Chemical Characterization of DNA Adducts Derived from the Configurationally Isomeric Benzo[c]phenanthrene-3,4-diol 1,2-Epoxides

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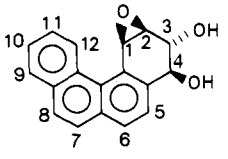
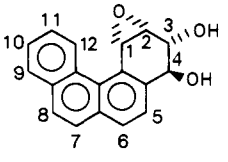
Abstract: The chemical structures of 16 principal adducts formed from the deoxyadenosine and deoxyguanosine residues of DNA upon reaction in vitro with the four configurationally isomeric 3,4-diol 1,2-epoxides derived from benzo[*c*]-phenanthrene-*trans*-3,4-dihydrodiol have been elucidated. These adducts (one cis and one trans addition product derived from each of the four configurationally isomeric diol epoxides and either deoxyguanosine or deoxyadenosine) were prepared in quantities sufficient for structural studies via the reactions of the diol epoxides with deoxyguanylic and deoxyadenylic acids, followed by enzymatic removal of the phosphate group. The site of covalent attachment of the diol epoxide moiety to the nucleoside residue in these adducts is at the exocyclic amino group. For most of the deoxyguanosine adducts, the linkage between this nitrogen and C_1 of the tetrahydrobenzo[*c*]phenanthrene system was established directly by NMR decoupling experiments using the pentaacetate esters of the adducts. Since the lack of observable NMR signals for the exocyclic N-H of adenine in the pentaacetates of the deoxyadenosine adducts made such decoupling experiments impossible, the site of attack of deoxyadenosine was deduced to be at the exocyclic N^6 by a combination of chemical stability considerations and pH titration (pK = 3.8 for a representative unacetylated adduct). The stereochemistry (cis or trans opening of the epoxide) of each adduct was assigned on the basis of the ^1H NMR spectrum of the corresponding pentaacetate ester. An empirical correlation has been found between *R*-absolute configuration at the benzylic carbon of the tetrahydroaromatic moiety and negative ellipticity of the major CD band for these benzo[*c*]phenanthrene diol epoxide adducts of deoxyguanosine and deoxyadenosine, as well as for analogous adducts derived from purine nucleosides and benzo[*a*]pyrene-7,8-diol 9,10-epoxides.

As predicted by the bay-region theory,[1] diol epoxides-1 and -2 are ultimate carcinogens derived from the weakly carcinogenic[2] environmental pollutant[3] benzo[*c*]phenanthrene. Although these diol epoxides are only minor metabolites from the hydrocarbon in rat liver microsomal systems,[4,5] they are of interest because of their exceptionally high tumorigenicity. Both diol epoxide-1 (in which the benzylic hydroxyl group and the epoxide oxygen are cis) and diol epoxide-2 (in which these groups are trans) are the most active diol epoxide tumor initiators tested to date on mouse skin.[6] In the newborn mouse tumor model, diol epoxide-2, but not diol epoxide-1, is also a potent tumorigen.[7] Reaction of the



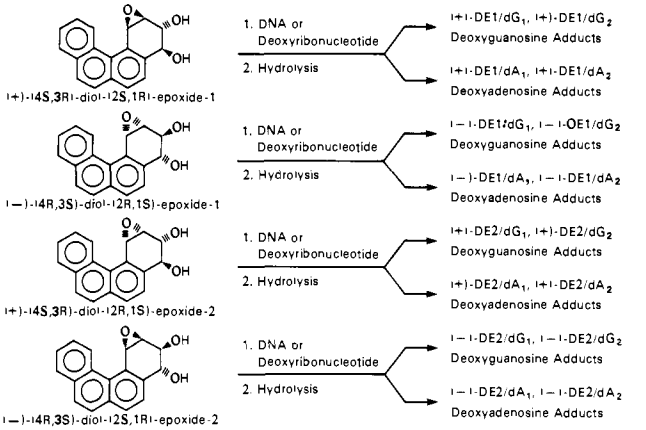
Diol epoxide-1



Diol epoxide-2

four possible configurational isomers of these diol epoxides with calf thymus DNA in vitro yields covalent DNA adducts with very high efficiency, relative to DNA-catalyzed epoxide hydrolysis.[8] Up to 60-70% of the epoxide was converted to such adducts, whereas <6% of the analogous benzo[*a*]pyrene diol epoxide-2 was converted to DNA adducts under the same conditions. Unlike the benzo[*a*]pyrene-7,8-diol 9,10-epoxides, the benzo[*c*]-phenanthrene-3,4-diol 1,2-epoxides react extensively with deoxyadenosine as well as deoxyguanosine residues of DNA in vitro,[8,9] and also when generated metabolically from the hydrocarbon in rodent embryo cell cultures.[10] Notably, the potent carcinogen 7,12-dimethylbenz[*a*]anthracene also reacts extensively with

Scheme I



deoxyadenosine residues in DNA upon metabolic activation.[11] In order to identify the chemical structures of the adducts formed,

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(1) 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.; Daly, J. W. In *Drug Metabolism-From Microbe to Man*; Parke, D. V., Smith, R. L., Eds.; Taylor and Francis Ltd.: London, 1976; pp 13-22.
(2) Stevenson, J. L.; Von Haam, E. *Am. Ind. Hyg. Assoc. J.* **1965**, *26*, 475-478.
(3) Lunde, G.; Bjorseth, A. *Nature (London)* **1977**, *268*, 518-519.
(4) Ittah, Y.; Thakker, D. R.; Levin, W.; Croisy-Delcey, M.; Ryan, D. E.; Thomas, P. E.; Conney, A. H.; Jerina, D. M. *Chem.-Biol. Interact.* **1983**, *45*, 15-28.
(5) Thakker, D. R.; Levin, W.; Yagi, H.; Yeh, H. J. C.; Ryan, D. E.; Thomas, P. E.; Conney, A. H.; Jerina, D. M. *J. Biol. Chem.* **1986**, *261*, 5404-5413.

we have prepared eight cis and eight trans addition products by reaction of the racemic benzo[c]phenanthrene diol epoxides-1 and -2 with deoxyguanylic and deoxyadenylic acids. These products have been correlated chromatographically with the corresponding adducts obtained from the optically active diol epoxides and DNA.[8,9] The present study reports the chemical and spectroscpoic characterization of these adducts.

Experimental Section

2′-Deoxyguanosine-5′-monophosphate and 2′-deoxyadenosine-5′-monophosphate were obtained as the sodium salts from Sigma Chemical Co. and used without further purification. *E. coli* alkaline phosphatase, type III, was obtained from Sigma as a suspension in 2.5 M ammonium sulfate. Optically active[12] and racemic[13] benzo[c]phenanthrene-3,4-diol 1,2-epoxides (cf. Scheme I for structures) were prepared as previously described.

Proton NMR spectra were recorded at 300 MHz in acetone-d_6 (Varian XL-300 for deoxyguanosine adducts and GE-NMR NT-300 for deoxyadenosine adducts). Chemical ionization (NH_3 gas) mass spectra were measured on a Finnigan MAT 4500 quadrupole mass spectrometer with a direct exposure probe. Circular dichroism (CD) spectra were measured on a JASCO Model J500A spectropolarimeter equipped with a data processing system for signal averaging. CD spectra of the free adducts in methanol were normalized to 1.0 absorbance unit at λ_{max}. UV spectra of chromatographic eluates were monitored on-line on a Hewlett-Packard Model 1090 liquid chromatograph equipped with a diode array detector.

Titration curves for adducts (−)-DE1/dG_1 and (−)-DE1/dA_2 (obtained from the reaction of (−)-benzo[c]phenanthrene-(4*R*,3*S*)-diol (2*R*,1*S*)-epoxide-1 with calf thymus DNA) were determined at 25 °C by measurement of the CD spectra upon addition of aliquots of a stock solution of each adduct to solutions whose pH was maintained with either HCl (1–100 mM), NaOH (10 mM), or buffers (5 mM). The final composition of the mixture was 1:9 methanol/water. 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 redetermining the CD spectra, which were found to be identical with those measured directly in buffer solutions near neutrality. The p*K* values were determined from plots of Θ_{obsd} vs pH at 259 nm (for (−)-DE1/dG_1) or 250 and 278 nm (for (−)-DE1/dA_2). In the latter case, measurements at the two different wavelengths gave values of the p*K* that were identical within experimental error (~0.1 p*K* unit).

