Synthesis and Identification of Benzo[a]pyrene-Guanine Nucleoside Adducts Formed by Electrochemical Oxidation and by Horseradish Peroxidase Catalyzed Reaction of Benzo[a]pyrene with DNA

E. G. Rogan,*,†,‡ E. L. Cavalieri,†,‡ S. R. Tibbels,†,‡ P. Cremonesi,†,‡ C. D. Warner,§ D. L. Nagel,†,‡ K. B. Tomer,∥ R. L. Cerny,∥ and M. L. Gross∥

Contribution from the Eppley Institute for Research in Cancer and Department of Pharmaceutical Sciences, University of Nebraska Medical Center, 42nd Street and Dewey Avenue, Omaha, Nebraska 68105-1065, Department of Chemistry, Hastings College, Hastings, Nebraska 68901, and Midwest Center for Mass Spectrometry, Department of Chemistry, University of Nebraska—Lincoln, Lincoln, Nebraska 68588-0362. *Received July 29, 1987*

Abstract: One-electron oxidation plays an important role in the metabolism of many substrates and their covalent binding to macromolecules. This mechanism of activation can be demonstrated by elucidation of the structure of DNA adducts. In this paper, we report the synthesis of adducts by anodic oxidation of benzo[a]pyrene (BP) in the presence of deoxyguanosine (dG) or guanosine (G). By using ^{1}H and two-dimensional NMR spectroscopy as well as fast atom bombardment and collisionally activated decomposition (CAD) mass spectrometry, adducts were identified as BP bound at C-6–C-8 of guanine (Gua), dG, and G and to N-7 of Gua. Loss of deoxyribose from the N-7 adduct was anticipated, but it was unexpectedly found that about 30% of the C-8 adduct with dG lost the deoxyribose moiety. The C-8 adduct of G almost entirely retained the ribose moiety. These compounds were used as markers for high-pressure liquid chromatography (HPLC) to identify adducts formed in the horseradish peroxidase catalyzed binding of BP to DNA. By use of HPLC in two solvent systems, adducts were identified in the supernatant fraction obtained after ethanol precipitation of the DNA and in an enzymatic digest of the DNA. The supernatant, containing adducts lost by depurination, afforded 95% of the N-7 adduct and about half of the C-8 adduct. The major adduct identified in the DNA digest was the C-8 of dG. The structure of the N-7 adduct in the supernatant was confirmed by CAD mass spectrometry. These results demonstrate that horseradish peroxidase catalyzes binding of BP to DNA by one-electron oxidation.

One fundamental concept in chemical carcinogenesis is that covalent binding of chemicals to cellular macromolecules, DNA, RNA, and protein, is the first critical step in the multistage process leading to tumor formation.[1,2] Most chemicals require metabolic activation to electrophilic intermediates that react with cellular nucleophiles.[1,2] For polycyclic aromatic hydrocarbons (PAH), metabolic activation can occur by two main pathways: one-electron oxidation to yield reactive intermediate radical cations[3-5] and monooxygenation to produce bay-region diol epoxides.[6,7] Peroxidases, including horseradish peroxidase (HRP) and prostaglandin H synthase (PHS), catalyze one-electron oxidation,[8-14] whereas cytochrome P-450 catalyzes both one-electron oxidation and monooxygenation.[15]

The most potent PAH, which include benzo[a]pyrene (BP), 7,12-dimethylbenz[a]anthracene, and 3-methylcholanthrene, have two main features in common: (1) a relatively low ionization potential (IP), which allows metabolic removal of one electron with formation of a radical cation, and (2) appreciable charge localization in the PAH radical cation, which renders this intermediate specifically and efficiently reactive toward nucleophiles.[3-5]

A correlation of IP with binding of PAH to DNA catalyzed by HRP[16] and PHS[17] has been established, suggesting that a low IP is necessary for binding to occur by one-electron oxidation. When the PAH radical cation has a charge partially localized at one or a few carbon atoms, specific binding to nucleophiles can occur. This was demonstrated by studying one-electron oxidation of various PAH with iodine/pyridine[18,19] and $Mn(OAc)_3$.[20,21]

One-electron oxidation of BP produces a radical cation with charge localized at C-6.[20-27] Experiments using ^{3}H/^{14}C-labeled BP indicate that this position is involved in the binding of BP to DNA in vivo,[28] but these adducts have not been isolated. Similar

* To whom correspondence should be addressed.
† Eppley Institute for Research in Cancer, University of Nebraska Medical Center.
‡ Department of Pharmaceutical Sciences, University of Nebraska Medical Center.
§ Department of Chemistry, Hastings College.
∥ Midwest Center for Mass Spectrometry, Department of Chemistry, University of Nebraska—Lincoln.

(1) Miller, J. A. *Cancer Res.* **1970**, *30*, 559-576.
(2) Miller, E. C.; Miller, J. A. *Cancer (Philadelphia)* **1981**, *47*, 2327-2345.
(3) Cavalieri, E. L.; Rogan, E. G. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic: New York, 1984; Vol. VI, pp 323-369.
(4) Cavalieri, E.; Rogan, E. In *Polycyclic Hydrocarbons and Carcinogenesis*; Harvey, R. G., Ed.; ACS Symposium Series 283; American Chemical Society: Washington, DC, 1985; pp 290-305.
(5) Cavalieri, E.; Rogan, E. *EHP Environ. Health Perspect.* **1985**, *64*, 69-84.
(6) Sims, P.; Grover, P. L. In *Polycyclic Hydrocarbons and Cancer*; Gelboin, H. V., Ts'o, P. O. P., Eds.; Academic: New York, 1981; Vol. 3, pp 117-181.
(7) Conney, A. H. *Cancer Res.* **1982**, *42*, 4875-4917.
(8) Zenser, T. V.; Mattamal, M. B.; Armbrecht, H. J.; Davis, B. B. *Cancer Res.* **1980**, *40*, 2839-2845.
(9) Wise, R. W.; Zenser, T. V.; Kadlubar, F. F.; Davis, B. B. *Cancer Res.* **1984**, *44*, 1893-1897.
(10) Josephy, P. D.; Eling, T. E.; Mason, R. P. *J. Biol. Chem.* **1983**, *258*, 5561-5569.
(11) Degen, G. H.; Eling, T. E.; McLachlan, J. A. *Cancer Res.* **1982**, *42*, 919-923.
(12) Kalyamaraman, B.; Sivarajah, K.; Eling, T. E. *Carcinogenesis (London)* **1983**, *4*, 1341-1343.
(13) Boyd, J. A.; Eling, T. E. *J. Biol. Chem.* **1984**, *259*, 13885-13896.
(14) Josephy, P. D.; Eling, T. E.; Mason, R. P. *Mol. Pharmacol.* **1983**, *23*, 461-466.
(15) Ortiz de Montellano, P. R. In *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, R. P., Ed.; Plenum: New York, 1986; pp 217-271.
(16) Cavalieri, E. L.; Rogan, E. G.; Roth, R. W.; Saugier, R. K.; Hakam, A. *Chem.-Biol. Interact.* **1983**, *47*, 111-122.
(17) Devanesan, P.; Rogan, E.; Cavalieri, E. *Chem.-Biol. Interact.* **1987**, *61*, 89-95.
(18) Cavalieri, E.; Roth, R. *J. Org. Chem.* **1976**, *41*, 2679-2684.
(19) Cavalieri, E.; Roth, R.; Rogan, E. G. In *Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis*; Freudenthal, R. I., Jones, P. W., Eds.; Raven: New York, 1976; Vol. 1, pp 181-190.
(20) Rogan, E.; Roth, R.; Cavalieri, E. In *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*; Bjorseth, A., Dennis, A. J., Eds.; Battelle: Columbus, OH, 1980; pp 259-266.
(21) Cremonesi, P.; Cavalieri, E.; Rogan, E. *Abstracts of Papers*, 192nd National Meeting of the American Chemical Society, Anaheim, CA; American Chemical Society: Washington, DC, 1986; ORGN 11.
(22) Rochlitz, J. *Tetrahedron* **1967**, *23*, 3043-3048.

