay-region diol epoxide isomers from three other hydrocarbons (carcinogenicity data for DBajA diol epoxide isomers unpublished), has not provided any clear correlations between the comparative carcinogenicities of the diol epoxide isomers of each hydrocarbon and either (i) the extent of their bonding to DNA bases *versus* hydrolysis, (ii) the ratio of cis *versus* trans opening of the epoxide group (both carcinogenic and noncarcinogenic DE2 isomers strongly prefer trans epoxide opening), (iii) the ratio of bonding to dG *versus* dA, or (iv) the propensity for forming adducts at dC or the N-7 position of dG.[26]

Table IV summarizes the relative amounts of each type of adduct formed by each diol epoxide. The numbers shown are normalized for a DNA that is 50% GC (calf thymus DNA is 42.5% GC[27]) and are based on the assumption that a DNA with a 50% GC content forms the same overall amount of adduct as does calf thymus DNA. Several generalizations may be made based on Table IV. The BA bay-region diol epoxides bond covalently to DNA more efficiently than the corresponding BP and DBajA diol epoxides but considerably less efficiently than the diol epoxides of BcPh. The BA bay-region diol epoxides bond more extensively to dG than to dA in DNA, a preference similar to the bay-region diol epoxides of BP and DBajA, but opposite from the preferences shown by the bay-region diol epoxides of BcPh. Although the extent of covalent adduct formation is greatest for the (*R,S,S,R*)-diol epoxide isomers derived from all four hydrocarbons, the magnitude of the differences observed (1.2- to less than 3-fold) would seem to be insufficient to explain the far greater carcinogenicities associated with (*R,S,S,R*)-absolute configuration.

The DE2 isomers derived from each hydrocarbon preferentially undergo trans epoxide ring opening, regardless of whether the nucleophile is an exocyclic amino group of dG, dA, or dC or an N-7 dG ring nitrogen. In most cases, the preference for trans addition is very strong (10-fold or greater). In contrast, appreciable quantitites of cis adducts are formed from the DE1 isomers. The majority of the DE1 isomers shown in Table IV form more cis adduct than trans adduct; in only a few cases, though, is the preference 10-fold or greater. The difference in preference for cis *versus* trans addition exhibited by diol epoxides-1 and -2 may reflect at least in part a mechanistic preference of DE1 for reaction *via* a carbocation intermediate (to give both cis and trans products) in contrast to the tendency of the DE2 isomer to react more readily *via* a nucleophilic substitution pathway (to give trans product). Such a difference in mechanism has been observed for solvolysis reactions of the diastereomeric BP diol epoxides.[28] In any event, the extent of trans *versus* cis addition does not explain the far greater carcinogenicities of the (*R,S,S,R*)-isomers compared to the (*S,R,R,S*)-isomers, both of which undergo predominantly trans addition.

The (*S,R,R,S*)-isomers are unique in forming appreciable amounts of a trans dC adduct. The same isomer of BA also forms significant amounts of an N-7 dG adduct; small

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(26) Comparisons of carcinogenicities of diol epoxides derived from *different* hydrocarbons suggest that ability to form dA adducts may be associated with greater potency; for example, see ref 5c.
(27) The GC content of calf thymus DNA was taken to be the average of the values reported in the following. (a) 45%: Mathews, C. K.; van Holde, K. E. *Biochemistry*; The Benjamin/Cummings Publishing Company, Inc.: Redwood City, CA, 1990; p 102. (b) 42% and 41.9%: Rawn, J. D. *Biochemistry*; Harper & Row, Publishers: New York, 1983; p 354. (c) 40% and 44%: Adams, R. L. P., Knowler, J. T.; Leader, D. P. *The Biochemistry of the Nucleic Acids, Tenth Edition*; Chapman and Hall: London, 1986; pp 13 and 23.
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Table IV. Adduct Formation by the Bay-Region Diol Epoxide Isomers of Four Polycyclic Aromatic Hydrocarbons[a]