Adducts with 2′-Deoxyguanosine. In typical experiments, racemic diol epoxide-1 or diol epoxide-2 (8.5 mg in 10–20 mL of acetone) was added in four equal portions at 2-h intervals to a solution of 500 mg of 2′-deoxyguanosine-5′-monophosphate (sodium salt) in 50 mL of 50 mM Tris-hydrochloride buffer (pH 7), and the mixture was stored at 37 °C for 20 h. In an experiment utilizing the enantiomers of diol epoxide-2, 2.5 mg of either optically active diol epoxide in 5 mL of acetone was added in one portion to 50 mL of the buffered nucleotide solution, and the mixture was allowed to react for 3 h at 37 °C. Upon completion of reaction, the cooled reaction mixtures were extracted 3–5 times with 20-mL portions of ethyl acetate and then 2–3 times with 20-mL portions of ether. Traces of organic solvents were removed under a stream of nitrogen, and the aqueous solution was passed through a C_{18} Sep-pak (Waters Associates). The Sep-pak was washed with water, and the desired deoxyribonucleotide adducts were then eluted from the Sep-pak with methanol (10 mL). This step removed a substantial proportion of the unreacted deoxyribonucleotide from the product mixture. The methanolic eluate was concentrated to dryness and dissolved in 10 mL of 125 mM Tris-hydrochloride buffer, pH 8.7, to which was added 3–5 units of *E. coli* alkaline phosphatase. After incubation for 3–6 h at 37 °C, the reaction mixture was cooled and passed through a C_{18} Sep-pak which was washed with water (20 mL) and 25% methanol in water (20 mL) and then eluted with methanol (5 mL). Yields of adducts were in the range of 210 absorbance units from 1400 absorbance units (at 254 nm) of diol epoxide. Adducts corresponding to those formed from DNA[8,9] were identified chromatographically as follows. The adducts from racemic diol epoxide-1 were eluted from a Du Pont Zorbax ODS column (4.6 × 250 mm) with 48% methanol in 50 mM Tris-acetate buffer, pH 7, at a flow rate of 1.2 mL/min. Four compounds, with rt 9.6, 10.2, 14.7, and 16.2 min, were shown to be derived from the (−)-, (+)-, (+)-, and (−)-enantiomers of diol epoxide-1, respectively, by comparison with standards prepared from the optically active diol epoxides. In the order of their elution, these compounds were labeled (−)-DE1/dG_1, (+)-DE1/dG_1, (+)-DE1/dG_2, and (−)-DE1/dG_2. The composition of the adduct mixture, determined by integration of the chromatographic peaks at 254 nm, was as follows: (−)-DE1/dG_1, 10.5%; (+)-DE1/dG_1, 8.7%; (+)-DE1/dG_2, 24.4%; (−)-DE1/dG_2, 18.9%. The adducts from racemic diol epoxide-2 were eluted from a Du Pont Zorbax ODS column (9.4 × 250 mm) with 52% methanol in 50 mM Tris-acetate buffer, pH 7, at a flow rate of 2.5 mL/min. Four compounds, with rt 14.7, 16.1, 18.8, and 22.8 min, were shown to be derived from the (−)-, (+)-, (+)-, and (−)-enantiomers of diol epoxide-2, respectively, and were labeled (−)-DE2/dG_1, (+)-DE2/dG_1, (+)-DE2/dG_2, and (−)-DE2/dG_2, in the order of their elution from the column. Integration of the chromatographic peaks at 254 nm gave the following composition: (−)-DE2/dG_1, 3.3%; (+)-DE2/dG_1, 2.7%; (+)-DE2/dG_2, 36.8%; (−)-DE2/dG_2, 14.1%. The identified adducts did not sum to 100% because of the presence of residual tetraols, as well as unidentified products, in the mixtures. Attempts to identify these latter products are in progress. A portion of the methanolic solution containing the deoxyguanosine adducts was saved for chromatographic separation of the free adducts, and the remainder was acetylated with pyridine and acetic anhydride for 16 h at room temperature.

Separation of Acetylated Deoxyguanosine Adducts. The mixture of acetylated deoxyguanosine adducts from the reaction of racemic benzo[c]phenanthrene diol epoxide-1 was subjected to a preliminary purification by preparative HPLC on a Du Pont Zorbax SIL column (9.4 × 250 mm) eluted with methylene chloride:ethyl acetate:methanol (80:15:5) at a flow rate of 5.5 mL/min.

The fraction that was eluted at 4.6–5.8 min was collected and separated on a Du Pont Zorbax ODS column (9.4 × 250 mm) eluted with 75% methanol in water at a flow rate of 4 mL/min. Four peaks, rt 7.22, 8.50, 10.92, and 13.12 min, were collected and identified as acetates of adducts (−)-DE1/dG_1, (+)-DE1/dG_1, (+)-DE1/dG_2, and (−)-DE1/dG_2, respectively, by chromatographic comparison with the individual acetates formed from the adducts that had been separated on a Du Pont Zorbax ODS column prior to acetylation.

The acetylated deoxyguanosine adducts from racemic benzo[c]-phenanthrene diol epoxide-2 were separated into two fractions by HPLC on a Du Pont Zorbax SIL column (9.4 × 250 mm) eluted with methylene chloride:ethyl acetate:methanol (90:7:3) at a flow rate of 5.5 mL/min: early fraction, rt 8.3 min, late fraction, rt 10.2 min. The early fraction was rechromatographed on a DuPont Zorbax ODS column (9.4 × 250 mm) eluted with 80% methanol in water at a flow rate of 4 mL/min. From the early SIL fraction was obtained two compounds, rt 5.06 and 6.57 min, that were shown to correspond to the acetates of (+)-DE2/dG_2 and (−)-DE2/dG_2, respectively, by chromatographic comparison with the acetates prepared from the individual adducts chromatographically purified prior to acetylation. The late SIL fraction was found to contain two compounds corresponding to the acetates of (−)-DE2/dG_1 (rt 5.04 min) and (+)-DE2/dG_1 (rt 5.35 min). Quantities of these compounds sufficient for spectroscopic analysis were prepared from reactions of the individual enantiomers of diol epoxide-2 with deoxyguanosine-5′-monophosphate. After workup, adducts from each enantiomer were subjected to chromatography on the SIL column, and the late peaks that corresponded to (+)-DE2/dG_1 and (−)-DE2/dG_1 were collected. In the case of adduct (−)-DE2/dG_1 further purification was effected by rechromatography on a Perkin-Elmer HS-3 C_{18} column, 4.6 × 83 mm, eluted with 60% methanol in water at a flow rate of 0.8 mL/min: rt 18.6 min. Because these adducts constitute such a small fraction of the total products formed and because of some anomalies observed in the NMR spectra of their pentaacetates (see Results and Discussion), we verified

(6) Jerina, D. M.; Sayer, J. M.; Yagi, H.; Croisy-Delcey, M.; Ittah, Y.; Thakker, D. R.; Wood, A. W.; Chang, R. L.; Levin, W.; Conney, A. H. In *Biological Reactive Intermediates IIA*; Snyder, R., Parke, D. V., Kocsis, J. J., Jollow, D. J., Gibson, C. G., Witmer, C. M., Eds.; Plenum Publishing Corp.: New York, 1982; pp 501–523. See also: Levin, W.; Wood, A. W.; Chang, R. L.; Ittah, Y.; Croisy-Delcey, M.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1980**, *40*, 3910–3914.

(7) Levin, W.; Chang, R. L.; Wood, A. W.; Thakker, D. R.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1986**, *46*, 2257–2261.

(8) Jerina, D. M.; Sayer, J. M.; Agarwal, S. K.; Yagi, H.; Levin, W.; Wood, A. W.; Conney, A. H.; Pruess-Schwartz, D.; Baird, W. M.; Pigott, M. A.; Dipple, A. In *Biological Reactive Intermediates III*; Kocsis, J. J., Jollow, D. J., Witmer, C. M., Nelson, J. O., Snyder, R., Eds.; Plenum Publishing Corp.: New York, 1986; pp 11–30.

(9) Dipple, A.; Pigott, M. A.; Agarwal, S. K.; Yagi, H.; Sayer, J. M.; Jerina, D. M., submitted for publication.

(10) Pruess-Schwartz, D.; Baird, W. M.; Yagi, H.; Jerina, D. M.; Pigott, M. A.; Dipple, A., submitted.

(11) Dipple, A.; Sawicki, J. T.; Moschel, R. C.; Bigger, C. A. H. In *Extrahepatic Drug Metabolism and Chemical Carcinogenesis*; Rydstrom, J., Montelius, J., Bengtsson, M., Eds.; Elsevier: Amsterdam, 1983; pp 439–448. Bigger, C. A. H.; Sawicki, J. T.; Blake, D. M.; Raymond, L. G.; Dipple, A. *Cancer Res.* **1983**, *43*, 5647–5651.

(12) Yagi, H.; Thakker, D. R.; Ittah, Y.; Croisy-Delcey, M.; Jerina, D. M. *Tetrahedron Lett.* **1983**, *24*, 1349–1352.