0002-7863/88/1510-4023$01.50/0

experiments conducted in vitro in which HRP catalyzed binding of BP to DNA indicate that virtually all of the binding occurs at C-6.[29]

Determination of the structure of adducts formed with cellular macromolecules provides evidence on mechanisms of activation leading to covalent binding. Because DNA may be the cellular macromolecule critical to initiation of carcinogenesis, we have studied the BP–DNA adducts formed in the HRP/H_2O_2 system. HRP was used to bind BP to DNA because the catalytic ability of the enzyme is often predictive of activation by mammalian peroxidases.[30] This in vitro binding system also allows identification of both stable and labile DNA adducts by comparison with synthetic adducts.

Anodic oxidation of BP in the presence of guanine (Gua) nucleosides was investigated as a suitable synthetic system for obtaining adducts formed by one-electron oxidation. Gua nucleosides were chosen because this base is most frequently modified by chemical carcinogens in biological systems.[31]

In this paper, we report the electrochemical synthesis of BP adducts formed with deoxyguanosine (dG) and guanosine (G), their structure elucidation, and their use in identification of adducts formed in HRP-catalyzed binding of BP to DNA in vitro.

Experimental Section

General Procedures. UV absorbance spectra were recorded in methanol on an Aminco DW-2C.

Proton and homonuclear two-dimensional chemical shift correlation spectroscopy (COSY) NMR spectra were recorded on a Varian XL-300 at 299.938 MHz as solutions in DMSO-d_6 at temperatures of 22, 35, and 45 °C. Spectra run at temperatures above 22 °C are indicated in the figure legends. Chemical shifts (δ) are reported relative to tetramethylsilane, which was employed either as a primary internal reference or as a secondary reference relative to residual DMSO at 2.50 ppm. Typical instrument parameters used were as follows: flip angle = 45°, SW = 4000 Hz, word length = 32 bit; data size = 32K; recycle time = 3.5–4.0 s. Water supression was accomplished with presaturation at the frequency of the water resonance for 1–2 s prior to the 45° observation pulse. For the COSY experiments a D_1–90°–t_1–90° acquisition sequence was used. A spectral width of 500–900 Hz was used with quadrature detection to collect a 128 or 256 fid × 1K data matrix. The matrix was zero-filled in the t_1 dimension and sine-bell weighted prior to transformation. The digital resolution of the resulting 1K × 1K matrix was 1.5 Hz per point.

Collisionally activated decomposition (CAD) spectra were obtained with a Kratos MS-50 triple analyzer tandem mass spectrometer, an MS/MS instrument that was previously described.[32] This instrument consists of a high-resolution MS-I of Nier–Johnson geometry, followed by an electrostatic analyzer used as MS-II. Samples were dissolved in a minimal amount of DMSO and a 1-μL aliquot was added to the matrix (dithiothreitol/dithioerythritol). We can only estimate that quantities in the range of 1–5 μg were used for each analysis. Fast atom bombardment (FAB) by 7-keV xenon atoms was used to desorb the preformed ions from the matrix, which was supported on a copper probe held at 8 kV.

CAD spectra were obtained in an MS/MS or tandem mass spectrometer mode by activating the ions in the third field free region. Collisions with helium gas (sufficient to suppress the ion beam by 50%) were used, and CAD spectra were obtained by scanning MS-II. Detection was with a postacceleration detector at 15 kV with respect to ground. Ten to thirty scans were signal averaged for each spectrum by using software developed at the Midwest Center for Mass Spectrometry. B/E_1 linked scans were obtained by activating ions in the first field free region and scanning both B and E_1 so that the ratio of B/E_1 was held constant.[33]

High-pressure liquid chromatography (HPLC) was conducted on a Spectra Physics 8100 or 8700 solvent delivery system. Effluents were monitored in series for UV absorbance (300 nm) and fluorescence, recording the data on a computing integrator. Where appropriate, radioactivity was monitored and recorded with a Ramona flow monitor (IN/US, Fairfield, NJ). Biologically prepared adduct samples were analyzed on an Altex Ultrasphere ODS 5-μm column (4.6 mm × 25 cm). After the column was eluted for 5 min with 30% methanol in water, a 70-min linear gradient to 100% methanol was run at 0.8 mL/min. A second solvent system for biologically prepared samples consisted of elution for 5 min with 20% acetonitrile in water, followed by an 80-min linear gradient to 100% acetonitrile, run at 1 mL/min. Preparative HPLC was conducted on a Du Pont Zorbax ODS column (21.2 mm × 25 cm) with a 100-min linear gradient from 50% methanol in water to 100% methanol at a flow rate of 5 mL/min.

Electrochemical syntheses were conducted with an apparatus (EG & G Princeton Applied Research, Princeton, NJ) composed of a potentiostat/galvanostat (Model 173), digital coulometer (Model 179), and cell system (Model 377A). The cell was equipped with a saturated calomel reference and a platinum counter electrode. Both the reference and counter electrodes were placed in bridge tubes separated from the bulk of the solution by a porous glass frit. The potentiostat was connected to a digital multimeter (Model 8060A, Fluke Manufacturing Co., Everett, WA) so that the electrolysis current could be read.

The electrolysis potential for BP was selected on the basis of the anodic peak potential measured for BP by cyclic voltammetry (Model CV27, Bioanalytical Systems, Lafayette, IN).

Commercially available DMF was purified by vacuum distillation over calcium hydride just prior to use and was stored under argon. $KClO_4$ (Aldrich) was used as obtained. BP was purified by column chromatography on aluminum oxide eluted with benzene/hexane (1:1). The product was recrystallized from benzene/hexane (mp 177–178 °C). The nucleosides (P-L Biochemicals, Milwaukee, WI) were desiccated under vacuum at 90 °C for 48 h prior to use.

Electrochemical Synthesis of BP–dG and BP–G Adducts. Coupling between $BP^{\cdot+}$ and nucleophilic groups of Gua nucleosides was obtained by selective anodic oxidation of BP. The only difference between experiments with dG and G stemmed from their solubility in DMF. G could be used in an amount up to 3-fold greater than dG, although this resulted in longer electrolysis time and more frequent occurrences of overcurrent, due to formation of impurities on the electrode surface.

In a typical preparation, DMF (35 mL, freshly distilled) containing 0.5 M $KClO_4$ as the supporting electrolyte was preelectrolyzed at +1.450 V, while argon was bubbled into the cell, until no appreciable current could be detected (ca. 30 min). The nucleoside (0.5 mmol of dG or 1.5 mmol of G) was added, and stirring was continued until the solution was clear. Bubbling of argon was stopped, and a positive pressure was maintained. BP (0.10 mmol of BP/1.0 mmol of nucleoside) was added as a solid; when it was dissolved, the cell was switched on. The electrode potential was gradually raised from 0 to 1.10 V, the anodic peak potential of BP, and kept constant at this value during the whole electrolysis. Both the output current (i) and the total charge (Q) were monitored throughout the experiment: at the beginning of the electrolysis i was usually 40–50 mA. The reaction was stopped when i had decreased to ca. $^1/_{20}$th of the initial value and a charge 3 times the theoretical charge expected (for a two-electron transfer) had accumulated. These two conditions were usually achieved in ca. 90 min.

This reaction afforded several adducts. To collect the adducts with BP bound to the C-8 of Gua, i.e., 8-(benzo[a]pyren-6-yl)deoxyguanosine (C8dG), 8-(benzo[a]pyren-6-yl)guanosine (C8G), and 8-(benzo[a]pyren-6-yl)guanine (C8Gua), 2 volumes of triple-distilled water was added to the dark brown solution at the end of the reaction. The resulting precipitate was removed by centrifugation. Small aliquots of the clear yellow supernatant were passed through two tandem C18 Sep-Paks (Waters, Milford, MA) and eluted with 2 volumes of water followed by 2 volumes of 40% methanol in water to remove unreacted nucleoside. The adducts were eluted in 2 volumes of 100% methanol and a final volume of acetone. The C-8 adducts were then purified by HPLC. In solution, these three yellow compounds showed characteristic greenish fluorescence.