	% bound to calf thymus DNA	exocyclic dG adducts				exocyclic dA adducts						% A adduct[b]
		(R) cis	(R) trans	(S) cis	(S) trans	(R) cis	(R) trans	(S) cis	(S) trans	dC	N-7	
BcPh[c]												
(−)-(R,S,R,S)-DE1	55		20	7.5			3.5	24				52
(+)-(S,R,S,R)-DE1	60	4.0			4.5	12			39			86
(+)-(S,R,R,S)-DE2	60		25	1.0			17	8.5		8.0		44
(−)-(R,S,S,R)-DE2	75	3.0			29	1.1			43			59
DBajA[d]												
(−)-(R,S,R,S)-DE1	3.5		1.3	1.3			0.12	0.89				30
(+)-(S,R,S,R)-DE1	3.5	1.9			0.81	0.19			0.61			24
(−)-(S,R,R,S)-DE2	3		1.9	0.17			0.36	0.14		0.42		17
(+)-(R,S,S,R)-DE2	9.5	1.9			6.1	<0.05			1.4			16
BP[e]												
(−)-(R,S,R,S)-DE1	5		0.23	4.4			<0.08	0.33				9
(+)-(S,R,S,R)-DE1	3.5	2.7			0.37	0.39			<0.05			13
(−)-(S,R,R,S)-DE2	2.5		1.5	0.26			0.73	0.06				33
(+)-(R,S,S,R)-DE2	14	0.35			13	<0.07			0.35			3
BA												
(−)-(R,S,R,S)-DE1	10		1.0	6.1			0.15	2.8				31
(+)-(S,R,S,R)-DE1	8	5.3			1.4	0.4			0.9			17
(−)-(S,R,R,S)-DE2	10		5.0	0.55			0.9	0.4		0.75	1.5	24
(+)-(R,S,S,R)-DE2	25	1.8			19	<0.2			3.6			16

[a] Except for the column showing % bound to calf thymus DNA, the numbers are based on equal numbers of GC and AT pairs in the DNA, corrected from a calf thymus DNA GC content of 42.5% (ref 24), as follows: The percent of each G and C adduct formed (from HPLC peak areas) was multiplied by 57.5/42.5 to correct for GC content, the resultant values (totaling more than 100%) were normalized to 100%, and the individual percentages were multiplied by the fraction of each individual isomer bound to DNA (column 1). Except in the column headed by %A adduct, the numbers show the percent of added diol epoxide that was converted to that type of adduct. [b] Percent of adducts found on dA. [c] Reference 4b. [d] Reference 4c. [e] Reference 4a.

amounts of this adduct have also been tentatively identified from reaction of DNA with the (S,R,R,S)-diol epoxide of BP.[4a] It is unknown whether depurination occurred during the initial incubation of diol epoxide with DNA or during the subsequent enzymatic digestion or both. Thus, the amount of this adduct indicated in Table IV represents a lower limit, since some of this adduct may have been lost in the organic solvent extraction of the adducted DNA. Formation of N-7 dG adduct(s) from the same isomer derived from BcPh is inferred by the observation of greater numbers of alkali-labile sites in DNA upon reaction with the (S,R,R,S)-diol epoxide in comparison with the its (R,S,S,R) enantiomer.[18]

Recently, we reported that the dA adducts of BA and BcPh diol epoxides might have opposite orientations relative to the DNA axis based on (R)- or (S)-configuration at the attachment site (C-1) on the hydrocarbon moiety.[8] Computational evidence[29] suggests that N^2 dG adducts of the diol epoxides of BaP and three other hydrocarbons have specific orientations in DNA, whereas NMR data and energy minimization calculations indicate that the trans N^2 dG adducts of the (+)-(R,S,S,R)-[30] and (−)-(S,R,R,S)-isomers[31] of BaP diol epoxide lie in opposite directions in the DNA minor groove. The trans adduct derived from the (+)-(R,S,S,R)-isomer ((S)-configuration at the attachment site on the hydrocarbon moiety) points toward the 5′-end of the modified strand, and the trans adduct derived from the (−)-(S,R,R,S)-isomer ((R)-configuration at the attachment site on the hydrocarbon moiety) points toward the 3′-end. It is possible that adduct orientation within DNA could influence biological responses such as carcinogenesis; Table IV shows the (R)- and (S)-adduct configurations at the point of attachment to the hydrocarbon moiety. The most carcinogenic diol epoxide isomer (R,S,S,R) of BcPh, BaP, or BA gives rise to the greatest amount of dG adducts with (S) absolute configuration, about 3–5-fold more than the other three isomeric diol epoxides. Further investigation of whether (S)- *versus* (R)-adduct configuration is linked to subsequent biological effects is warranted.

It is clear that other factors besides absolute configuration at the N-substituted benzylic carbon atom influence the structure of adducts in DNA. The position of the hydroxyl group derived from the epoxide oxygen will depend on whether the epoxide ring opening was cis or trans, and the overall orientation within the DNA structure is different for a cis adduct, relative to the corresponding trans adducts.[32,33] The most carcinogenic (R,S,S,R)-isomers are distinguished by their ability to form adducts with (S)-trans configuration (Table IV). Further investigation of the possible influence of cis *versus* trans adduct formation on subsequent biological effects thus also merits attention.

Supplementary Material Available: Experimental procedures for preparative HPLC of adducts, yields of trans dC adduct formed upon reaction of BA (−)-(S,R,R,S)-DE2 with several oligomers and polymers of dC, circular dichroism spectra in methanol for the eight dG and dA adducts derived from (−)-BA diol epoxides-1 and -2, as well as a tabulation of the calculated methine H–C–C–H dihedral angles and corresponding coupling constants for the acetates of adenine adducts depicted schematically in Figure 5 (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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