(13) Sayer, J. M.; Yagi, H.; Croisy-Delcey, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1981**, *103*, 4970–4972.

that they were indeed identical with the corresponding adducts formed from DNA. Thus, adducts (+)-DE2/dG_1 and (−)-DE2/dG_1 were also isolated after treatment of calf thymus DNA with (±)-benzo[*c*]phenanthrene diol epoxide-2, followed by enzymatic hydrolysis.[14] When the phosphodiesterase and alkaline phosphatase enzymes were added simultaneously, it was noted that substantial amounts (up to 40%) of adducts containing the typical benzo[*c*]phenanthrene chromophore were eluted from C_{18} Sep-paks by 25% methanol in water and consisted primarily of compounds that were not retained on ODS columns eluted with 50% methanol in water. These adducts presumably corresponded to phosphate-containing DNA fragments that were resistant to further enzymatic hydrolysis. In order to assess the possible significance of incomplete enzymatic hydrolysis, small-scale binding reactions were carried out with weight ratios of (±)-benzo[*c*]phenanthrene diol epoxide-2 to DNA of 1:8 and 1:160. When the phosphodiesterase and alkaline phosphatase were added sequentially, as described in ref 14, the enzymatic hydrolysis appeared to be essentially complete, and virtually identical distributions of deoxyribonucleoside adducts were obtained at both the high and low diol epoxide:DNA ratios.

All of the acetylated deoxyguanosine adducts from both diol epoxides-1 and -2 gave mass spectra (CI, NH_3) with molecular ions at m/z 756 ($M^+ + 1$) corresponding to the pentaacetates.

Adducts with 2′-Deoxyadenosine. For adducts from racemic diol epoxide-1, 1.46 g 2′-deoxyadenosine-5′-monophosphate in 146 mL of 50 mM Tris-hydrochloride buffer (pH 7) was mixed with 7.1 mg of diol epoxide in 14.2 mL of acetone and kept at 37 °C for 5 h. For adducts from racemic diol epoxide-2, 1.28 g of 2′-deoxyadenosine-5′-monophosphate in 64 mL of 50 mM Tris-hydrochloride buffer (pH 7) was mixed with 6.3 mg of diol epoxide in 12.6 mL of acetone and kept at 37 °C for a total of 24 h. Reaction mixtures in each case were extracted 4 times with ethyl acetate (1 volume) and once with an equal volume of ether and then purged of organic solvent by bubbling nitrogen through the solutions. The resultant aqueous solutions were diluted with an equal volume of 200 mM Tris-hydrochloride buffer, pH 9, *E. coli* alkaline phosphatase was added (1 unit for each 17 mg of nucleotide), and the solutions were incubated at 37 °C overnight. Thereafter, the reaction mixtures were passed in portions through C_{18} Sep-paks such that six Sep-paks were used for the diol epoxide-1 reaction and three were used for the diol epoxide-2 reaction. Each Sep-pak was washed with water (20 mL) and 25% methanol in water (30 mL) and the adducts were then eluted with methanol (2.6 mL) to yield 178 absorbance units (at 255 nm) of adducts from the diol epoxide-1 reaction and 196 absorbance units of adducts from the diol epoxide-2 reaction. These represent yields in terms of absorbance of about 15 and 18%, respectively, based on starting diol epoxide.

Separation of Deoxyadenosine Adducts. The methanol solution of diol epoxide-1 adducts with deoxyadenosine was separated by chromatography on an Altex Ultrasphere ODS column (5 μm, 4.6 × 250 mm) eluted with 52% methanol in water at 1 mL/min. Four peaks with rt of 15.3, 17.4, 28.5, and 33.4 min were collected and shown, by chromatographic comparison with products formed from the optically active diol epoxides, to be derived from the (+)-, (−)-, (−)-, and (+)-enantiomers of the diol epoxide, respectively, and were labeled (+)-DE1/dA_1, (−)-DE1/dA_1, (−)-DE1/dA_2, and (+)-DE1/dA_2. One product, (−)-DE1/dA_1, required further chromatography in 55% methanol in water to remove a UV absorbing contaminant. The absorbance units at 255 nm in each of the four products so obtained were 21.6, 11.2, 41.4, and 33.3, corresponding to 12%, 6.3%, 23.3%, and 18.7% of the initial product mixtures, respectively.

The methanol solution of the diol epoxide-2 adducts was separated, using the Altex Ultrasphere column above, by elution with 48% methanol in water at 1 mL/min, and peaks with rt 16.4, 18.6, 25.3, and 29.9 min were collected and shown to correspond to products from the (+)-, (−)-, (−)-, and (+)-enantiomers of diol epoxide-2, respectively, and were labeled (+)-DE2/dA_1, (−)-DE2/dA_1, (−)-DE2/dA_2, and (+)-DE2/dA_2. Yields were ~10, 5.3, 55, and ~60 absorbance units at 255 nm for each of these products, respectively, corresponding to 5%, 3%, 28%, and 31% of initial products.

Each solution of purified adduct was evaporated to dryness, dissolved in pyridine (0.5 mL), and treated with acetic anhydride (0.15 mL). After standing at room temperature overnight, solutions were reduced to dryness, and the peracetylated products were recovered by chromatography on a Du Pont Zorbax SIL column (4.6 × 250 mm) eluted with methylene chloride:ethyl acetate:methanol (160:30:1). All of the acetylated deoxyadenosine adducts from both diol epoxides-1 and -2 gave mass spectra (CI, NH_3) with molecular ions at m/z 740 ($M^+ + 1$) corresponding to the pentaacetates.

(14) Baird, W. M.; Brookes, P. *Cancer Res.* **1973**, *33*, 2378–2385. Sawicki, J. T.; Moschel, R. C.; Dipple, A. *Cancer Res.* **1983**, *43*, 3212–3218.

Results and Discussion

Reactions of the four configurationally isomeric 3,4-diol 1,2-epoxides derived from benzo[*c*]phenanthrene with calf thymus DNA in vitro, followed by DNA hydrolysis, have been shown to yield at least four deoxyribonucleoside adducts from each configurational isomer (Scheme I).[8,9] Chromatographic and UV spectral comparison of these adducts with adducts prepared from the diol epoxide isomers and deoxyribonucleotides identified the DNA products as pairs of adducts (numbered in order of their elution from C_{18} columns)[8,9] derived from deoxyguanosine and deoxyadenosine residues. Ultraviolet spectra in methanol of the free deoxyguanosine adducts exhibit λ_{max} as follows: (+)-DE1/dG_1, 253 nm; (−)-DE1/dG_1, 253 nm; (+)-DE1/dG_2, 252 nm; (−)-DE1/dG_2, 252 nm; (+)-DE2/dG_1, 255 nm; (−)-DE2/dG_1, 256 nm; (+)-DE2/dG_2, 251 nm; (−)-DE2/dG_2, 251 nm. The early eluting adducts (dG_1) derived from each enantiomeric pair of diol epoxides had essentially identical UV spectra, as did the late eluting adduts (dG_2). Small differences in the UV spectra of the early and late eluting adducts from a given diol epoxide (viz., DE1/dG_1 vs. DE1/dG_2, DE2/dG_1 vs. DE2/dG_2) were consistently observed; notably, λ_{max} for the late eluting adducts from diol epoxide-2 was at 4–5 nm shorter wavelength than for the corresponding early eluting adducts. The difference in λ_{max} between the early and late eluting adducts from diol epoxide-1 was much smaller: ~1 nm. A similar pattern was observed for the deoxyadenosine adducts, whose λ_{max} values in methanol are the following: (+)-DE1/dA_1, 253 nm; (−)-DE1/dA_1, 253 nm; (+)-DE1/dA_2, 252 nm; (−)-DE1/dA_2, 252 nm; (+)-DE2/dA_1, 255 nm; (−)-DE2/dA_1, 255 nm; (+)-DE2/dA_2, 251 nm; (−)-DE2/dA_2, 251 nm. For these adducts, also, there was little difference between the λ_{max} values for the early and late eluting diol epoxide-1 adducts, and a more substantial spectral difference between the early and late eluting adducts derived from diol epoxide-2.

^{1}H NMR spectra of the unmodified adducts were unsatisfactory because of extreme peak broadening observed in the only suitable solvent, Me_2SO-d_6. This difficulty was avoided by acetylation of the adducts, which also facilitated the purification of the deoxyguanosine adducts by permitting the use of silica gel chromatography. Mass spectra of the acetates confirmed the introduction of five acetyl groups. This observation, in conjunction with their NMR spectra (vide infra), indicated that acetylation of the five hydroxyl groups, and not N-acetylation of the purine base, had occurred.