To collect the 7-(benzo[a]pyren-6-yl)guanine (N7Gua) adduct and an adduct containing two molecules of BP (BPBPGua) a different purification procedure was followed. The reaction mixture was dried under vacuum, the solid material was dissolved in 0.5 mL of DMSO, and 3 mL

(23) Jeftic, L.; Adams, R. N. *J. Am. Chem. Soc.* **1970**, *92*, 1332–1337.
(24) Wilk, M.; Girke, W. *J. Natl. Cancer Inst.* (*U.S.*) **1972**, *49*, 1585–1597.
(25) Cavalieri, E.; Auerbach, R. *J. Natl. Cancer Inst.* (*U.S.*) **1974**, *53*, 393–397.
(26) Johnson, M. D.; Calvin, M. *Nature* (*London*) **1973**, *241*, 271–272.
(27) Blackburn, G. M.; Taussig, P. E.; Will, J. P. *J. Chem. Soc., Chem. Commun.* **1974**, 907–908.
(28) Rogan, E.; Roth, R.; Katomski, P.; Benderson, J.; Cavalieri, E. *Chem.-Biol. Interact.* **1978**, *22*, 35–51.
(29) Rogan, E.; Katomski, P. A.; Roth, R. W.; Cavalieri, E. *J. Biol. Chem.* **1979**, *254*, 7055–7059.
(30) Marnett, L. J.; Weller, P.; Battista, J. R. In *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, R. P., Ed.; Plenum: New York, 1986; pp 29–76.
(31) Sage, E.; Haseltine, W. A. *J. Biol. Chem.* **1984**, *259*, 11098–11102.
(32) Gross, M. L.; Chess, E. K.; Lyon, P. A.; Crow, F. W.; Evans, S.; Tudge, H. *Int. J. Mass Spectrom. Ion Phys.* **1982**, *42*, 243–254.
(33) Jennings, K. R.; Mason, R. S. In *Tandem Mass Spectrometry*; McLafferty, F. W., Ed.; Wiley: New York, 1983; pp 197–222.

of acetone was added. The solution was then applied to a preparative silica TLC plate (Merck). DMSO was removed under vacuum. The plate was eluted with acetone/benzene/water (70:20:10), and the following products were separated: BP dimer (R_f 0.89), BPBPGua (R_f 0.52), C-8 adducts (R_f 0.46), N7Gua (R_f 0.43). The bands of the products were removed from the plate, and final purification of BPBPGua and N7Gua was conducted on HPLC. BPBPGua eluted as a broad peak with a retention time similar to that of BP with the methanol/water gradient. N7Gua was virtually insoluble in any solvent and sparingly soluble in DMSO. It displayed a great tendency to adhere to glassware and HPLC packing materials. Both compounds were yellow. N7Gua fluoresced purple, whereas BPBPGua fluoresced yellow.

Typical yields from the BP and dG reaction were 4.1% C8dG, 1.8% C8Gua, 47% N7Gua, 5.4% BPBPGua, and <0.5% BP dimer. From the reaction of BP and G, typical yields were 24% C8G, 1.6% C8Gua, 3.6% N7Gua, 1.6% BPBPGua, and <0.3% BP dimer.

BP: UV, λ_{max} (nm) 266, 274 (s), 285, 297, 349, 365, 385, 404; NMR, 7.83–7.92 (m, 2 H, 8-H, 9-H), 8.04–8.10 (m, 3 H, 2-H, 4-H, 5-H), 8.23 (d, 1 H, 3-H), 8.37–8.42 (m, 2 H, 1-H, 7-H), 8.45–8.49 (d, 1 H, 12-H), 8.72 (s, 1 H, 6-H), 9.22–9.28 (m, 2 H, 10-H, 11-H).

N7Gua: UV, λ_{max} (nm) 266, 277 (s), 288, 301, 357, 375, 395, 406; NMR, 6.20 (s, 2 H, 2-NH_2[Gua]), 7.38 (d, 1 H, 5-H), 7.58 (d, 1 H, 7-H), 7.85 (t, 1 H, 8-H), 7.96 (t, 1 H, 9-H), 8.11 (d, 1 H, 4-H), 8.14 (t, 1 H, 2-H), 8.30 (d, 1 H, 3-H), 8.34 (s, 1 H, 8-H[Gua]), 8.49 (d, 1 H, 1-H), 8.61 (d, 1 H, 12-H), 9.37 (d, 1 H, 10-H), 9.37 (d, 1 H, 11-H), 10.72 (s, 1 H, 1-H[NH Gua]).

C8Gua: UV, λ_{max} (nm) 266, 277, 289, 301, 357, 375, 395, 406; NMR, 6.49 (s, 2 H, 2-NH_2[Gua]), 7.75–7.78 (m, 1 H, 8-H), 7.85 (d, 1 H, 5-H), 7.92–8.00 (m, 2 H, 7-H, 9-H), 8.07–8.15 (m, 2 H, 2-H, 4-H), 8.28 (d, 1 H, 3-H), 8.46 (d, 1 H, 1-H), 8.58 (d, 1 H, 12-H), 9.33–9.39 (m, 2 H, 10-H, 11-H), 10.79 (br s, 1 H, 1-H[NH Gua]), 13.0 (br s, 1 H, 9-H[NH Gua]).

C8dG: UV, λ_{max} (nm) 267, 277 (s), 289, 301, 358, 376, 396, 406; NMR, 1.63–1.74 (m, 1 H, 2′-H), 2.83–2.92 (m, 1 H, 2′-H), 3.10–3.45 (m, 3 H, 4′-H, 5′-H_2), 3.96–4.00 (m, 1 H, 3′-H), 4.74 (s, 1 H, 3′-OH), 5.42 (t, 1 H, 1′-H), 5.58–5.85 (2 br s, 1 H, 5′-OH), 6.69 (br s, 2 H, 2-NH_2[Gua]), 7.45 (d, ~$^1/_2$ H, 5-H), 7.59 (d, ~$^1/_2$ H, 5-H), 7.65 (d, ~$^1/_2$ H, 7-H), 7.80–7.85 (m, ~1–$^1/_2$ H, 7-H, 8-H), 7.91–7.99 (m, 1 H, 9-H), 8.08–8.17 (m, 2 H, 2-H, 4-H), 8.23 (d, 1 H, 3-H), 8.50 (d, 1 H, 1-H), 8.62 (d, 1 H, 12-H), 9.35–9.41 (m, 2 H, 10-H, 11-H), 10.95 (s, 1 H, 1-H[NH Gua]).

C8G: UV λ_{max} (nm) 267, 277, 290, 303, 359, 377, 398, 407; NMR, 3.46–3.79 (m, 7 H, 3′-H, 4′-H, 5′-H_2, 2′-OH, 3′-OH, 5′-OH), 4.79 (q, 1 H, 2′-H), 4.96 (d, ~$^1/_2$ H, 1′-H), 5.03 (d, ~$^1/_2$ H, 1′-H), 6.61–6.67 (2 br s, 2 H, 2-NH_2[Gua]), 7.49 (d, ~$^1/_2$ H, 5-H), 7.57 (d, ~$^1/_2$ H, 5-H), 7.69–7.81 (m, 2 H, 7-H, 8-H), 7.85–7.90 (m, 1 H, 9-H), 7.98 (d, ~$^1/_2$ H, 4-H) 8.04–8.11 (m, ~1–$^1/_2$ H, 2-H, 4-H), 8.23–8.27 (m, 1 H, 3-H), 8.41–8.45 (m, 1 H, 1-H), 8.56 (d, 1 H, 12-H), 9.26–9.35 (m, 2 H, 10-H, 11-H).