Site of Purine Substitution. Neither chloroform-*d* nor benzene-d_6 proved to be a satisfactory solvent for measurement of the NMR spectra of the pentaacetates of the adducts, because of line broadening and/or solvent-induced decomposition. However, excellent resolution was obtained in acetone-d_6 (Figure 1 and Table I). Listings of the NMR line positions for the sugar protons are given in the supplementary material. The C_8-H proton of guanine (δ between 7.8 and 7.9 ppm in the adducts; 8.11 ppm in the triacetylated derivative of deoxyguanosine) was observed in all of the adducts from deoxyguanosine. Several of the deoxyguanosine adducts exhibited peaks that were identified by decoupling experiments as consistent with a proton on the exocyclic N^2 of guanine, covalently bound to C_1 of the tetrahydrobenzo[*c*]phenanthrene moiety: (−)-DE1/dG_1, δ 7.72 ppm; (+)-DE1/dG_2, δ 7.74 ppm; (−)-DE1/dG_2, δ 7.75 ppm; (+)-DE2/dG_1, δ 6.06 ppm; (−)-DE2/dG_1, δ 6.08 ppm; (+)-DE2/dG_2, δ 7.01 ppm; (−)-DE_2/dG_2, δ 7.21 ppm. Analogies between the spectra (both NMR and CD; vide infra) of these adducts and the spectrum of adduct (+)-DE1/dG_1, in which this N-H proton was not distinguishable, strongly support the structural assignment of all the deoxyguanosine adducts as resulting from attack of the same nitrogen upon the benzylic C_1 position of the diol epoxide. In the case of adduct (−)-DE1/dG_1, this structural assignment was also supported by measurement of changes in the CD band at 259 nm of the free adduct upon titration with acid and with base.[15] Observed pK values[8] (Figure 2) of 2.4 and 9.7 (in 10% methanol

(15) Kasai, H.; Nakanishi, K.; Traiman, S. *J. Chem. Soc., Chem. Commun.* **1978**, 798–800.

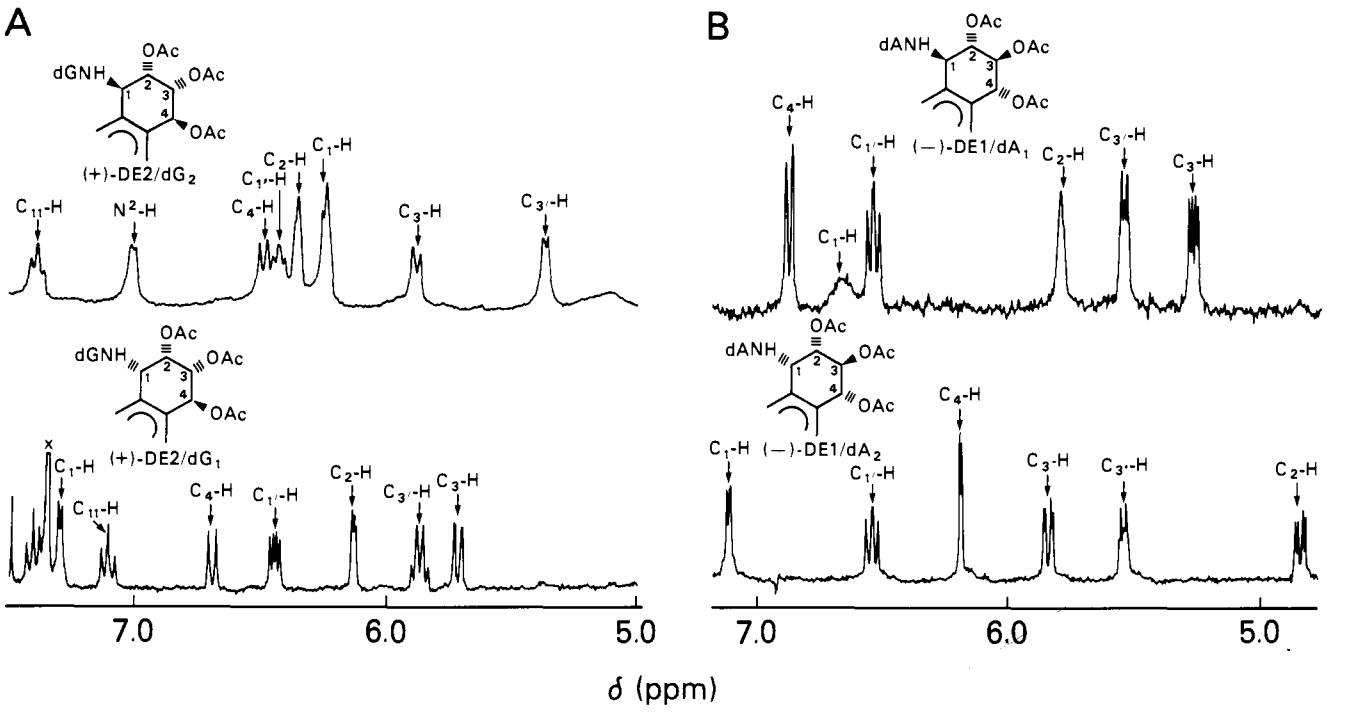


Figure 1. Partial NMR spectra (300 MHz, acetone-d_6) showing the benzo-ring methine proton resonances of typical acetylated adducts derived from benzo[*c*]phenanthrene diol epoxides and deoxyguanosine (A) or deoxyadenosine (B). For brevity, the deoxyguanosine and deoxyadenosine moieties are designated as dG and dA, respectively. Carbon atoms designated with a prime symbol are derived from the sugar.

in water, 25 °C) for this adduct are in good agreement with values of 1.4–2.1 and 9.1–9.8 for guanosine adducts at N^2 formed from benzo[*a*]pyrene diol epoxides.[15,16] In particular, the existence of the amide ionization at high pH excludes the possibility of alkylation at N_1 or O^6.

In the case of the deoxyadenosine adducts, an NMR signal corresponding to the exocyclic N^6-H proton of adenine was not detected, although mass spectra indicated the presence of only five acetyl groups, and the chemical shifts for the methine protons (Table I and supplementary material) were consistent with acetylation of the five hydroxyl groups. For all eight adducts, signals corresponding to protons at C_2 and C_8 of adenine were identified at δ 8.5–8.6 and 7.9–8.3 ppm, respectively. CD spectra of unacetylated adduct (−)-DE1/dA_2 (10% methanol in water 25 °C) showed an increase in the absolute magnitude of both the positive band at 250 nm and the negative band at ~280 nm with increasing pH, indicative of a single ionization with a pK of 2.8 (Figure 2). The value of this pK is consistent with substitution at N^6 (cf. pK values of 2–3 for adducts with benzo[*a*]pyrene-7,8-diol 9,10-epoxide)[17] and not with substitution at N_1, since reported pK values[18] for 1-methyladenosine in water are in the range of 8.3–8.8. Treatment of adducts (+)-DE1/dA_2 and (+)-DE2/dA_2 with base (0.15 M NaOH in ~20% water in methanol) for 1.5 h at 100 °C, which should induce rearrangement of a possible N_1-adduct to the isomeric N^6-adduct,[18,19] followed by HPLC resulted in each case in the recovery of a predominant peak that was chromatographically identical with that for the starting material. The chemical stability of the isolated adducts is also not consistent with substitution at N_3 or N_7 of adenine. Deoxyadenosine residues of DNA methylated at the 3- and 7-positions undergo cleavage of the sugar–nitrogen bond with half-lives of 15–38[20,21] and 3.3 h,[21] respectively, at neutral pH and

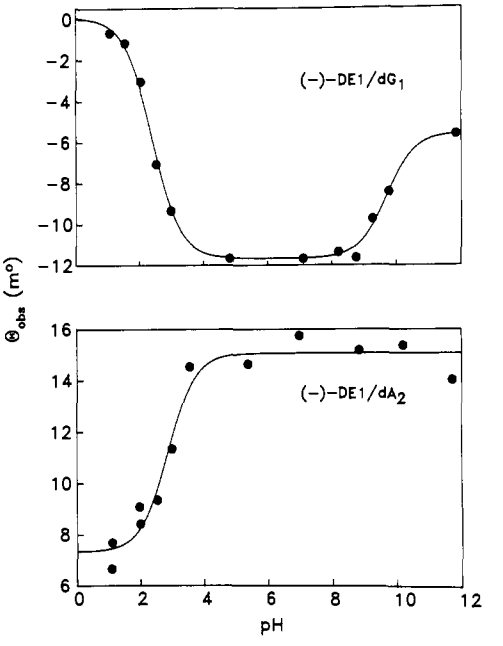


Figure 2. Dependence on pH of the observed ellipticity of nucleoside adducts in 1:9 methanol/water solutions at 25 °C. The upper graph corresponds to Θ_{obsd} at 259 nm for adduct (−)-DE1/dG_1. The curve is a theoretical titration curve for a compound with pK values of 2.4 and 9.7. The lower graph corresponds to Θ_{obsd} at 250 nm for adduct (−)-DE1/dA_2. A theoretical curve is shown for titration of a compound with pK 2.85.

37 °C. Methylation of deoxyadenosine-5′-monophosphate appears to result in even more facile ($T_{1/2}$ ~1.5 h) deglycosylation, under these conditions, of the 3- and 7-methyl derivatives formed.[22]

(16) Moore, P. D.; Koreeda, M.; Wislocki, P. G.; Levin, W.; Conney, A. H.; Yagi, H.; Jerina, D. M. In *Drug Metabolism Concepts*; ACS Symposium Series, No. 44; Jerina, D. M., Ed.; American Chemical Society: Washington, D.C., 1977; pp 127–154.
(17) Jeffrey, A. M.; Grzeskowiak, K.; Weinstein, I. B.; Nakanishi, K.; Roller, P.; Harvey, R. G. *Science* **1979**, *206*, 1309–1311.
(18) Macon, J. B.; Wolfenden, R. *Biochemistry* **1968**, *7*, 3453–3458. Martin, D. M. G.; Reese, C. B. *J. Chem. Soc.* (*C*) **1968**, 1731–1738.
(19) Dipple, A.; Brookes, P.; Mackintosh, D. S.; Rayman, M. P. *Biochemistry* **1971**, *10*, 4323–4330.
(20) 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. *Ibid.* **1973**, *331*, 349–356. Margison, G. P.; Margison, J. M.; Montesano, R. *Biochem. J.* **1976**, *157*, 627–634.
(21) 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.

Table I. ^{1}H NMR Spectra of Acetates of Deoxyribonucleoside Adducts and of Tetraols from Benzo[*c*]phenanthrene Diol Epoxides-1 and -2[a]

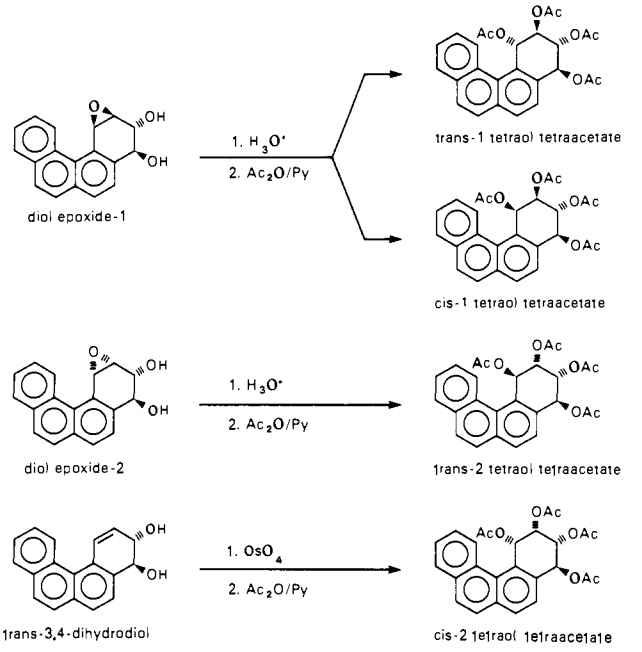
compounds (as acetates)	methine hydrogens			
	C_1–H	C_2–H	C_3–H	C_4–H
cis-1 tetraol	7.33	5.06	5.91	6.43
	$J_{1,2}$ = 2.4;	$J_{2,3}$ = 10.5;	$J_{3,4}$ = 5.4	
(−)-DE1/dG$_2$	7.00	5.07	5.97	6.32
	$J_{1,2}$ = 3.5;	$J_{2,3}$ = 10.2;	$J_{3,4}$ = 4.6	
(+)-DE1/dG$_2$	6.93	5.01	5.96	6.31
	$J_{1,2}$ = 3.4;	$J_{2,3}$ = 10.1;	$J_{3,4}$ = 4.3	
(−)-DE1/dA$_2$	7.12	4.85	5.85	6.20
	$J_{1,2}$ = 3.4;	$J_{2,3}$ = 8.7;	$J_{3,4}$ = 2.2	
(+)-DE1/dA$_2$	7.13	4.85	5.84	6.20
	$J_{1,2}$ = 3.2;	$J_{2,3}$ = 8.5;	$J_{3,4}$ = 2.0	
trans-1 tetraol	6.99	5.45	5.16	6.66
	$J_{1,2}$ = 3.8;	$J_{2,3}$ = 3.2;	$J_{3,4}$ = 8.1	
(−)-DE1/dG$_1$	6.23	5.93	5.17	6.68
	$J_{1,2}$ = 3.2;	$J_{2,3}$ = 2.8;	$J_{3,4}$ = 8.0	
(+)-DE1/dG$_1$	6.22	5.93	5.17	6.68
	$J_{1,2}$ = 3.5;	$J_{2,3}$ = 3.2;	$J_{3,4}$ = 8.2	
(−)-DE1/dA$_1$	6.66	5.79	5.25	6.87
	$J_{1,2}$ = ~2.5;	$J_{2,3}$ = 3.4;	$J_{3,4}$ = 7.8	
(+)-DE1/dA$_1$	6.66	5.79	5.25	6.87
	$J_{1,2}$ = ~2.8;	$J_{2,3}$ = 3.5;	$J_{3,4}$ = 7.8	
cis-2 tetraol	7.55	5.97	5.73	6.69
	$J_{1,2}$ = 3.9;	$J_{2,3}$ = 2.3;	$J_{3,4}$ = 8.6	
(−)-DE2/dG$_1$	7.00	6.30	5.71	6.72
	$J_{1,2}$ = 4.3;	$J_{2,3}$ = 2.0	$J_{3,4}$ = 8.6	
(+)-DE2/dG$_1$	7.30	6.14	5.72	6.70
	$J_{1,2}$ = 4.7;	$J_{2,3}$ = 1.9;	$J_{3,4}$ = 9.3	
(−)-DE2/dA$_1$	7.40	6.10	5.76	6.71
	$J_{1,2}$ = 4.0;	$J_{2,3}$ = 1.8;	$J_{3,4}$ = 7.7	
(+)-DE2/dA$_1$	7.40	6.11	5.76	6.72
	$J_{1,2}$ = 3.9;	$J_{2,3}$ = ~1.9;	$J_{3,4}$ = 7.8	
trans-2 tetraol	6.75	5.97	5.69	6.53
	$J_{1,2}$ = 4.9;	$J_{2,3}$ = 2.5;	$J_{3,4}$ = 8.3	
(−)-DE2/dG$_2$	6.26	6.34	5.91	6.49
	$J_{1,2}$ = 4.1;	$J_{2,3}$ = 2.6;	$J_{3,4}$ = 8.4	
(+)-DE2/dG$_2$	6.25	6.36	5.88	6.49
	$J_{1,2}$ = 4.5;	$J_{2,3}$ = 1.6;	$J_{3,4}$ = 8.5	
(−)-DE2/dA$_2$	6.61	6.25	5.99	6.43
	$J_{1,2}$ <4;	$J_{2,3}$ = 2.7;	$J_{3,4}$ = 8.3	
(+)-DE2/dA$_2$	6.62	6.26	5.99	6.43
	$J_{1,2}$ <4;	$J_{2,3}$ = 2.6;	$J_{3,4}$ = 8.3	

[a] Spectra were measured in acetone-d_6 at 300 MHz. Line positions are reported in δ (ppm) and coupling constants (J) are given in hertz.

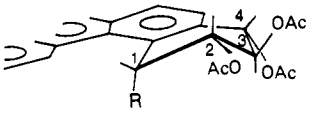
Thus, the sugar–nitrogen bonds in deoxyadenosine adducts alkylated at the 3- or 7-position would not have been expected to survive the conditions used in this study for their preparation and workup. Furthermore, relatively vigorous acid treatment (incubation for 15 h at 37 °C in 0.2 M ammonium acetate buffer, pH 4.5) of (+)-DE1/dA$_2$, (−)-DE2/dA$_2$ and (+)-DE2/dA$_2$ resulted in each case in the recovery of a single chromatographic peak that was identical with that for the untreated adduct.

Stereochemistry of Addition. The NMR signals corresponding to the methine protons on the benzo ring of the acetylated adducts (Table I) were assigned on the basis of direct decoupling or COSY (homonuclear 2-D J-correlation) experiments, which distinguished these protons from $C_{1'}$-H and $C_{3'}$-H of the sugar moiety (cf. Figure 1), and by comparison with NMR spectra of the tetraacetates derived from the tetraols produced upon cis and trans hydration of the benzo[*c*]phenanthrene-3,4-diol 1,2-epoxides (Scheme II). Three of these tetraacetates have been reported.[13] Tetraol cis-2, which was not detected upon hydrolysis of diol epoxide-2, was prepared by oxidation of benzo[*c*]phenanthrene-*trans*-3,4-dihydrodiol with osmium tetroxide, and its tetraacetate was formed by reaction with acetic anhydride in pyridine for 48 h at room temperature. For the diol epoxide-1 adducts, a clear distinction between products resulting from either cis or trans

Scheme II

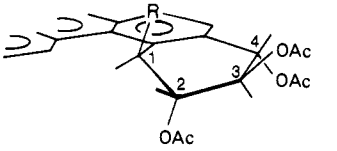


addition of the purine amino group to the epoxide at C_1 was possible on the basis of the coupling constants $J_{2,3}$ and $J_{3,4}$. In the case of the cis adducts, pseudoaxial orientation of the substituent at C_1 (constrained by steric hindrance in the bay region) requires that the acetoxy groups at C_2 and C_3 be pseudoequatorial in the conformation assumed by these molecules; thus, $J_{2,3}$ is large



cis-1 Adducts

(9–10 Hz). For the trans adducts, a conformation is preferred in which the substituent at C_1 is again pseudoaxial, the angle between C_2-H and C_3-H is ~120° ($J_{2,3}$ ~ 2–3 Hz), and the angle between C_3-H and C_4-H is large ($J_{3,4}$ ~ 8 Hz). Thus, for the



trans-1 Adducts

diol epoxide-1 adducts, the late eluting (DE1/dG$_2$ and DE1/dA$_2$) adducts result from cis opening of the epoxide whereas the early eluting adducts (DE1/dG$_1$ and DE1/dA$_1$) result from trans opening.