BPBPGua: UV λ_{max} (nm) 266, 291, 303, 363 (s), 380, 403; NMR, 6.48 (s, 2 H, 2-NH_2[Gua]), 7.57–7.68 (m, 4 H, 5-H, 7-H), 7.86–8.26 (m, 13 H, 12 ar, 8-H[Gua]), 8.39–8.44 (m, 1 H, ar), 8.81–8.92 (m, 4 H, 10-H, 11-H), 10.99 (s, 1 H, 1-H[NH Gua]).

HRP-Catalyzed Synthesis of BP–DNA Adducts. [^{3}H]BP (100 μM, 15 Ci/mmol, Amersham Corp., Arlington Heights, IL) was bound to 6 mM calf thymus DNA (P-L Biochemicals, Milwaukee, WI) in the presence of 0.5 mM H_2O_2 and 0.1 mg/mL HRP (type II, Sigma Chemical Co., St. Louis, MO) in 0.067 M sodium potassium phosphate, pH 7.0.[29] Following the reaction period of 1 h at 37 °C, the DNA was precipitated by addition of 2 volumes of ethanol and wound out on a glass rod. The supernatant was saved, and the DNA was redissolved in 0.05 M Tris/0.005 M $MgCl_2$, pH 7.1, and extracted twice with an equal volume of $CHCl_3$. The supernatant and $CHCl_3$ extracts were combined, evaporated, lyophilized, redissolved in methanol/DMSO (1:1), and analyzed by HPLC. The DNA was digested to deoxyribonucleosides by using the method of Baird and Brookes.[34] In this procedure, DNA was digested first with deoxyribonuclease I, followed by snake venom phosphodiesterase and alkaline phosphatase. Adducts were recovered from the digest by six extractions with an equal volume of $CHCl_3$ and analyzed by HPLC. A number of preparations were analyzed, and reproducible chromatograms were routinely obtained. To collect N7Gua for MS analysis, six 40-mL reactions were incubated as described above and the supernatants combined. A total of 10 μg of N7Gua was obtained after purification by HPLC and was analyzed by CAD/MS.

Results

Electrochemically Synthesized Adducts. Electrochemical synthesis of adducts from BP and dG or G consistently yielded three major isolated adducts, C8dG or C8G, C8Gua, and N7Gua, plus BPBPGua. Coupling of BP with dG afforded predominantly the N7Gua adduct (80% of isolated products), whereas reaction of BP with G gave predominantly C8G (70% of isolated products). A second feature distinguishing the two reactions was the relative stability of C8G vs C8dG. Approximately 30% of the C8dG formed lost the deoxyribose moiety to yield C8Gua, whereas only 6% of C8G lost the ribose moiety.

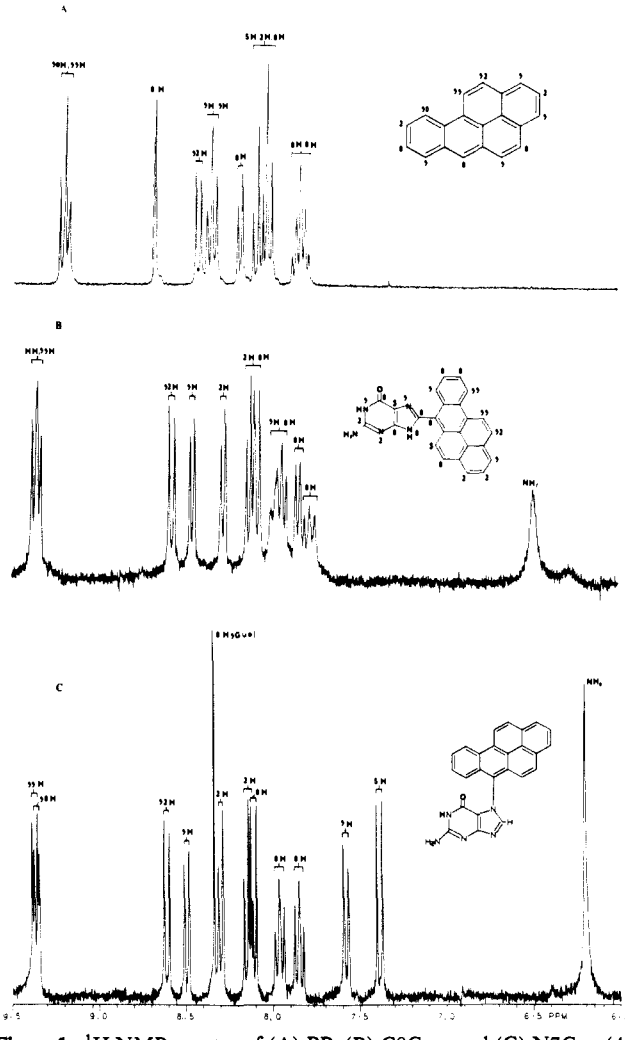

Figure 1. ^{1}H NMR spectra of (A) BP, (B) C8Gua, and (C) N7Gua (45 °C).

With the standard methanol/water HPLC gradient, C8dG eluted at 55 min, C8Gua at 57 min, and N7Gua at 59.5 min. In the acetonitrile/water gradient, C8dG eluted at 32 min, C8Gua at 37 min, and N7Gua at 39 min. These two gradients provided different conditions under which small quantities of adducts from biological samples could be separated to prove identity with the electrochemically synthesized adducts.

The structures of the synthesized adducts were elucidated by a combination of UV, NMR, and MS, which included both FAB and CAD spectra. FAB[35] and FAB coupled with CAD[36] have been used in the structure determination of modified nucleosides and nucleotides. Above 300 nm, UV spectra of the adducts showed maxima red-shifted by 8–10 nm, which is characteristic of substitution at C-6 of BP.[37] Evidence for structure determination

(34) Baird, W. M.; Brookes, P. *Cancer Res.* **1973**, *33*, 2378–2385.

(35) (a) Slowikowski, D. L., Schram, K. H. *Nucleosides & Nucleotides* **1985**, *4*, 309–345. (b) Budzikiewicz, H. *Mass. Spectrom. Rev.* **1985**, *4*, 145.

(36) (a) Crow, F. W.; Tomer, K. B.; Gross, M. L.; McCloskey, J. A.; Bergstrom, D. E. *Anal. Biochem.* **1984**, *139*, 243–262. (b) Tomer, K. B.; Gross, M. L.; Deinzer, M. L. *Anal. Chem.* **1986**, *58*, 2527–2534. (c) Gross, M. L.; Tomer, K. B.; Cerny, R. L.; Giblin, D. E. In *Mass Spectrometry in the Analysis of Large Molecules*; McNeal, C. J., Ed.; Wiley: Chichester, UK, 1986; pp 171–190.

(37) Cavalieri, E.; Calvin, M. *Photochem. Photobiol.* **1971**, *14*, 641–653.

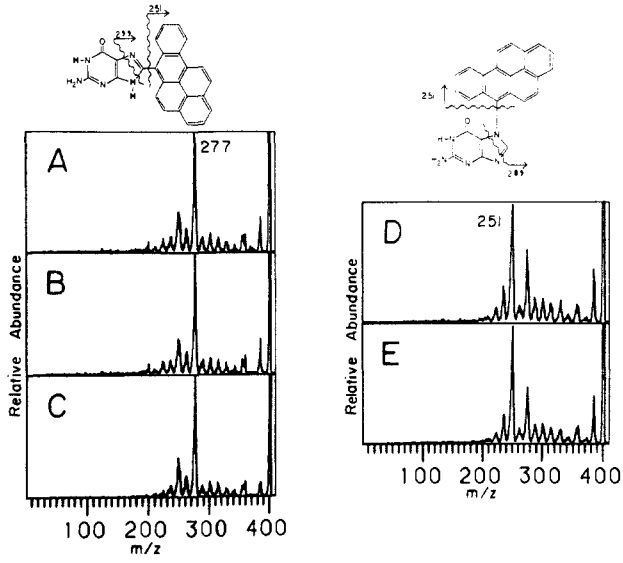

Figure 2. CAD spectra of (A) C8Gua, (B) C8dG, (C) C8G, (D) N7Gua (2-μg sample), and (E) N7Gua (10 μg) isolated from the HRP-catalyzed reaction.

by NMR and MS is discussed below.

N7Gua. Determination of the structure was accomplished by using NMR and FAB coupled with tandem mass spectrometry. The NMR spectrum (Figure 1C) shows the absence of the singlet proton at C-6 of the BP moiety, indicating that BP is substituted at position 6. This observation is further substantiated by the shielding effect of the protons 5-H and 7-H due to the peri effect of Gua substituted at C-6. The remaining protons of BP were assigned by comparing the chemical shifts with those of the parent compound BP. The presence of the sharp singlet of the proton at C-8 of Gua (8.34 ppm) indicates that Gua was not substituted at C-8. In addition, the two protons that give a resonance at 6.20 ppm demonstrate that no substitution occurred at the amino group. Thus, it appears that substitution of the Gua moiety occurred at N-7. Loss of deoxyribose provides further evidence for an N-7 adduct, because substitution at this position destabilizes the glycosidic bond.

The mass spectrum of ions desorbed by FAB shows an $(M + H)^+$ ion of m/z 402 (observed 402.1360, theoretical 402.1355, error 1.2 ppm). Upon collisional activation (Figure 2D), the $(M + H)^+$ ion decomposes to produce a most abundant ion of m/z 251 (probably $C_{20}H_{11}^+$ of the BP moiety). The m/z 251 ion is abundant because protonation probably occurs readily at N-7, followed by loss of neutral Gua. A second major fragment ion of m/z 277 is probably BP-$NC^{\bullet+}$. A third fragment ion of m/z 385 is assigned as $(M + H - NH_3)^+$. Ions of intervening masses constitute the series formed by losses of C_nH_{n-4}, although losses of C_nH_{n-5} and C_nH_{n-3} also occur. Similar series of ions are found in the CAD spectra of unsubstituted PAH.[38]

C8Gua. Elucidation of the structure of the C8Gua adduct by NMR rests on the absence of both the C-6 proton in the BP moiety and the sharp singlet of C-8 in Gua (Figure 1B). Assignment of the other protons was obtained by comparison of the chemical shifts with those of the parent compound and by COSY.

An $(M + H)^+$ ion of m/z 402 (observed 402.1336, theoretical 402.1355, error 4.9 ppm) is again desorbed by FAB, and collisional activation yields the same three major fragments as N7Gua (Figure 2A), although the $(M + H)^+$ ion of this isomer has a significantly different CAD spectrum from that of N7Gua. The most abundant fragment is likely BP-$CN^{\bullet+}$, at m/z 277. The greater abundance of the fragment of m/z 277 in the spectrum of C8Gua as compared with N7Gua is attributed to the greater stability of BP-$CN^{\bullet+}$ vs BP-$NC^{\bullet+}$. The relative abundances of the m/z 251 and 277 ions are reproducible under the conditions

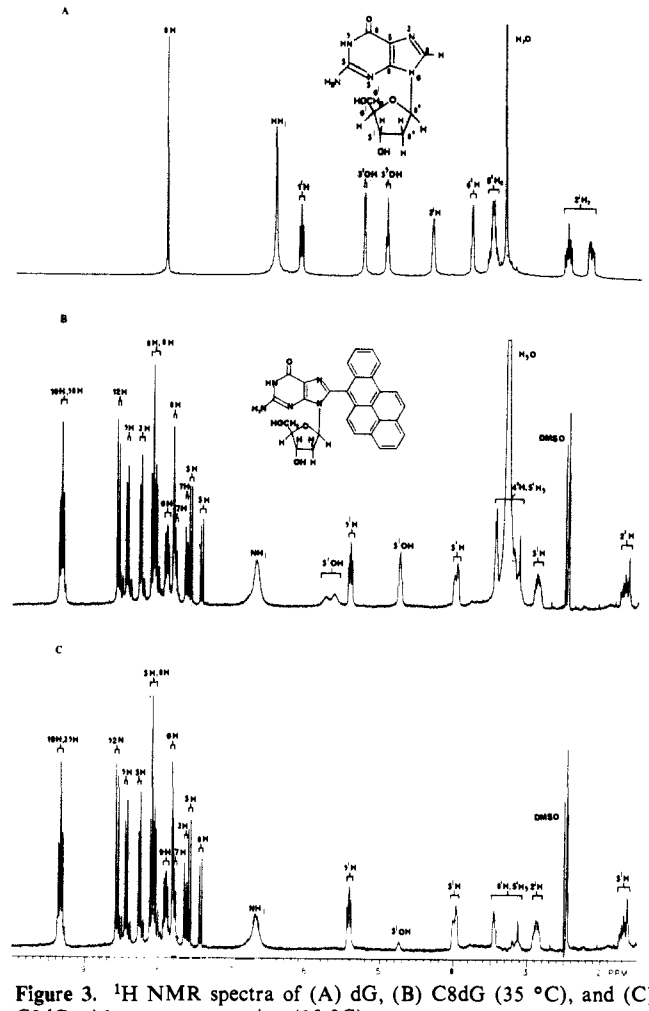

Figure 3. ^1H NMR spectra of (A) dG, (B) C8dG (35 °C), and (C) C8dG with water suppression (35 °C).

of high-energy collisional activation used here and provide a criterion for rapidly distinguishing the N-7 and C-8 adducts at the microgram level.

A second isomer of the C8Gua adduct cochromatographed with the C8dG adduct under the conditions of both gradients. Formation of the two isomers arises from hindered rotation of the Gua moiety bound at C-6 due to the peri effect of 5-H and 7-H of the BP moiety. To demonstrate that this was indeed a C8Gua adduct, the "C8dG" peak was isolated from an electrochemical preparation and dissolved in methanol/water (1:1). The mixture was hydrolyzed in 1 M HCl for 1 h at 37 °C, neutralized, and chromatographed to give two peaks, one with a retention time of C8Gua and the other with a retention time of C8dG. The apparent C8dG was analyzed by FAB mass spectrometry, and the highest mass ion observed was of m/z 402, indicating the presence of a BP adduct without any sugar moiety. The C8Gua peak at 57 min in the methanol/water gradient HPLC contains only one isomer. This is corroborated by its NMR spectrum (Figure 1B), in which the proton at C-5 of the BP moiety shows only one chemical shift, in contrast to the same proton of C8dG (Figure 3B), which exhibits two chemical shifts arising from two unseparated isomers.

C8dG. The NMR spectrum of C8dG is shown in Figure 3B, and the spectrum with water suppression is shown in Figure 3C. Both spectra are compared with that of dG (Figure 3A). The C8dG NMR spectrum shows the absence of the characteristic singlet of the proton at C-6 of BP. The remaining aromatic proton resonances were assigned by using COSY and by comparing their chemical shifts with those of the parent BP. In this case, the two shielded protons 5-H and 7-H each exhibit two different chemical shifts, as evidenced by COSY, suggesting the presence of the two isomers generated by hindered rotation of dG at the C-6 bond of BP. COSY spectroscopy of the aliphatic region allows us to

(38) Pachuta, S. J.; Kenttämaa, H. I.; Sack, T. M.; Cerny, R. L.; Tomer, K. B.; Gross, M. L.; Pachuta, R. R.; Cooks, R. G. *J. Am. Chem. Soc.* **1988**, *110*, 657–665.

assign unequivocally the 1′-H and the two 2′-H protons, as well as the 3′-H and 3′-OH. The 3′-OH signal almost disappears with water suppression (Figure 3C). The broad signal at 5.7 ppm, which totally disappears with water suppression, has been tentatively assigned as the 5′-OH of dG. The remaining protons, 4′-H and 5′-H_2, are thought to be under the broad signal of water (Figure 3B). With water suppression, they are tentatively designated as shown in Figure 3C. With the exception of one of the 2′-H protons and the tentatively assigned 5′-OH, all protons of the deoxyribose moiety are shifted upfield compared with the corresponding protons of dG (Figure 3A), presumably due to the anisotropic shielding effect of the π electrons of BP.