It was not possible to distinguish between the products of cis and trans addition to diol epoxide-2 on the basis of coupling constants (cf. cis-2 and trans-2 tetraol tetraacetates, Table I). However, when pairs of adducts (DE2/dG$_1$ vs. DE2/dG$_2$, DE2/dA$_1$ vs. DE2/dA$_2$) from the same diol epoxide enantiomer were compared, a marked downfield shift (0.8–1.0 ppm) was consistently observed for the C_1-H signal of the acetates derived from the early eluting adducts (DE2/dG$_1$, DE2/dA$_1$), relative to the acetates of the late eluting adducts (DE2/dG$_2$, DE2/dA$_2$). This resembles the downfield shift of 0.8 ppm of the signal for C_1-H in the cis-2, relative to the trans-2, tetraol tetraacetate. A similar, though less pronounced, downfield shift (0.2–0.5 ppm in the isomer-2 series) is observed for C_1-H in other cis vs. trans tetraol tetraacetates derived from bay-region diol epoxides.[23,24]

(22) Lawley, P. D.; Brookes, P. *Biochem. J.* **1964**, *92*, 19c–20c.

(23) Yagi, H.; Thakker, D. R.; Hernandez, O.; Koreeda, M.; Jerina, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 1604–1611.

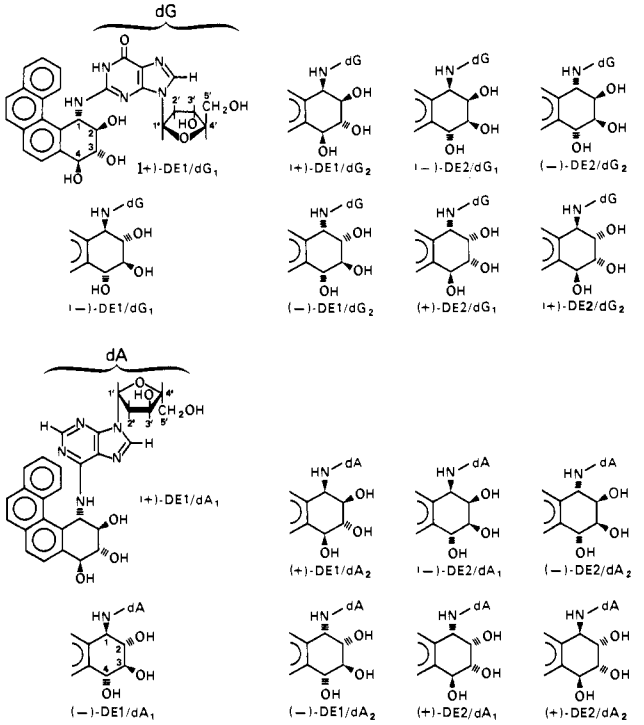


Figure 3. Structures of the adducts derived by addition of the exocyclic nitrogen of deoxyguanosine and deoxyadenosine to the four configurationally isomeric benzo[*c*]phenanthrene diol epoxides. For brevity, the deoxyguanosine and deoxyadenosine moieties are designated as dG and dA, respectively.

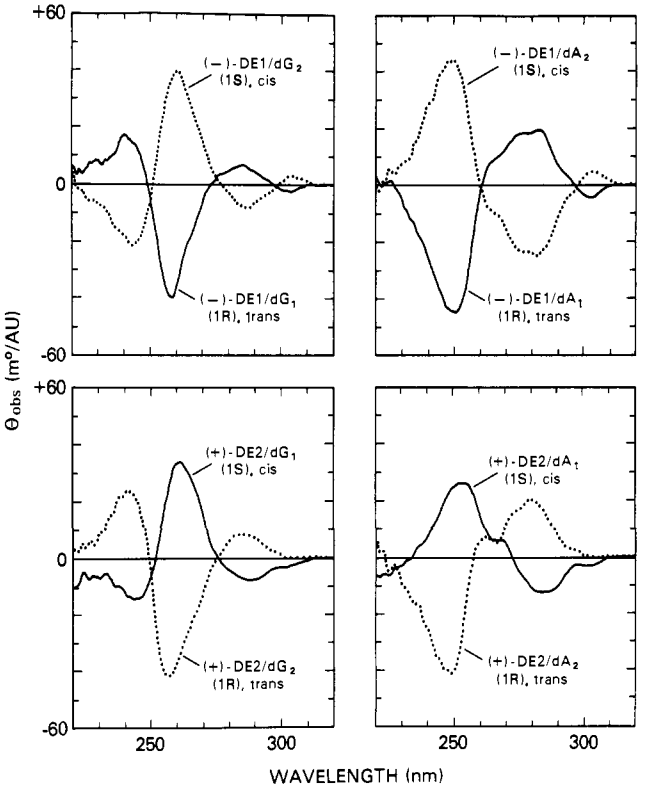


Figure 4. Circular dichroism spectra in methanol for the adducts derived from deoxyguanosine and deoxyadenosine with (−)-benzo[*c*]-phenanthrene-(4*R*,3*S*)-diol (2*R*,1*S*)-epoxide-1 and (+)-benzo[*c*]-phenanthrene-(4*S*,3*R*)-diol (2*R*,1*S*)-epoxide-2. The spectra have been normalized to 1 absorbance unit at λ_{max}. Corresponding derivatives of the enantiomers of these diol epoxides exhibited CD spectra that were essentially mirror images of those shown (see supplementary material).

Thus, based on this empirical relationship of chemical shifts, we assign the stereochemistry of addition to the epoxide as cis for the earlier eluting, DE2/dG$_1$ and DE2/dA$_1$, adducts in each pair. An analogous downfield shift of the C$_1$-H signal (0.5–0.8 ppm) is also observed for the acetylated products of cis addition of the purine nucleosides to diol epoxide-1 (Table I), whose structures are unequivocally assigned on the basis of coupling constants. Partial structures of the adducts are shown in Figure 3.

The chemical shift of the C$_{3'}$-H proton of the sugar (δ 5.86–5.87 ppm; cf. Figure 1) in the acetylated cis adducts (−)-DE2/dG$_1$ and (+)-DE2/dG$_1$ is anomalous, when these compounds are compared with all the other deoxyguanosine adducts (δ 5.3–5.4 ppm) as well as the deoxyadenosine adducts ($\delta \sim 5.5$ ppm). Furthermore, the chemical shift for C$_1$-H of the tetrahydro benzo ring of acetylated cis adduct (−)-DE2/dG$_1$ appears about 0.3 ppm farther upfield than the chemical shift of the corresponding proton in its pseudoenantiomer (+)-DE2/dG$_1$. Decoupling experiments have shown these assignments to be unambiguous. Isolation of these adducts from DNA, followed by acetylation, resulted in a pair of compounds whose NMR spectra were identical with those of the corresponding adducts formed by reaction of (+)- and (−)-diol epoxide-2 with deoxyguanylic acid; thus, the anomalous NMR spectra are not a result of any artefact of the preparation or isolation of these compounds. The CD spectra of these two acetates were essentially mirror images of each other. They were identical in shape to, and about 1.5 times as intense as, the corresponding spectra (vide infra) of the unacetylated adducts. We are unable at present to account for the conformational features of these two cis adduct acetates that are presumably responsible for their observed NMR spectral differences.

In the present study, we observed that trans adducts from deoxyadenosine or deoxyguanosine and diol epoxide-1 were eluted earlier from C$_{18}$ columns by methanol–water mixtures than were their cis-adduct counterparts. The opposite elution order was observed for the diol epoxide-2 adducts; i.e., the cis adducts were eluted earlier. For a given diol epoxide diastereomer, we observed the same elution order for the cis vs. trans adduct derived from each enantiomer; however, this result is probably fortuitous, since such a relationship was previously shown not to exist for analogous guanosine adducts of benzo[*a*]pyrene diol epoxides; e.g. for the (−)-benzo[*a*]pyrene diol epoxide-2 adducts, the trans adduct was eluted before the cis adduct, whereas for the (+)-benzo[*a*]pyrene diol epoxide-2 adducts, the cis adduct was eluted before the trans adduct.[16]

As expected, UV spectra of the adducts derived from a given nucleoside and the two enantiomers of a given diol epoxide diastereomer are superimposable. In the case of diol epoxide-2 adducts, the early eluting *cis adducts* formed from either (+)- or (−)-diol epoxide-2 and deoxyguanosine or deoxyadenosine exhibit UV maxima in methanol at 4–5 nm longer wavelengths than the corresponding late eluting trans adducts. For diol epoxide-1 adducts, there is little ($\sim$1 nm) difference between the UV maxima for the cis and trans adducts, and the early eluting *trans adducts* exhibit maxima at the longer wavelengths.