The FAB mass spectrum of C8dG contains two major ions of m/z 518 $(M + H)^+$ and of m/z 402. Accurate mass determination by peak matching yields a value of 518.1830; the correct value for $C_{30}H_{24}N_5O_4$ is 518.1828, indicating an error of 0.2 ppm. The m/z 402 ion arises by H transfer from the sugar moiety to the base followed by loss of $C_5H_8O_3$. Also present in the spectrum are ions of m/z 540 $(M + Na)^+$ and of m/z 424 $(M + Na - C_5H_8O_3)^+$. Collisional activation of the two ions of m/z 540 and 518 causes production of fragments of m/z 424 $(BP - Gua + Na)^+$ and 402 $(BP - Gua + H)^+$, respectively. Collisional activation of the ion of m/z 424 produces three major fragments in decreasing abundance: ions of m/z 385, 277, and 251. More importantly, the CAD spectrum of the ion of m/z 402 (Figure 2B) once again shows that the BP is bound at C-8 and not at N-7.

C8G. The same criteria adopted for elucidation of the structure of C8dG were used to determine the structure of C8G. The FAB mass spectrum of C8G contains major ions of m/z 556 $(M + Na)^+$, 534 $(M + H)^+$ (observed 534.1787, theoretical 534.1777, error 1.8 ppm), 424 $(BP - Gua + Na)^+$, and 402 $(BP - Gua + H)^+$. The latter two ions result from losses of $C_5H_8O_4$ moieties. Collisional activation of the ion of m/z 424 again produces ions of m/z 385, 277, and 251. The collisionally activated decompositions of the ion of m/z 402 show conclusively that the BP is bound to C-8 and not N-7 (Figure 2C).

The site of binding is corroborated by the lack of the sharp singlet of the proton at C-8 in Gua and the singlet at C-6 of BP in the NMR spectrum of C8G (Figure 4B). The aromatic protons were assigned following the same procedure as for the other adducts. In this case, the compound contained two isomers, as evidenced by the characteristic two shielded doublets of 5-H and the two coupled doublets of 4-H, as assigned by COSY. The presence of two isomers is further corroborated by the two doublets at 4.96 and 5.03 ppm corresponding to 1′-H. All chemical shifts of the ribose moiety are shifted upfield compared with G (Figure 4A), with the exception of the proton 2′-H. The deshielded 2′-H proton was tentatively designated on the basis that the same proton is shifted downfield in the NMR spectrum of C8dG (Figure 3B). The conclusive assignment of the 2′-H proton has been obtained by COSY, which shows that this proton is coupled with the two 1′-H doublets. The broad multiplet at 3.46–3.79 ppm contains six protons, three of which correspond to the hydroxylic groups, as determined by exchange with D_2O (Figure 4C).

BPBPGua. The presence of two BP moieties in this adduct is demonstrated by the $(M + H)^+$ ion at m/z 652 obtained by FAB (see below) and by the characteristic NH_2 signal at 6.48 ppm of the Gua moiety, which by integration relative to the other aromatic protons shows that the latter are twice that of a single BP moiety.

The NMR spectrum of this adduct shows no singlet in the region of 8.7 ppm, corresponding to the proton 6-H of the BP moiety. This suggests that both C-6's of the two BPs are involved in chemical bonds. Other recognizable features in this spectrum are the exchangeable proton at 10.99 ppm, corresponding to 1-H of Gua, and multiplets at 8.81–8.92 ppm, corresponding to four protons, namely the angular 10-H and 11-H. Furthermore, a multiplet in the high field at 7.57–7.68 ppm corresponding to four protons has been tentatively designated as the protons 5-H and 7-H due to the peri effect of substitution at C-6.

The MS data below suggest that this adduct contains two BPs linked to one another. Thus, it can be formed by electrochemical oxidation of BP, which reacts at C-6 with the BP moiety of

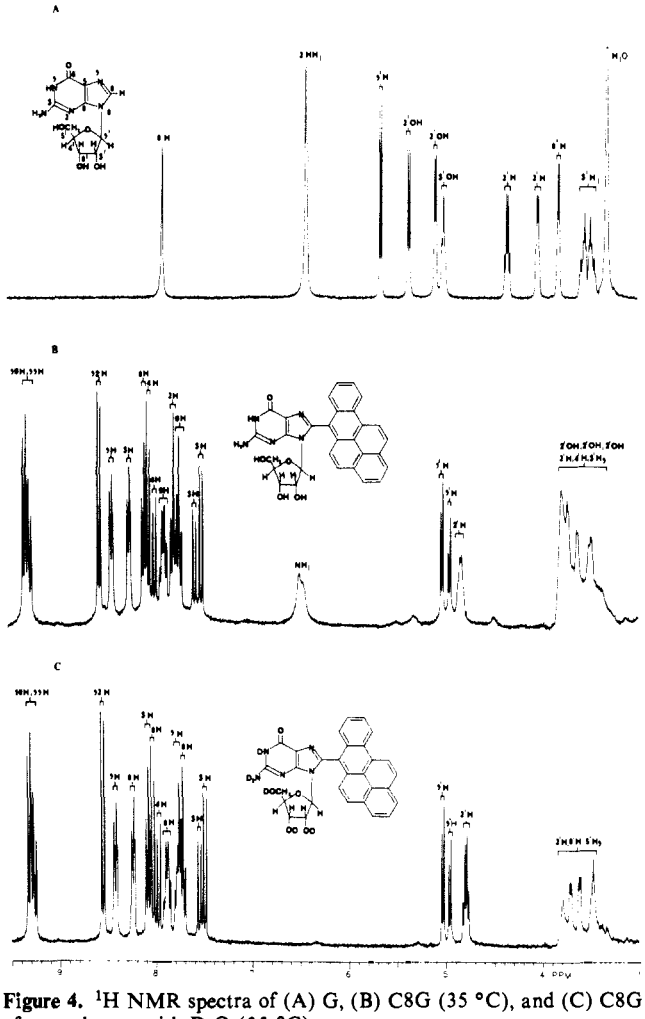

Figure 4. ^1H NMR spectra of (A) G, (B) C8G (35 °C), and (C) C8G after exchange with D_2O (35 °C).

N7Gua, or, alternatively, by electrochemical oxidation of BP dimer, which reacts with dG or G. Incidentally, BP dimer, which is produced by anodic oxidation of BP with dG or G and when BP alone is electrochemically oxidized in DMF, was identified by chromatographic analysis, HPLC, and TLC with an authentic sample of BP dimer obtained as a byproduct in the synthesis of the radical cation perchlorate of BP[39] (and unpublished results). This BP dimer is a mixture of isomers tentatively designated as linked at 6–6′, 6–1′, and 6–3′.

Electrochemical oxidation of BP (12 mg) in the presence of N7Gua (1 mg) in 25 mL of DMF did not yield any BPBPGua. Therefore, we suggest that this adduct is formed by electrochemical oxidation of BP dimer, which reacts with dG or G.

FAB of the BPBPGua adduct leads to desorption of an $(M + H)^+$ of m/z 652 (observed 652.2162, theoretical 652.2137, error 3.7 ppm), suggesting that the adduct contains two molecules of BP and one of Gua. The mass spectrum of the desorbed ions shows a peak at nearly every m/z value in the mass region m/z 350–700. The m/z 652 ion is seen with a signal-to-background ratio of 7:1. No obvious fragment ions can be assigned on the basis of the full mass spectrum.