The preference for cis addition to diol epoxide-1, and trans addition to diol epoxide-2, upon reaction with deoxyadenylic and deoxyguanylic acids is qualitatively similar to that observed for water addition upon hydronium ion catalyzed solvolysis of the epoxides.[13] These results could not, however, have been predicted a priori, since general acid catalysis[13,25,26] of ring opening by the phosphate group, followed by attack of the purine moiety from the same side of the epoxide within the catalytic complex, would favor cis addition products from both diol epoxides. Such a cis

(24) Whalen, D. L.; Ross, A. M.; Yagi, H.; Karle, J. M.; Jerina, D. M. *J. Am. Chem. Soc.* **1978**, *100*, 5218–5221. Yagi, H.; Thakker, D. R.; Lehr, R. E.; Jerina, D. M. *J. Org. Chem.* **1979**, *44*, 3439–3442. 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. Adams, J. D.; Sayer, J. M.; Chadha, A.; Shirai, N.; Lehr, R. E.; Kumar, S.; Levin, W.; Jerina, D. M., in preparation.

(25) Whalen, D. L.; Ross, A. M.; Montemarano, J. A.; Thakker, D. R.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1979**, *101*, 5086–5088.

(26) Gupta, S. C.; Pohl, T. M.; Friedman, S. L.; Whalen, D. L.; Yagi, H.; Jerina, D. M. *J. Am. Chem. Soc.* **1982**, *104*, 3101–3104.

addition mechanism is predominant for reactions of several phenolic compounds with benzo[*a*]pyrene diol epoxide-2[23,27] and might have been predicted for the addition of nucleotides to the benzo[*c*]phenanthrene diol epoxides as well, due to the possibility of complexation between the aromatic moieties of the reactant molecules. Under the conditions of this study, however, this mechanism appears not to have been a major pathway for the nucleotide addition reactions. Prediction of the extent of cis vs. trans addition of nucleotide residues in DNA itself, as opposed to the free nucleotides, is complicated still further by the unknown nature of possible modes of noncovalent binding of the diol epoxides to the DNA prior to the covalent binding step.

Circular Dichroism Spectra. Figure 4 shows circular dichroism (CD) spectra in methanol of the free adducts derived from deoxyguanosine and deoxyadenosine with (−)-benzo[*c*]-phenanthrene-(4*R*,3*S*)-diol (2*R*,1*S*)-epoxide-1 and (+)-benzo-[*c*]phenanthrene-(4S,3R)-diol (2*R*,1*S*)-epoxide-2. The corresponding eight spectra for the adducts from (+)-benzo[*c*]-phenanthrene-(4*S*,3*R*)-diol (2*S*,1*R*)-epoxide-1 and (−)-benzo-[*c*]phenanthrene-(4*R*,3*S*)-diol (2*S*,1*R*)-epoxide-2 are included in the supplementary material. They are essentially mirror images of those shown, indicative that the hydroxyl groups of the sugar contribute little or nothing to the observed CD spectra. Each cis/trans pair of adducts is a pair of epimers differing in configuration at C_1. Cis attack of the purine amino group results in retention of absolute configuration at C_1 of the epoxide, whereas trans attack leads to inversion. Because of this diastereomeric relationship, the CD spectra of the cis and trans adducts are not expected to be strict mirror images of each other (cf. (+)-DE2/dA_1 and (+)-DE2/dA_2); however, the major CD bands are opposite in sign and approximately comparable in intensity for each cis/trans pair. This observation suggests that a large contribution to the observed CD spectra results from the interaction between the purine and phenanthrene moieties and that the relative orientations of these ring systems are primarily determined by the absolute configuration at the benzylic C_1. Of particular interest is the observation that the major CD band at 250–260 nm is negative in all those adducts that have (1*R*)-absolute configuration and positive in those adducts that have (1*S*)-absolute configuration. A similar relationship was observed for the guanosine adducts from optically active benzo[*a*]pyrene-7,8-diol-9,10 epoxide-2, such that the CD band at ~250 nm was negative for the cis adduct from (+)-benzo[*a*]pyrene-(7*R*,8*S*)-diol (9*S*,10*R*)-epoxide-2 and for the trans adduct from (−)-benzo[*a*]pyrene-(7*S*,8*R*)-diol (9*R*,10*S*)-epoxide-2, both of which adducts have (10*R*)-absolute configuration.[16] An apparent discrepancy in the CD spectra for the guanosine adducts of optically active benzo[*a*]pyrene diol epoxide-1 in the earlier study[16] was found to result from a confusion in the elution order of the adducts from cis vs. trans addition to this epoxide. Reinvestigation has now shown[28] that the early peak (on reverse-phase HPLC) derived from guanosine and (+)-benzo-[*a*]pyrene-(7*S*,8*R*)-diol (9*S*,10*R*)-epoxide-1, whose major CD band at 250 nm is negative, is a cis-(10*R*)-adduct and that the early peak derived from guanosine and (−)-benzo[*a*]pyrene-(7*R*,8*S*)-diol (9*R*,10*S*)-epoxide-1, whose CD band at 250 nm is also negative, is a trans-(10*R*)-adduct.

Although the CD spectra of adenosine and deoxyadenosine adducts derived from benzo[*a*]pyrene diol epoxide-2 are more complex,[17,28] the signs of the two most prominent bands (at 244 and 279 nm) bear the same relationship to the absolute configuration at C_{10}, such that these peaks are negative in adducts with (10*R*)-absolute configuration. Thus, for adducts derived from benzo[*a*]pyrene and benzo[*c*]phenanthrene, the two parent hydrocarbons extensively investigated to date, there is an empirical correlation between negative major CD bands exhibited by the products of addition of the exocyclic nitrogen of purine nucleosides to the diol epoxides and (*R*)-absolute configuration at the benzylic position of nitrogen attack. We consider it unsafe to extend this generalization concerning the sign of the CD bands to structural assignment of purine nucleoside adducts formed from diol epoxides of other hydrocarbons, in the absence of further structural information, since the sign of these bands may depend in part on the structure of the hydrocarbon. Thus, we cannot at present predict the relationship between absolute configuration and the sign of the transitions when the parent hydrocarbon is varied. However, it appears reasonable to assume that, for adducts derived from attack of the exocyclic nitrogen of purines on the diol epoxides from a *single parent hydrocarbon*, there will be a roughly opposite relationship between the signs of the CD transitions exhibited by such adducts when they have opposite absolute configurations at the benzylic position of attack. This generalization may be useful in tentatively assigning structures of minor adducts if the absolute configuration of one or more major adducts in a series derived from a given parent hydrocarbon can be unequivocally established by independent means.

Summary. Like the corresponding products from benzo[*a*]-pyrene-7,8-diol 9,10-epoxides, the major isolated products from the reaction in vitro of DNA with the benzo[*c*]phenanthrene diol epoxides result from attack of exocyclic amino groups of purine bases upon the benzylic carbon of the epoxide. However, unlike the benzo[*a*]pyrene diol epoxides, the benzo[*c*]phenanthrene diol epoxides react extensively with deoxyadenosine as well as deoxyguanosine residues in DNA. This has prompted us to isolate and identify a complete set of four cis and four trans adducts derived from attack of a deoxyadenosine moiety on the four configurationally isomeric benzo[*c*]phenanthrene-3,4-diol 1,2-epoxides, as well as the eight analogous adducts from deoxyguanosine. Previous investigation of products involving the addition of adenine-containing residues of nucleic acids to benzo-[*a*]pyrene diol epoxides had been limited by the small amounts of products available, and adducts resulting from the covalent modification of adenine nucleosides by diol epoxide-1 enantiomers had not been characterized. The present study thus represents the first instance in which all possible cis and trans adducts of both adenine and guanine nucleosides to a set of four optically active diol epoxides have been isolable in sufficient quantity to permit their complete spectroscopic characterization and comparison.

With these results in hand, it is possible to compare the distributions of cis vs. trans adducts from reaction of DNA with the configurationally isomeric diol epoxides, since such data may eventually provide a better understanding of the detailed molecular interactions between these chiral molecules and DNA. In the reaction between the enantiomers of benzo[*c*]phenanthrene diol epoxide-2 and calf thymus DNA in vitro,[8,9] trans adducts are the major products from both deoxyguanosine and deoxyadenosine residues. The direction of this selectivity resembles that observed for diol epoxide-2 in reactions with the free mononucleotides (see above) and in hydronium ion catalyzed[13] solvolysis. Although trans attack is preferred by both diol epoxide-2 enantiomers, the absolute configuration of the diol epoxide exerts a substantial influence upon the *extent* to which this trans attack by deoxyadenosine residues in DNA is favored. Approximately 40 times more trans (DE2/dA_2) than cis (DE2/dA_1) adduct is formed from (−)-benzo[*c*]phenanthrene diol epoxide-2, whereas only twice as much trans as cis adduct is formed from the enantiomeric (+)-diol epoxide-2. Absolute configuration appears to exert much less of an influence on cis vs. trans adduct formation from diol epoxide-2 with deoxyguanosine residues as nucleophiles.