The $(M + H)^+$ ion was submitted to CAD in the MS/MS experiment (Figure 5). The most abundant fragment ion is of m/z 400. A linked B/E_1 scan of the m/z 652 ion yields higher mass resolution than in the spectrum presented in Figure 5 and shows that the peak at m/z 400 is principally a singlet. This fragment originates by hydrogen transfer and expulsion of a neutral BP moiety. The two predominant fragment ions in the lower mass region are of m/z 251 and 277 and are probably BP^+

(39) Cremonesi, P.; Warner, C.; Cavalieri, E.; Rogan, E. *Abstracts of Papers*, 193rd National Meeting of the American Chemical Society, Denver, CO; American Chemical Society: Washington, DC, 1987; ORGN 167.

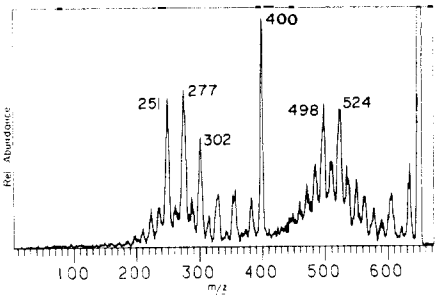

Figure 5. CAD spectrum of BPBPGua.

$(C_{20}H_{11})^+$ and BP-NC$^{\bullet+}$. These ions are also seen in the CAD spectra of the BP–Gua adducts and in the BP–dG or BP–G adducts (Figure 2). A comparison of the mass region m/z 300–400 in Figure 5 and in Figure 2 reveals that the more abundant fragments are formed from BPBPGua with m/z values in this mass region. These fragments are tentatively assigned as arising from disruption of a BP ring linked to an intact BP ring system (e.g., the ion m/z 302 is likely BP-$C_4H_3^{\bullet+}$. Thus, formation of these fragments is consistent with two BPs attached to one another. There is also a complex pattern of fragment ions in the mass region 450–600. The ions that give rise to these and adjoining peaks are formed by extensive disruption of one of the BP moieties. For example, the ions of m/z 498 and 524 may be produced by losses of $C_{10}H_8$ and $C_{10}H_{10}$, respectively, and are similar to fragmentation of simpler polycyclic aromatic compounds.[38]

HRP/H_2O_2-Catalyzed Binding of BP to DNA. When BP was bound to DNA in the HRP/H_2O_2 system, the level of binding ranged between 60 and 90 μmol/mol DNA-P. Adducts were analyzed in the ethanol/water supernatant obtained by precipitation of the DNA and in the DNA itself, digested enzymatically to deoxyribonucleosides. A number of preparations were analyzed, and reproducible chromatograms were obtained. With the acetonitrile/water gradient (Figure 6A), the supernatant, which contains adducts lost from DNA by depurination (C8Gua, N7Gua), exhibits a small amount of material chromatographing with the C-8 markers and a larger peak with N7Gua. The predominance of the N7Gua peak is expected from the destabilization of the glycosidic bond by substitution at N-7. The material chromatographing with the C8dG marker is presumably the second identified C8Gua isomer (see above).

When the supernatant was chromatographed in the methanol/water gradient (Figure 6B), unknown material with the same retention time as C8dG was detected, producing an artefactual profile. This peak was collected and analyzed by UV spectroscopy and FAB/MS. The UV spectrum showed no absorption corresponding to BP or another polycyclic aromatic moiety. No identifiable adduct peaks were observed by FAB/MS. This radioactive peak presumably corresponded to a compound obtained by tritium exchange from the [^{3}H]BP.

Proof of the structure of the N7Gua formed in the HRP/H_2O_2-catalyzed reaction was obtained by collecting the adduct from the supernatants of several large incubations and analyzing it by CAD/MS. The fragmentation pattern of the m/z 402 (Figure 2E) is identical with that of authentic N7Gua (Figure 2D). This method of analysis is sensitive enough to require only 2 μg of adduct (Figure 2D).

The profile of adducts in the DNA digest was the same in both gradient systems (Figure 7). C8dG is the largest peak observed, with small amounts of the depurinated adducts C8Gua and N7Gua. The aqueous fraction remaining after $CHCl_3$ extraction of the DNA digest was also analyzed by HPLC. Aside from a small peak eluting with the solvent front and a peak for BP, no other peaks were ever observed in the chromatography of this fraction.

By use of the peak areas observed in Figures 6A (supernatant) and 7A (DNA digest), the approximate amounts of the three adducts are compared in Table I. About 80% of the total identified adducts were found in the supernatant. The major

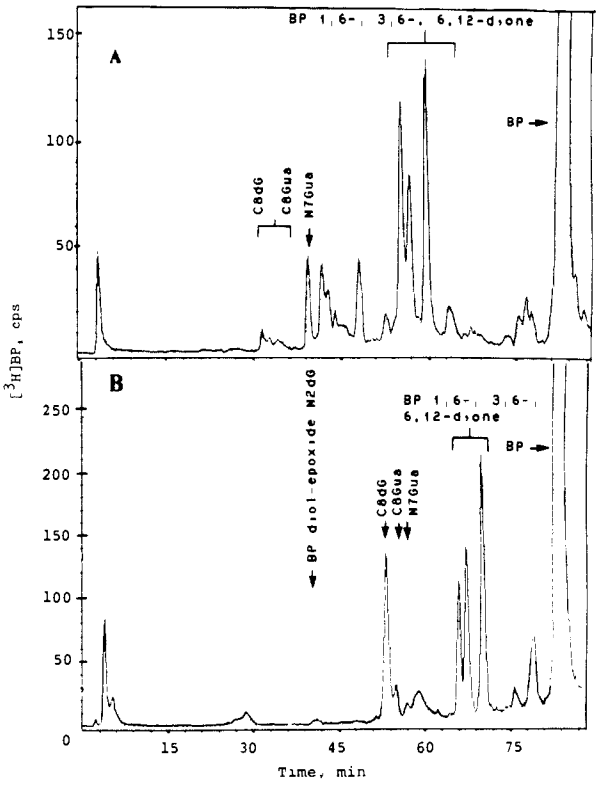

Figure 6. Profile of adducts in the supernatant fraction from HRP-catalyzed binding of BP to DNA: (A) acetonitrile/water gradient; (B) methanol/water gradient.

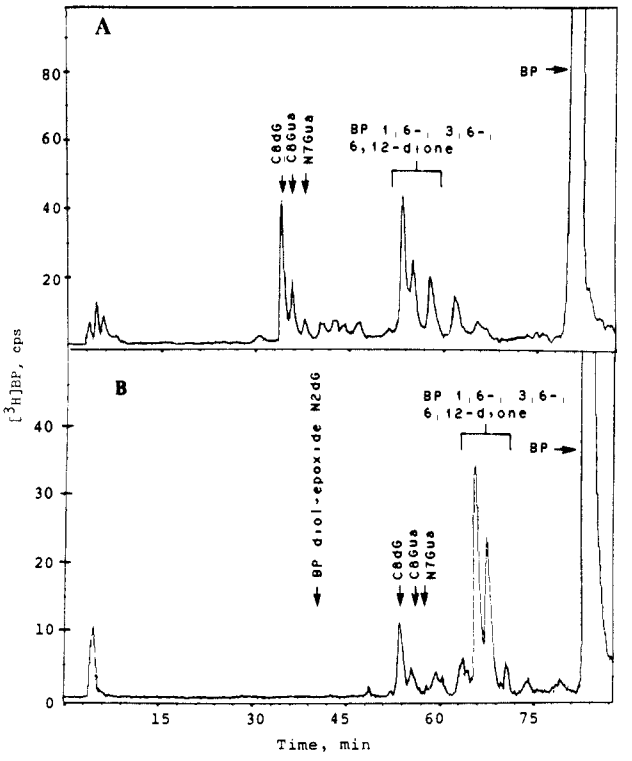

Figure 7. Profile of adducts in the DNA digest from HRP-catalyzed binding of BP to DNA; (A) acetonitrile/water gradient; (B) methanol/water gradient.

adduct formed was N7Gua, which was almost entirely lost by depurination, as expected because substitution at N-7 is known to destabilize the glycosidic bond.

Discussion

The structures of DNA adducts of chemical carcinogens provide evidence on the mechanism by which a procarcinogen is activated.

Table I. Adducts Identified in HRP-Catalyzed Binding of BP to DNA

fraction[a]	adducts, pmol		
	C8dG + C8Gua	N7Gua	total
supernatant	14 (54)[b]	43 (96)	57 (81)
DNA digest	12 (46)	2 (4)	14 (19)

[a] The aliquot of supernatant chromatographed was 2% of the total sample, whereas the aliquot of DNA digest was 9% of the total sample. [b] Percentage of adduct in each fraction.

Although the presence of particular adducts indicates that a mechanism of activation occurs, this is not sufficient to imply its role in carcinogenesis.

BP diol epoxide was found to form a DNA adduct at the exocyclic 2-amino group of dG after treatment of the target tissue mouse skin with BP.[40-42] This adduct has been interpreted as a major piece of evidence in proposing the diol epoxide as the ultimate carcinogenic form of BP. This hypothesis has been extended to include formation of bay-region diol epoxides as the ultimate carcinogenic forms of other PAH.[6,7] Further studies on the formation of adducts between diol epoxides and DNA in vitro have led to the discovery of labile adducts that are lost by depurination and depyrimidination.[31,43]

Several lines of evidence suggest that covalent binding of PAH to nucleophiles can be obtained by one-electron oxidation of the hydrocarbon.[3-5] According to this mechanism, binding of BP occurs at position 6.[20-27] Evidence for binding of BP to DNA at C-6 in mouse skin in vivo[28] and in several in vitro systems in which BP is activated by cytochrome P-450[28] and HRP/H_2O_2[29] was obtained by specific loss of tritium from C-6, although the structures of the adducts were not determined.

Identification of such adducts requires preparation of model adducts with BP bound at C-6. Anodic oxidation of BP with dG leads to binding at N-7 and C-8 of Gua. Although the N-7 adduct was anticipated to be recovered without the deoxyribose moiety, the C-8 adduct also shows some unexpected loss of the sugar. This indicates that substitution at C-8 leads to some instability of the glycosidic link. In contrast, the reaction of BP and G produces the anticipated N-7 adduct without the ribose, and the C-8 adduct is isolated almost entirely as the nucleoside. Furthermore, the N-7 and C-8 adducts can now be distinguished by the different ratios of the two fragments of m/z 277 and 251, as seen in CAD spectra (Figure 2).

BP–DNA adducts formed in the HRP-catalyzed reaction have been identified as containing BP bound at C-6 to the N-7 or C-8 position of Gua, and they are mostly lost from DNA by depurination (Table I). These adducts confirm previous results indicating binding of BP to DNA at C-6[29] and demonstrate that HRP activates BP by one-electron oxidation.

Cytochrome P-450 also catalyzes one-electron oxidation of a variety of compounds. This mechanism has been demonstrated in the metabolism of sulfides and sulfoxides, which are oxygenated to sulfoxides and sulfones, respectively, via an initial formation of a sulfinium radical intermediate.[44,45] Similarly, quadricyclane is metabolized by initial formation of a radical cation.[46] We have demonstrated that the BP quinone metabolites are obtained from BP radical cation.[47,48] Dihydropyridine[49] and cyclopropylamine[50,51] induce suicide inactivation of cytochrome P-450 via one-electron oxidation of the substrate. The same mechanism is thought to play a significant role in the hepatotoxicity of acetaminophen.[52,53]

When BP is bound to DNA by HRP[29] or cytochrome P-450,[28] loss of tritium from C-6 suggests activation of BP by one-electron oxidation. The results of this paper confirm the initial evidence that HRP catalyzes binding of BP to DNA by one-electron oxidation. We have recently obtained N7Gua from the supernatant fraction when BP and DNA are incubated with uninduced or 3-methylcholanthrene-induced rat liver microsomes (manuscript in preparation).

Identification of DNA adducts in target tissues of experimental animals treated with a carcinogen can disclose only adducts that remain as part of the DNA during the treatment and isolation procedures. Because a large portion of the BP–DNA adducts formed by one-electron oxidation is depurinated, they are very difficult to identify in the DNA of target tissues by the method of DNA isolation, digestion to nucleosides, and HPLC analysis. This method can be used in vitro because both stable and labile adducts can be recovered. Identification of labile, such as N7Gua, and moderately stable, such as C8dG, adducts formed in vivo requires other techniques, which are very sensitive and/or involve less manipulation of the DNA. For example, preparation of monoclonal antibodies reactive with specific adducts can be used to identify carcinogen–DNA adducts. For aromatic hydrocarbons, use of fluorescene line-narrowing spectrometry also represents a very specific, sensitive method of analysis[54,55] (manuscript in preparation).

Acknowledgment. This research was supported by Grants CA 25176, 32376, and 36727 from the National Cancer Institute and NIH Shared Instrumentation Grant 1S10RR01968. The mass spectrometry experiments were conducted at the Midwest Center for Mass Spectrometry, National Science Foundation Regional Facility (Grant CHE-8620177).

(40) Koreeda, M.; Moore, P. D.; Wislocki, P. G.; Levin, W.; Conney, A. H.; Yagi, H.; Jerina, D. M. *Science (Washington, D.C.)* **1978**, *199*, 778–781.
(41) Ashurst, S. W.; Cohen, G. M. *Int. J. Cancer* **1981**, *27*, 357–364.
(42) Alexandrov, K.; Rojas, M.; Bourgeois, Y.; Chouroulinkov, I. *Carcinogenesis (London)* **1983**, *4*, 1655–1657.
(43) Osborne, M.; Merrifield, K. *Chem.-Biol. Interact.* **1985**, *53*, 183–195.
(44) Watanabe, Y.; Iyanagi, T.; Oae, S. *Tetrahedron Lett.* **1980**, *21*, 3685–3688.
(45) Watanabe, Y.; Iyanagi, T.; Oae, S. *Tetrahedron Lett.* **1982**, *23*, 533–536.
(46) Stearns, R. A.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1985**, *107*, 4081–4082.
(47) Cavalieri, E.; Wong, A.; Cremonesi, P.; Warner, C.; Rogan, E. In *Polynuclear Aromatic Hydrocarbons: Chemistry, Characterization and Carcinogenesis*; Cooke, W. M., Dennis, A. J., Eds.; Battelle: Columbus, OH, 1986; pp 131–143.
(48) Cavalieri, E.; Rogan, E.; Devanesan, P.; Cremonesi, P. *Biochem. Pharmacol.*, in press.
(49) Augusto, O.; Beilan, H. S.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1982**, *257*, 11288–11295.
(50) Hanzlik, R. P.; Tullman, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 2048–2050.
(51) MacDonald, T. L.; Zirvi, K.; Burka, L. T. Peyman, P.; Guengerich, F. P. *J. Am. Chem. Soc.* **1982**, *104*, 2050–2052.
(52) Mason, R. P.; Fischer, V. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1986**, *45*, 2493–2499.
(53) Potter, D. W.; Hinson, J. A. *J. Biol. Chem.* **1987**, *262*, 966–973.
(54) Heisig, V.; Jeffrey, A. M.; McGlade, M. J.; Small, G. J. *Science (Washington, D.C.)* **1984**, *223*, 289–291.
(55) Sanders, M. J.; Cooper, R. S.; Jankowiak, R.; Small, G. J.; Heisig, V.; Jeffrey, A. M. *Anal. Chem.* **1986**, *58*, 816–820.