In the case of diol epoxide-1, the two enantiomers actually prefer to undergo attack from different directions (cis vs. trans) upon reaction with deoxyadenosine residues in DNA: the (−)-diol epoxide-1 enantiomer yields mainly (7:1) the cis adduct, whereas the (+)-diol epoxide-1 enantiomer yields mainly (3:1) the trans adduct. The latter case, as well as the preference (~3:1) for trans addition of deoxyguanosine residues to (−)-diol epoxide-1, represents a reversal of the selectivity observed both in hydronium ion catalyzed solvolysis and in the reactions of the free nucleotides

(27) Sayer, J. M.; Yagi, H.; Wood, A. W.; Conney, A. H.; Jerina, D. M. *J. Am. Chem. Soc.* **1982**, *104*, 5562–5564. Huang, M.-T.; Wood, A. W.; Chang, R. L.; Yagi, H.; Sayer, J. M.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1986**, *46*, 558–566.

(28) Agarwal, S. K.; Sayer, J. M.; Yeh, H. J. C.; Yagi, H.; Chadha, A.; Koreeda, M.; Jerina, D. M., in preparation.

with both diol epoxide-1 enantiomers, in which cis attack on the epoxide predominates. The influence of absolute configuration on selectivity for cis vs. trans addition of deoxyribonucleotide residues in DNA to the benzo[c]phenanthrene diol epoxide enantiomers must reflect differences in interactions of these chiral diol epoxides with the asymmetric DNA molecule. Details of these interactions are presumably significant in bringing about the observed differences among the tumorigenic[7] as well as mutagenic[29] responses elicited by the enantiomeric diol epoxides.

(29) Wood, A. W.; Chang, R. L.; Levin, W.; Thakker, D. R.; Yagi, H.; Sayer, J. M.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1984**, *44*, 2320-2324.

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Supplementary Material Available: Listing of the ^{1}H NMR chemical shifts for the sugar hydrogens of the acetylated deoxyribonucleoside adducts, as well as CD spectra of the adducts derived from (+)-benzo[c]phenanthrene-(4*S*,3*R*)-diol (2*S*,1*R*)-epoxide-1 and (-)-benzo[c]phenanthrene-(4*R*,3*S*)-diol (2*S*,1*R*)-epoxide-2 (2 pages). Ordering information is given on any current masthead page.

Communications to the Editor

Nucleophilic Additions to Thionolactones. New Synthetic Technology for the Construction of Medium- and Large-Ring Ethers

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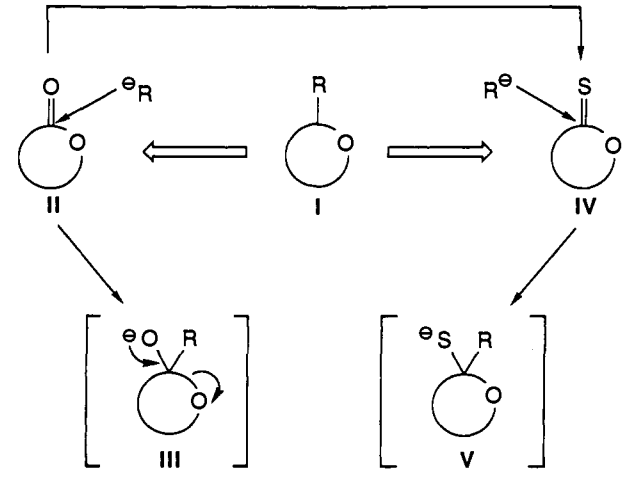
Medium- and large-ring molecular frameworks are abundant in nature[1] and are increasingly becoming targets for total synthesis. While a plethora of methods are now available for the synthesis of medium lactones and macrolactones, technology for the construction of the corresponding cyclic ethers is still emerging.[2] In this paper we report a new method for the construction of medium and large cyclic ethers from the readily available lactones[3] via the corresponding thionolactones.[4]

Scheme I outlines the general concepts that led to the development of the present technology. In view of their ready availability, lactones II were considered as excellent potential precursors to their ether counterparts I. Unfortunately, nucleophilic additions to medium and large macrolactones generally result in rupture of the ring, due to the high reactivity of the tetrahedral intermediates III obtained from the initial addition. On the other hand, nucleophilic addition to thionolactones IV (available from the corresponding lactones)[4] was expected[5,6] to lead to a more stable, and yet more nucleophilic, tetrahedral intermediate V due to the

Scheme I



unique properties of the thiolate anion. Facile alkylation of V followed by further elaboration of the resulting mixed thioketals was then expected to furnish the requisite cyclic ethers I with varying substitution. These expectations were fully realized resulting in a practical, flexible, and wide-scope method for the generation of medium and large cyclic ethers as the following results illustrate.

Thionocaprolactone[7] reacted smoothly with a variety of organometallic reagents, including methyllithium, allyllithium, and 2-furyllithium in THF at -78 °C, producing, after quenching with MeI, the corresponding addition products in good to excellent yields according to eq. 1 (Table I, entries 1-4). Furthermore, the methylthio group was easily and efficiently removed from these adducts by reduction with tin hydride reagents (e.g., Ph_3SnH, n-Bu_3SnH) as indicated in Table I.

To explore the generality and scope of this method in terms of ring size and substitution, a series of thionolactones were prepared[7] and subjected to the described sequence (organometallic addition-MeI trapping and Ph_3SnH-AIBN reduction) leading to a series of substituted oxocyclic systems in good to excellent yields. Table I includes some of these results illustrating the potential of the present technology in the construction of medium

(1) For some excellent recent reviews on such compounds from marine origins, see: Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 251, 551; **1986**, *3*, 1.

(2) For some recent examples see: (a) Nicolaou, K. C.; Hwang, C.-K.; Duggan, M. E.; Reddy, K. B.; Marron, B. E.; McGarry, D. G. *J. Am. Chem. Soc.* **1986**, *108*, 6800. (b) Nicolaou, K. C.; Duggan, M. E.; Hwang, C.-K. *J. Am. Chem. Soc.* **1986**, *108*, 2468. (c) Overman, L. E.; Blumenkoph, T. A.; Castaneda, A.; Thompson, A. S. *J. Am. Chem. Soc.* **1986**, *108*, 3516. (d) Carling, R. W.; Holmes, A. B. *J. Chem. Soc., Chem. Commun.* **1986**, 565. (e) Kocienski, P. *J. Chem. Soc. Perkin Trans. 1* **1985**, 2093. (f) Schreiber, S.; Kelly, S. E. *Tetrahedron Lett.* **1984**, *25*, 1757. (g) Jackson, W. P.; Ley, S. V.; Morton, J. A. *Tetrahedron Lett.* **1981**, *22*, 2601.

(3) For reviews on methods of constructing medium lactones and macrolactones, see: (a) Nicolaou, K. C. *Tetrahedron* **1977**, *33*, 683. (b) Back, T. G. *Tetrahedron* **1977**, *33*, 3041. Masamune, S.; Bates, G. S.; Corcoran, J. W. *Angew Chem. Int. Ed. Engl.* **1977**, *16*, 585. Paterson, I.; Mansuri, M. M. *Tetrahedron* **1985**, *41*, 3569.

(4) For reviews on the synthesis of thionolactones, see: (a) Cava, M. P.; Levinson, M. I. *Tetrahedron* **1985**, *41*, 5061. (b) Jones, B. A.; Bradshaw, J. S. *Chem. Rev.* **1984**, *84*, 17.

(5) For addition of organolithium reagents to thionoesters, see: Narasimhau, L.; Sanitra, R.; Swenton, J. S. *J. Chem. Soc., Chem. Commun.* **1978**, 719.

(6) In line with these properties is our recent successful bridging of macrodithionolactones to bicyclic systems.[2a]

(7) Thionocaprolactone (**1**) was prepared from caprolactone and Lawesson's reagent in 80% yield. The other thionolactones reported in this work were obtained similarly and in the following yields. Table I (entry, yield): 5, 40%; 6, 32%; 7, 56%; 9, 62%; 10, 80%; 11, 78%; Scheme II, compound **1**, 80%; Scheme III, compound **6**: 72%. The lactones were obtained from the corresponding hydroxy acids by lactonization: Corey, E. J.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1974**, *96*, 5614. Further details will be given in a forthcoming full account of this work.

0002-7863/87/1509-2504$01.50/0